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The Ecology of *Neoechinorhynchus rutili* (Acanthocephala) in Scottish Freshwater Lochs

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1986.

Lassiere, O.L. (1988a). Host parasite relationships between larval *Sialis lutaria* (Megaloptera) and *Neoechinorhynchus rutili* (Acanthocephala). *Parasitology*, 97, 331-338.

Lassiere, O.L. (1988b). *Gladhouse Reservoir: brown trout health survey*. Report submitted to Lothian Regional Council, Planning Department, pp.66.

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I hereby declare that apart from the acknowlegements made above, this dissertation is my own original work.

Olivia Lassiere

9.X.89.

Olivia L. Lassiere

C O N T E N T S

Tables (appear within each chapter)

Figures (appear within each chapter)

GENERAL SUMMARY

1. The ecology of *Neoechinorhynchus rutili* (Acanthocephala) has been investigated in a Scottish population of brown trout (*Salmo trutta*) inhabiting a small highland loch, in Central region. The results have been compared with data from other hosts and localities. In addition, a complementary study on the ecology of *Echinorhynchus truttae* in brown trout was carried out at a reservoir in Lothian Region.

2. A review of the knowledge of the classification, morphology of developmental stages, life cycle, ecology and distribution and biochemistry and physiology of *Neoechinorhynchus rutili* was undertaken.

3. Examination of 1189 fish (11 species) from 8 Scottish regions and collation of available records indicated that *Neoechinorhynchus rutili* infects 8 species of freshwater fish (*Esox lucius*, *Gasterosteus aculeatus*, *Perca fluviatilis*, *Phoxinus phoxinus*, *Salmo gairdneri*, *Salmo salar*, *Salmo trutta* and *Salvelinus alpinus*) at 41 sites in 6 Scottish regions. These sites encompass a wide spectrum of aquatic environments in terms of size, water quality and faunal community structure. This wide distribution of *Neoechinorhynchus rutili* is explained through the multifarious habits of the definitive host species and possibly interactions with human, avian or insect factors. There is evidence for temporal stability of the infection at some of these sites.

4. The main field site for the examination of the ecology of *Neoechinorhynchus rutili* was Loch Maragan (Grid ref. NN 402278), Central Region. This small loch (surface area 7.3 ha, maximum depth 10.2 m, volume 153943 m^3) lies at 472 m above sea level and had slightly acid water conditions (p.H. 6.44, October 1986). Three

species of fish (*Anguilla anguilla*, *Phoxinus phoxinus* and *Salmo trutta*) were found to inhabit the loch. In August 1987 the brown trout population size was estimated, by a simple mark and recapture technique, as 2641 (1 to 3 year olds) (Maximum value).

5. Two benthic faunal surveys, carried out in November 1986 and May 1987 respectively, did not reveal the species of invertebrate which was acting as intermediate host for *Neoechinorhynchus rutili* at Loch Maragan.

6. A total of 226, between 1 and 5 years old were caught at Loch Maragan over a 25 month period between July 1986 and August 1988 and examined for visceral and gut macroparasites. Minnows (n = 207) and alder flies (*Sialis lutaria*) (larvae and adults) were also examined. *Sialis lutaria* larvae were infected with *Neoechinorhynchus rutili* and and unidentified trematode metacercaria. The minnows harboured *Neoechinorhynchus rutili*, *Ligula intestinalis* and *Crepidostomum* spp. The brown trout harboured *Capillaria salvelini*, *Crepidostomum* spp., *Diphyllobothrium ditremum* and *D.dendriticum* and *Neoechinorhynchus rutili*. The distributions of these parasitic infections amongst their hosts are described.

7. The collection of 4992 *Neoechinorhynchus rutili* from 226 brown trout, revealed the overdispersed nature of the infection (k = 0.7893), individual fish harbouring between 1 and 324 worms. The overall prevalence and mean intensity of infection were 87.6% and 21.5 respectively. Monthly prevalence values (of samples) never fell below 50%. Trout age, but not sex, influenced the infection parameters.

8. *Neoechinorhynchus rutili* exhibited a definite seasonal cycle of intensity and maturation in brown trout at Loch Maragan. Although recruitment apparently occurred throughout the year, worm intensities,

particularly of gravid females, peaked in summer when water temperatures and host feeding rates were maximal. Acanthor production occurred between March and November. In summer 1987 the *Neoechinorhynchus rutili* metapopulation (in trout aged 1 to 3 years) was estimated to be 36154, of which 5566 were gravid females. These females were estimated to have produced 1.6×10^8 shelled acanthors which represents a reproductive success rate of 3.5×10^{-3} % i.e. 1 in 38030 shelled acanthors becoming a reproductively active female in the next parasite generation. No evidence for density dependent effects upon worm fecundity were found. Similar seasonal patterns were found at other Scottish locations.

9. *Neoechinrhynchus rutili* was typically found in the ileal and rectal regions of the trout gut, but the distribution was dynamic with respect to season, worm sex, state of maturity and infection intensity.

10. No adverse effect upon the condition factor of brown trout in Loch Maragan could be attributed to the presence of *Neoechinorhynchus rutili*.

11. The diet of brown trout at Loch Maragan was analysed and *Sialis lutaria* larvae formed an important element, especially in spring. Larger trout were found to be piscivorous.

12. Acanthocephalan specimens found in the haemocoel of *Sialis lutaria* larvae collected from 2 Scottish sites (Loch Maragan and Loch Monzievaird) were identified, on the basis of their morphology, as the eoacanthocephalan *Neoechinorhynchus rutili*.

13. *Sialis lutaria* larvae were found to be infected with *Neoechinorhynchus rutili* at Loch Maragan and 3 other Scottish sites

where fish were also infected (Bridge of Weir fish farm, Drumore Loch and Loch Monzievaird). The distribution of infection was typically overdispersed (k values ranged from 0.27 to 1.25). Overall prevalence values ranged from 4.2 to 40.7%. Infection parameters varied with insect size and season.

14. Establishment of *Neoechinorhynchus rutili* in experimental infections of rainbow trout (*Salmo gairdneri*) via feeding upon infected *Sialis lutaria* larvae was up to 33% successful. Experiments to infect ostracods (*Herpetocypris reptans*) and *Sialis lutaria* larvae via feeding with shelled acanthors were unsuccessful.

15. Postcyclic transmission of *Neoechinorhynchus rutili* occurred in the laboratory when rainbow trout were exposed to worms established in the three-spined stickleback (*Gasterosteus aculeatus*) hosts. The re-establishment rate was estimated as 92.8%. There is circumstantial evidence to suggest that this form of transmission occurs in natural populations in Scotland.

16. In the complementary study at Gladhouse Reservoir, 4 visceral and gut parasites were identified in the brown trout: *Cystidicola farionis*, *Eustrongylides* sp. (Nematoda), *Eubothrium crassum* (Cestoda) and *Echinorhynchus truttae* (Acanthocephala). Both *E.crassum* and *Echinorhynchus truttae* were overdispersed in their hosts and the overall prevalences and intensities of infection were 61.9% and 92.9% and 1.48 and 156.4 respectively. *Echinorhynchus truttae* exhibited a seasonal pattern of maturation, females releasing shelled acanthors in the summer months. This study also considered the logistics of carrying out scientific research in collaboration with members of the public.

17. The appendices include a list of sites and references of the worldwide distribution of *Neoechinorhynchus rutili* in its definitive host species, a taxonomic list of these 116 species and a list of sites where one locality has several infected host species present.

CHAPTER ONE

GENERAL INTRODUCTION

The publication of the Biology of the Acanthocephala in 1985 which reviews the results of over one thousand scientific papers, theses and books, and the organisation of two acanthocephalan workshops, the first in Cambridge (1985) and the second in Exeter (1989) confirm the fact that this phylum of parasites has provided important subject material in parasitological research. Throughout the book and in the workshop discussions, the main underlying feature was that study of acanthocephalan species serves to demonstrate aspects of parasite biology in general. Although a significant volume of research has been carried out on this group there are still many areas which require further study. One area identified by both Dobson & Keymer (1985) and Kennedy (1985a) was research into the population biology and ecology of acanthocephalans particularly with respect to regulatory mechanisms and transmission dynamics. Nickol's (1985a) aspiration that future acanthocephalan research would lead to the resolution of puzzling instances of dispersal and distribution among hosts emphasised this as an important research area.

Dobson & Keymer (1985) formulated a simple theoretical model to describe acanthocephalan population dynamics and found that a major stumbling block was a scarcity of empirical data to work with. They stated that only through the development of models based on field observations and complementary laboratory studies examining temperature dependence of physiological processes would it be possible to test their quantitative predictions against long-term data sets collected under natural conditions. This objective can only be realistically fulfilled if the biology of the chosen model parasite species is well researched and all aspects of its life history fully elucidated.

Thus in October 1985, when this research began, the consensus was

that a more detailed study of the population dynamics of a specific host-parasite system was required to improve the understanding of acanthocephalan population dynamics. The host parasite system that was initially selected was one involving the eoacanthocephalan, *Neoechinorhynchus rutili* (parasite) and the three-spined stickleback, *Gasterosteus aculeatus*, (host). This was considered to be a suitable choice since *N.rutili* is one of the 6 species of acanthocephalan known to infect British freshwater fish (Brown, Chubb & Veltkamp, 1986) and *G.aculeatus* is widely distributed throughout the British Isles (Wheeler, 1969). Since *G.aculeatus* is a small, hardy fish it would be an ideal species with which to carry out laboratory based infection experiments. Additionally, background information was available from studies of the dynamics of this system in English populations (Walkey, 1967; Chappell, 1969b, c). In Scotland, there were records of the occurrence of *N.rutili* in *G.aculeatus* but no population studies had been carried out (Ritchie, 1915; Turnbull, 1958; Pike & Edwards, 1983). Although this autoecological approach to research has been criticised in the past (Kennedy, 1981) its value has been recently recognised (Brown & Roughgarden, 1989) in the context of a wider forum with respect to the monitoring of global change where they see such studies as the building blocks of integrated studies. In the context of the present study, this approach was deemed the only way possible by which the population dynamics could be feasibly examined. One common feature of previous examinations of acanthocephalan population dynamics in fish hosts was a dearth of information about the host population itself, in terms of its size and composition. This was considered to be an essential element of the proposed study.

Extensive fieldwork in the Glasgow area during the first year of study (October 1985 to October 1986) failed to reveal any sites of infection of *Gasterosteus aculeatus*. During this period several sites

of infection of brown trout, *Salmo trutta* were found, in addition to the ten sites reported in the literature, and so the emphasis of the project was switched to a host-parasite system involving brown trout and *N.rutili*. Two previous studies on the population dynamics of this system by Robertson (1953) and Bwathondi (1976), although limited in duration and to the consideration of infections of fairly low intensity in the definitive hosts, provided some background information. In addition, this switch of system provided the opportunity to examine any possible adverse effects of *N.rutili* infection upon natural brown trout populations which are considered to be a national resource in Scotland (Campbell, 1971; Ellis, 1989).

Unfortunately, none of the Scottish records of *N.rutili* in various definitive host species at number of locations included an indentification of the intermediate host species although Robertson (1953) did mention finding a single alder fly (*Sialis lutaria*) larva infected with a juvenile *N.rutili* specimen, that was collected in Lochan an Daim, Tayside. In the 1960's the work of Merritt & Pratt (1964) in Oregon, U.S.A. and Walkey (1967) in County Durham, England, led to the conclusion that the life cycle was of the typical simple eoacanthocephalan type, involving crustaceans, specifically ostracods, as the single intermediate hosts (Bullock, 1969). The finding of Valtonen (1979) of *N.rutili* utilizing ostracods in its life cycle involving whitefish (*Coregonus nasus*) in the Bothnian Bay, Finland, further consolidated this view. However, it was unclear as to whether ostracods were involved in Scotland. Population dynamic studies were started without the knowledge of which invertebrates were acting as the intermediate hosts for *N.rutili* in Scotland. Hence an infected population of *Salmo trutta* was selected and analysis of diet in conjunction with an analysis of the benthic fauna was carried out to

identify the intermediate hosts while monthly samples of the trout were collected to examine the population dynamics of the worm in its definitive host. Concurrently, it was the intention to make and estimate of the brown trout population (at Loch Maragan) such that the size of the host samples required to give a representative impression of the parasite population could be determined. The estimation of brown trout populations in small highland lochs had apparently not been done previously in Scotland.

As well as being an important element of the mechanism by which parasite populations are regulated, the effect that a parasite has on its host, in the case of brown trout, is also of interest in terms of the conservation and management of healthy stocks. Brown trout have been described as "the basis of a national pastime and a tourist attraction of considerable importance" (Campbell, 1971) in Scotland and it is therefore of interest to know the potential adverse effects of parasites on their survival and fecundity. In times of limited employment opportunities in Britain and the prospect of the reduced working week the maintenance of healthy stocks of fish available for leisuretime fishing is also an important social issue. From the economic viewpoint many trout in fish farms are also infected with *N.rutili* which are subsequently used to stock natural water bodies. It seems obvious that appropriate legislation with respect to the stocking of waters with trout can only be formulated when it is based on the results of apparently less applied academic research projects. Indeed, Mills (1989) identified the release of hatchery reared trout as one of a number of important management issues, but only from the point of view of maintaining local genetically different populations. Perhaps this should be extended to include the possible adverse effects of parasite dispersal. If trout stocks are to be maintained in Scotland, scientific advice should be more easily available and Mills

(1989) envisaged that this could be brought about by the setting up of a government fishery advisory service and the formation of a Brown Trout Trust.

During the course of this research the opportunity to examine the population dynamics of a second acanthocephalan species, *Echinorhynchus truttae*, infecting brown trout at Gladhouse Reservoir, Lothian Region, arose. At this site the brown trout were reported to be 100% infected with this parasite by the fishermen. Therefore a project was set up in conjunction with A.D. Jamieson of Lothian Regional Council, Planning Department in the summer of 1987 to investigate this claim. Our aim was to utilize the manpower provided by the fishermen to collect sample material in order to compare the overall pattern of the population dynamics of this parasite with that of *N.rutili* at other sites. This project also provided the opportunity to examine brown trout diets in a lowland reservoir and to examine the importance of the 500 introduced brown trout to the population dynamics of the acanthocephalan population and more generally to the quality of fishing. These fish were made specifically identifiable by a tattoo. In addition we hoped to assess the value and logistical problems associated with carrying out research with members of the public.

In summary, the main objectives of my research were:

1.1. To identify and study the intermediate host of *N.rutili* in Scottish fish populations through the analysis of the diets of infected definitive hosts from a number of localities over a number of seasons.

1.2. To examine experimentally the possibility of postcyclic transmission of *N.rutili* and to consider its potential in natural populations through the analysis of the diet of potential hosts in the

wild.

1.3. To examine the geographical distribution of *N.rutili* in Scotland and utilize information from 1.1 and 1.2 to assess the possible routes of transmission and dispersal which may have brought about the observed distribution.

1.4. To investigate the population dynamics of *N.rutili* and *Echinorhynchus truttae* infecting freshwater fish in Scotland.

CHAPTER TWO

NEOECHINORHYNCHUS RUTILI: GENERAL REVIEW

2.1. INTRODUCTION

The main part of my research concentrated on the ecology of *Neoechinorhynchus rutili* and possible modes for its transmission between hosts. However, there is a large body of research on other aspects of the biology of *N.rutili*. The aim of this chapter is to review and summarise the more important references.

2.2. CLASSIFICATION

Amin (1985a) discussed the chronology of the development of acanthocephalan taxonomy and classified *N.rutili* as follows:

Class:	Eoacanthocephala	Van Cleave 1936
Order:	Neoechinorhychida	Southwell & Macfie 1925 (Neoacanthocephala Van Cleave 1963)
Family:	Neoechinorhynchidae	Ward 1917 (Hebesomidae Van Cleave 1928, Hebesomitidae Yamaguti 1963)
Subfamily:	Neoechinorhynchinae	Travassos 1926
Genus:	*Neoechinorhynchus*	Stiles & Hassall 1905 (*Neorhynchus* Hamman 1892, *Eorhynchus* Hamman 1892, *Eosentis* Van Cleave 1914)
Species:	*rutili*	Muller (1780) Stiles & Hassall 1905 (type) (*Echinorhynchus rutili*, Muller 1780; *E.cobiditis* Gemilin, 1791, *E.clavaeceps* Zeder, 1800)

Additionally, *N.rutili* has a number of synonyms which appear in the literature and include:

Echinorhynchus rutili	Muller 1780
Neorhynchus rutili	Muller 1780, Van Cleave 1914
Eorhynchus rutili	Muller 1780
Neorhynchus clavaeceps	Zeder 1780
Echinorhynchus tuberosus	Zeder 1803
Echinorhynchus cobitinus	Schrank 1780
Echinorhynchus cobitidus	Gmelin 1781
Echinorhynchus clavaeceps	Zeder 1800

2.3. MORPHOLOGY OF DEVELOPMENTAL STAGES OF *NEOECHINORHYNCHUS RUTILI*

Neoechinorhynchus rutili is generally assumed to exhibit typical acanthocephalan life cycle involving 3 infrapopulations: shelled acanthors in the hosts' aquatic environment, larvae developing in arthropod intermediate hosts and dioecious adult worms in the alimentary tract of the fish definitive host. The morphology of each of these stages is discussed below.

2.3.1. Acanthor

According to Schmidt (1985), the only report of the early embryology of an eoacanthocephalan was by Meyer (1931) for *N.rutili*. He traced the cleavage and cell lineage up to the development of the shelled acanthor which is infective to intermediate hosts. He described the development of *N.rutili* as being characterized, in the initial period by distinct quadrant-type division, which then proceeded unevenly. Gastrulation is epibolic and the embryonic development results in an embryonic larva enclosed in 3 relatively delicate oval membranes. H. Taraschewski (p.c.) reported that there were polysaccharides between these layers in acanthors of other *Neoechinorhynchus* species and thought that this may encourage feeding by a potential intermediate host. Merritt & Pratt (1964) described the mature eggs as being oval and usually measuring 27 x 17 um with the acanthor itself measuring 24 x 9 um inside the shell. The acanthor has no spines or rostellar hooks (Petrochenko, 1956). Further measurements were given by Meyer (1932), Van Cleave & Lynch (1950), Petrochenko (1956) and Yamaguti (1963) and are all within the same range as Merritt & Pratt's (1964) description. Walkey (1963) described the eggs as being eliptical in shape and twice as long as broad with a mean length of 29.6 um with the freed acanthor measuring 30 um in length. Shelled acanthors from *N.rutili* from brown trout in Scotland were 31 x

20 um in dimensions (personal observation).

2.3.2. Acanthella

The name acanthella refers to the stage of the worm which has infected the intermediate host. Merritt & Pratt (1964) followed the infection of *N.rutili* acanthors in the ostracod *Cypria turneri*, which was the intermediate host at their site, Suttle Lake, Jefferson County, Oregon, U.S.A. They made a detailed study of the development of the acanthella up to the cystacanth stage and made measurments and drawings of acanthellae of different ages. They described the juvenile or cystacanth stage as being 400 to 1000 um long (proboscis inverted), as possessing all the structures characteristic of the adult and that there was some sexual dimorphism, females being noticably longer than males.

2.3.3. Adult

The body of adult *N.rutili* is divided into 2 major regions; the presoma (proboscis + neck) and the metasoma (main body/trunk). *Neoechinorhynchus rutili* has many of the features characteristic of the class Eoacanthocephala (or class Neoechinorhynchidea according to Yamaguti, 1963) which include; small body size, invaginable proboscis with a comparatively small number of hooks, trunk not spined, few hypodermic nuclei which are either large and amoeboid or fragmented, the proboscis receptacle with a single layered wall, no protonephridial organs and a syncytial cement gland. In addition, Bullock (1969) included aquatic habitat of host, a lacunar system with the main longitudinal vessel dorsal and ventral (at least anteriorly), ligament sacs dorsal and ventral and crustaceans as the intermediate hosts as characteristic of this class.

Many workers have described the adult morphology of *N.rutili* with

varying degrees of attention to detail (e.g. Meyer, 1932; Van Cleave & Lynch, 1950; Petrochenko, 1956; Golvan, 1959; Bykhovskaya *et al.*, 1962 and Yamaguti, 1963) and a representative cross-section of these are presented below.

2.3.3.1. *Body shape and dimensions*

Details of some of the published accounts of the measurements and description of *N.rutili* are summarized (Table 2.1). My own measurements of *N.rutili* from various Scottish hosts are shown in Table 7.3.

Table 2.1. *Neoechinorhynchus rutili*: body shape and dimensions

Length mm	Width mm	Shape	Reference
f: 5-10 m: 2- 6	0.4-1.0	Body curved, bent ventrally. Narrowing gradually from front to back.	Meyer (1932)
f:2.1-10 m:1.5- 6	f:0.3-0.8 m:0.25-0.63	Body arched, anterior and posterior extremities gradually bent ventrally.	Van Cleave & Lynch (1950)
f:4.4-7.7 m:4.1-4.5	f:0.74-0.82 m:0.44-0.45	Fusiform parasites. Greatest width in anterior third of body. Body slightly bent on one of its sides. No spines body wall.	Petrochenko (1956)
-	-	Body thornless, of small size almost always bent on the ventral side.	Golvan (1959)
f:2.1-10 m:1.5- 6	f:0.30-0.82 m:0.25-0.60	Fusiform body curving ventrally.	Bykhovskaya *et al* (1962)

(f = female, m = male)

These measurements and descriptions should be taken with caution since considerable modification due to shrinkage under different methods of preservation are possible (Van Cleave & Lynch, 1950). Therefore, a standardized method of preparation, as suggested by

Bullock (1969) would be useful to allow for comparisons between material of different origin.

2.3.3.2. *Proboscis and hooks*

Details of some of the published measurements and descriptions of the proboscis morphology of *N.rutili* are given (Table 2.2).

Table 2.2. *Neoechinorhynchus rutili*: proboscis morphology

Length um	Width um	Sheath um	Shape	Hooks Number/ length um	Reference
-	-	-	-	6 x 3	Meyer (1932)
f:90-132 m:79-120	f:93-132 m:70-120	- -	Short, cylindroid when fully protruded but approaching globular	18 T:64-84 M:31-46 B:22-34	Van Cleave & Lynch (1950)
f:146-152 m:123-136	f:100-123 m: 97-110	230-380	A rounded oval	T:52-61 M:29-42 B:23-32	Petrochenko (1956)
-	-	-	Short, small subglobular	6 x 3	Golvan (1959)
f:130-150 m: 80-140	f:100-120 m: 90-110	-	Small & round	T:45-79 M:26-42 B:12-32	Bykhovskaya *et al* (1962)

(f = female, m = male, T = terminal, M = median, B = basal)

Where hook measurements are given these are for female worms.

Meyer (1932), Van Cleave & Lynch (1950) and Petrochenko (1956) produced drawings of the lateral view of the proboscis and Golvan

(1959) of the anterior view showing the quincunxial arrangement of the hooks.

2.3.3.3. *Tegument*

No specific work has been carried out on the tegument of *N.rutili*, however it is likely that it conforms to the typical acanthocephalan pattern as first described by Crompton & Lee (1965) using the electron microscope for the palaeacanthocephalan *Polymorphus minutus*.

2.3.3.4. *Lacunar system*

In the genus *Neoechinorhynchus* the principle canals of the lacunar system are dorsal and ventral and united by transverse pseudometamerically arranged anastomoses (Golvan, 1959).

2.3.3.5. *Lemnisci*

The lemnisci, which are 2 diverticula formed by the invagination of the inner layers of the body wall, have been described for *N.rutili* by several authors (e.g. Petrochenko, 1956; Bykhovskaya *et al*, 1962). Bykhovskaya *et al* (1962) stated that the lemnisci contain 1-2 giant nuclei. In female worms the lemnisci are 1.30-1.33mm long and in males 0.70-1.52mm long.

2.3.3.6. *Giant nuclei*

The giant nuclei are so consistent in size, number and location they are used as a taxonomic character for *N.rutili*. They are found embedded in the radial layer of the hypodermis. Van Cleave & Lynch (1950) described their distribution as 'nuclei giant in trunk, 5 in mid-dorsal line and a single one ventrally in the sagittal plane at a position about a quarter of the trunk length from the anterior end'. Similarly, Petrochenko (1956) described their distribution as '5 on one side and 1 or 2 on the other'.

2.3.3.7. *Nervous system*

The fullest and best illustrations of the nervous system of the Acanthocephala, including that of *N.rutili*, were produced by Yarzhinskii (1868) (according to Petrochenko, 1956).

2.3.3.8. *Excretory system*

Neoechinorhynchus rutili has no discrete excretory organs or system and it must be assumed that metabolic waste products are removed by diffusion across the membranes of the cuticular pores. Little is known of their excretory products, but Dunagan (1964) reported the production of lactate in *Neoechinorhynchus* spp.

2.3.3.9. *Reproductive system*

2.3.3.9.1. *Female*

Van Cleave & Lynch (1950) described the female reproductive system of *N.rutili* as 'genital tract very short, in fully mature individuals only a little more than 0.3 mm long. Body cavity, especially in posterior region, clearly separated into a dorsal and ventral ligament sac holding the developing embryos. The uterine funnel opens directly into the ventral sac. Posterior extremity of females often somewhat obliquely truncated with the genital orifice near the ventral margin'. These authors also produced drawings of the female worms from different hosts and localities. The detailed morphology of the uterine bell was studied by Yarzhinskii (1868).

My own transmission electron micrographs from sections of adult female worms from brown trout revealed that the ovarian ball structure was similar to that described by Peura, Valtonen & Crompton (1982) for *Corynosoma semerme*, with the each individual oocyte being surrounded by a multinucleate syncytium. This aspect of *N.rutili* fine structure requires further study.

2.3.3.9.2. *Male*

Van Cleave & Lynch (1950) described the male reproductive system as follows: 'In small worms the testes reach nearly up to the proboscis receptacle but in large worms the genital organs occupy less than half the length of the trunk and in diameter practically fill the cavity. Testes in broad contact with each other. Cement glands relatively short, nearly the same diameter as the testes and slightly longer than broad. Cement reservoir conspicuously rounded, commonly surrounded for almost half of its length by the overhanging posterior margin of the cement gland. Cement ducts relatively short, not much longer that the length of the cement reservoir. Copulatory bursa relatively weak, without heavy musculature'.

Petrochenko (1956) describes the male reproductive system as 'testes slightly elongated, approximately equal, though sometimes the anterior slightly larger than the posterior, 0.55-0.67 mm long, 0.33-0.34 mm wide. Testes transparent, but clearly visible in preparations clarified in glycerin. Cement glands dark brown, syncytial, occupying about 0.78 mm of the body length. Bykhovskaya *et al.* (1962) described it as 'testes oval, about 0.16-0.67 x 0.24-0.34 mm. Dark syncytial cement glands with lengths of 0.24-0.78 mm. Bieler (1913) produced detailed drawings of the cement gland of *N.rutili* specimens from the burbot, *Lota lota*.

The fine structure of *N.rutili* spermatozoa has not been examined although Marchand & Mattei (1977, 1978 a,b & 1979) have considered that of the related species *Neoechinorhynchus agilis*. My own examinations of transmission electron micrographs of sections of ovarian tissue from inseminated adult female worms from brown trout hosts gave an impression of spermatozoan flagellar structure. The flagellae in transverse section had the typical 9:2 arrangement of

microtubules which differs from 9:3 structure in *N.agilis* as reported by Marchand & Mattei (1977, 1978 a,b & 1979). This obviously requires further investigation.

2.4. VARIABILITY IN MORPHOLOGY

The published literature indicates that there is variability in the morphology of *N.rutili* from different sources. Van Cleave & Lynch (1950) made drawings of the proboscides and whole adult male and female specimens of *N.rutili* from their North American and Finnish collections. They concluded that no consistent differences had been found which could safely be attributed as a response to a given host species. More recently, Skryabina (1978) studied the morphology of 602 specimens of *N.rutili* from 10 species of fish (*Leuciscus idus*, *Thymallus thymallus*, *Thymallus arcticus*, *Coregonus articus sardinella*, *C.nasus*, *C.peled*, *C.lavaretus*, *Esox lucius*, *Phoxinus percnurus* and *P.czekanowskii*) from water bodies of the Kola peninsula, the Ob, Yenisei, Lena, Kolyma and Chauna rivers in the U.S.S.R. He showed that all absolute specific characters considered (13 in females and 17 in males) have a considerable range of variations due to modificational, individual and age variability. Some differences were between members of the European and Siberian populations of *N.rutili* were probably due to the geographic variability of the species. The most constant characters were length of the proboscis and its hooks. By contrast, Amin & Redlin (1980) working on *Echinorhynchus salmonis* found that the sex and age of the various host fish (*Coregonus hoyi* and *Osmerus mordax*) considerably affected worm body form and size as well as size of proboscis, proboscis hooks, proboscis receptacle, lemnisci, testes and cement glands. The wide distribution of *Neoechinorhynchus rutili* in a number of fish species in Scotland (see Chapter 3) would provide excellent material for further investigation of this phenomenon.

2.5. LIFE CYCLE

As in all acanthocephalans, the adult worms of *N.rutili* are dioecious, living attached to the gut wall, posterior to the stomach in their definitive host. Following copulation the eggs are retained in the female body cavity until the shelled acanthor stage is reached. Traditionally it has been thought that these are liberated by the parent female and pass to the exterior in the faeces of the fish host. These acanthors are then fed upon by suitable intermediate hosts (see Table 4.7 p.80 for reported species) and develop as described by Merritt & Pratt (1964) into the cystacanth stage which is infective to the definitive host. Various times for this development have been quoted. Merritt & Pratt (1964) quote 48 to 57 days at 15°C in the ostracod *Cypria turneri*. Walkey (1967) found that between 20 and 30 days were necessary to complete development in the ostracods *Cypria ophthalmica* and *Candona candida* at 18°C.

At the start of this project the general concensus about the life cycle was that no demonstrable secondary intermediate hosts existed (Walkey, 1967) and was thus simple involving only ostracod intermediate hosts and fish definitive hosts (Fig. 2.1). The observation by Merritt & Pratt (1964) of cystacanth stages in the crayfish, *Pacifastacus trowbridgi* from Suttle Lake Oregon were discounted as representing paratenic hosts and not playing an essential role in the life cycle. The investigations into the life cycle (Merritt & Pratt, 1964; Walkey, 1967; Valtonen, 1979) indicated that no other forms of transmission besides that via ostracods took place in their respective localities. This aspect of the biology is dealt with in Chapters 7 and 8.

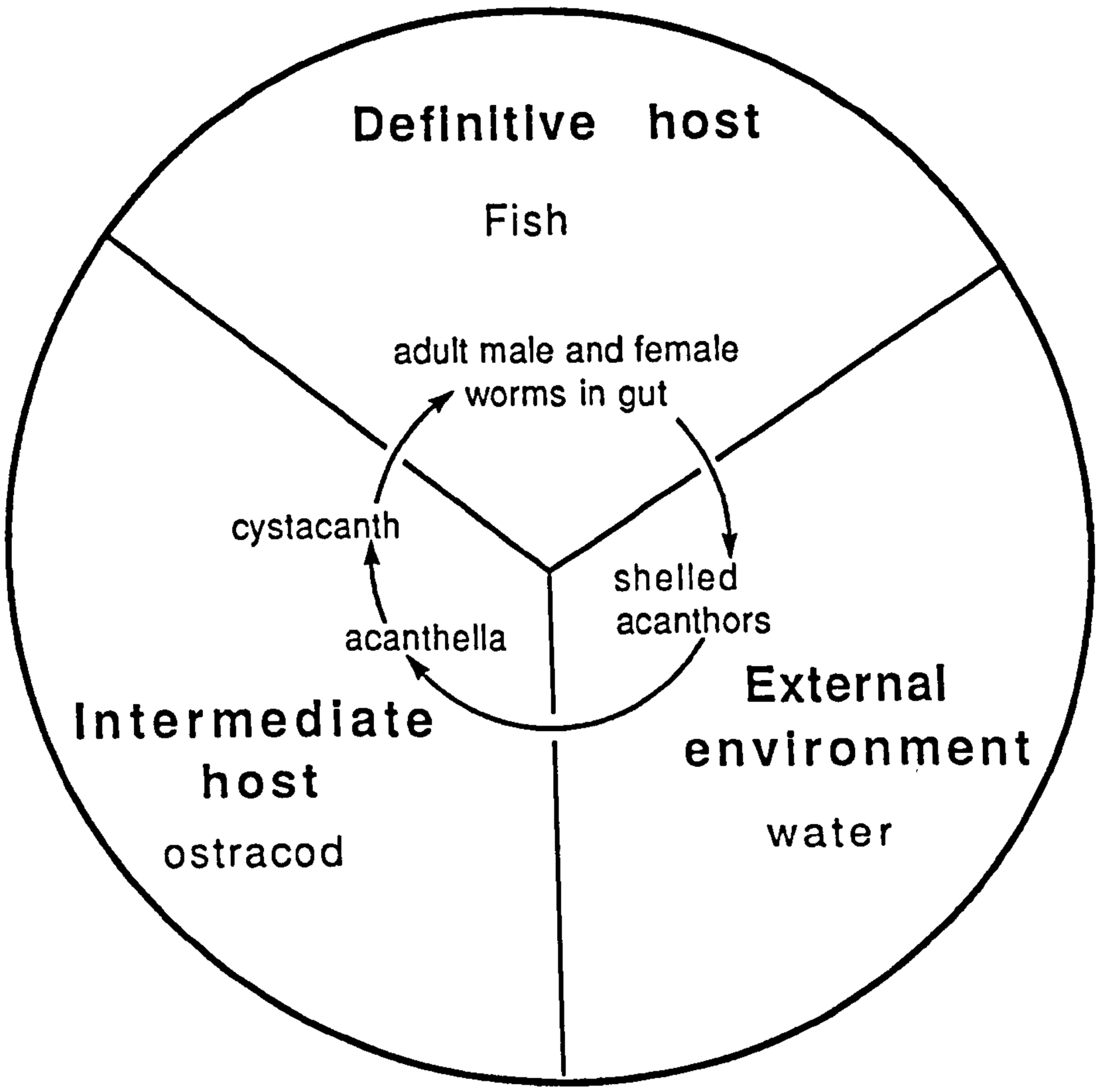


Fig 2.1 Diagrammatic representation of the life cycle of *Neoechinorhynchus rutili* as envisaged by Merritt & Pratt (1964) and Walkey (1967)

2.6. ECOLOGY AND DISTRIBUTION

In 1950 Van Cleave & Lynch published a definitive paper entitled 'The circumpolar distribution of *Neoechinorhynchus rutili*, an acanthocephalan parasite of fresh-water fishes' in the Transactions of the American Microscopical Society. From a large number of observations they had established that *N.rutili* was known to occur in freshwater fishes of Central Europe, extending northwards into Russia, Finland and Sweden. In the New World, it had been found in Wisconsin, in Washington, off the coast of Alaska and within the Arctic Circle of the Canadian Northwest Territories. Unfortunately at that time there was no proof as to whether the distribution between the 2 continental masses was uninterrupted or involved a corridor through either Siberia or Greenland. Petrochenko (1956) found *N.rutili* in the Siberian District of the U.S.S.R. which closes Van Cleave & Lynch's (1950) corridor and seems to confirm their opinion that *N.rutili* is a parasite which has circumpolar distribution in the Northern Hemisphere.

Van Cleave & Lynch (1950) described *N.rutili* as a characteristically freshwater species despite some of their records being from fish taken at the mouth of streams or in brackish coastal waters. Interestingly, Petrochenko (1956) indicated that *N.rutili* occurs in fish from both fresh and salt water in the U.S.S.R. Therefore, the impression that *N.rutili* is characteristic of freshwater species may be a false one simply due to the unequal distribution of ichthyoparasitolgists who study the freshwater and marine environments. *Neoechinorhynchus rutili* is not restricted to low lying geographical locations and there have been several reports of finding this worm in hosts at altitude. Heitz (1917, 1918) recorded its occurrence in salmon at an elevation of 1560m in a river in Switzerland and Datta (1936) claimed to have found it in *Noemacheilus*

spp. from Indian Tibet (alt. 16082 ft) and Kashmir (alt. 5160ft).

To date I have compiled over 100 references to the geographical distribution of *N.rutili* in 116 species of fish which further consolidates the original claim of Van Cleave & Lynch (1950) for its circumpolar distribution (see Appendices I & II). The possible mechanisms by which such a wide distribution has occurred are discussed in the context of Scottish distribution patterns in Chapter 3.

The reports of the seasonal distribution patterns of *N.rutili* have been reviewed by Chubb (1982) for definitive hosts inhabiting the climatic subregions humid warm summers, humid cool summers, marine west coast, semi-desert and subpolar in the mid latitude region and in the mountain climatic region. He did not find any seasonal data about hosts from the tropical, subtropical or polar world climatic regions. Despite variable patterns of prevalence and intensity within and between climatic regions he did find that where detailed assessments of the maturity stages were made, regardless of climatic zone, the majority were gravid in spring and summer. He attributed the local specific patterns to a combination of variable factors, both abiotic and biotic which influenced the population dynamics of the parasite either indirectly or directly. This aspect of the ecology of *N.rutili* in a population inhabiting brown trout is dealt with in Chapters 5 and 6.

2.7. BIOCHEMISTRY AND PHYSIOLOGY

Specific information about the biochemistry and physiology of *N.rutili* is virtually non-existent but there are a few scattered references to other members of the genus.

2.7.1. Feeding and nutritional physiology

Since all acanthocephalans including species of *Neoechinorhynchus* have no alimentary canal it seems logical that the parasite surface would be the major site of nutrient uptake. Various pieces of biochemical evidence have shown the surface of the tegument of *Neoechinorhynchus* spp. to be important in this process. For example, Uglem & Beck (1972) found aminopeptidase activities against a variety of artificial substrates in homogenates of *Neoechinorhynchus cristatus*, *N.crassus* and *N.emydis*. Aminopeptidase activity was detected in the apical region of the *N.cristatus* tegument histochemically, and hydrolysis of some peptides by intact *N.cristatus* *in vitro* suggested that the activity was exposed at the tegument surface (Uglem, 1972). Dunagan (1962) also obtained tenuous evidence suggesting dissaccharidase activities hydrolysing maltose and trehalose on the surface of a species of *Neoechinorhynchus* from turtles.

The question of how solutes are able to move across the plasma membrane of the parasite has not been fully answered. Evidence for absorption of carbohydrate was found by Dunagan (1962) but none has been found for absorption of amino acids, lipid or nucleosides in this genus. The best evidence for the existence of active cation transport in these worms seems to be their capacity to withstand a variety of osmotic conditions. Species of *Neoechinorhynchus* maintained in 85-145mM sodium chloride solutions containing small amounts of calcium chloride managed to retain normal tissue hydration levels for 2-4 days and to survive for as long as 2 weeks (Gettier, 1942; Van Cleave & Ross, 1944). Although species of *Neoechinorhynchus* are apparently equipped with various mechanisms for obtaining nutrients they are able to survive for long periods without food. Van Cleave & Ross (1944) found that *Neoechinorhynchus* spp. were able to survive in unfed

turtle hosts and Dunagan (1964) found that in a similar host-parasite system the worms retained at least marginal glycogen reserves for longer than 16 days. This may be reflect reduced metabolic rates in worms from poikilothermic hosts or an adaptation to the long periods of fasting undergone by such hosts.

2.7.2. Carbohydrate metabolism and energy production

Acanthocephalans depend upon anaerobic carbohydrate metabolism to obtain energy and therefore they store polysaccharides, most commonly glycogen, which can be metabolized without oxygen to yield some energy. There have been several reports of glycogen in worms from the genus *Neoechinorhynchus*. Glycogen levels approaching 20% of the dry mass of adult worms have been reported (Dunagan, 1964). Using histological techniques, Bullock (1949) found that in *N.cylindratus* and *N.emydis*, glycogen was concentrated in the subcuticula, the reproductive organs, the lacunar system, the ganglia and the lemnisci.

Acanthocephalans metabolize carbohydrates by glycolysis and a number of the enzymes in the glycolytic pathway have been isolated from members of the genus *Neoechinorhynchus* including aldolase (Dunagan, 1964) and lactate dehydrogenase (Saxon & Dunagan, 1976). A number of the enzymes involved in the pentose phosphate pathway in *Neoechinorhynchus* spp. from turtles have been investigated by Saxon & Dunagan (1976) and there is some indication that for some enzymes alternative summer and winter isomers exist.

2.7.3. Lipids

Bullock (1949) carried out some histological studies on the distribution of 'fatty substances' in *N.cylindratus* and *N.emydis* and found that the distribution in the worm was similar to that of glycogen (see section 2.7.2). Barrett & Butterworth (1973) found

lutein, a xanthophyll derivative of alpha carotene, dissolved in the lipid of *N.pseudemydis*.

2.7.4. Culturing *Neoechinorhynchus* species

2.7.4.1. *In vivo*

Merritt & Pratt (1964) developed a successful method of maintaining the life cycle of *N.rutili* in the laboratory. Gravid female worms were obtained from the intestines of a number of fish hosts (*Salmo trutta*, *S.gairdneri*, *Oncorhynchus nerka nerka* and *Prosopium transmontanus*). The acanthors were removed from the female worms, washed in tap water, centrifuged and rewashed. Acanthors remained viable for 6 months under refrigeration.

A supply of living ostracods (*Cypria turneri*) was maintained in refrigerated conditions in well aerated aquaria, along with original lake sediment as a substrate. Uninfected ostracods were placed in small finger bowls containing a suspension of shelled acanthors and allowed to feed for 6 to 8 hours after which the ostracods were removed to larger, well aerated finger bowls maintained at 15°C. Infected ostracods were introduced into uninfected cuttthroat trout (*Salmo clarkii*) via a pipette in the oesophagus which resulted in successful infections.

2.7.4.2. *In vitro*

There have been a number of attempts to maintain species of *Neoechinorhynchus in vitro*. Gettier (1942) found that *N.emydis* from terrapins, lived for up to 20 days in 0.5-0.7% sodium chloride solution. Solutions of potassium chloride, magnesium chloride and calcium chloride were less satisfactory, but the addition of traces of $CaCl_2$ to 0.5% NaCl prolonged survival. However, under these conditions normal tissue hydration levels were only maintained for 2 to 4 days. Van Cleave & Ross (1944) experimented with a number of different

concentrations of NaCl solution on *N.emydis*. In 0.75% NaCl the worms lived for 9-13 days, but they became turgid; in 0.85% NaCl the worms survived for 12-14 days but the body surface became wrinkled. In 0.8% NaCl they died after 10 days. They also noted that worms appeared to survive longer in solutions from which contact with free atmosphere was excluded. They concluded that a solution between 0.8 and 0.85% was required to maintain the normal flattened shape of the worm.

The technique by which Dunagan (1962) cultured *Neoechinorhynchus emydis* and *N.pseudemydis* is summarised in Crompton & Lassiere (1987). For example, *N.emydis* maintained in Eagle's HeLa medium (after sterilization) remained motile for between 26 and 96 days. No growth was observed, but copulation and egg maturation occurred.

There are apparently no reports of the *in vitro* culture of *N.rutili*. I carried out some simple observations of both *N.rutili* and *Echinorhynchus truttae in vitro* maintenance. Specimens of *N.rutili* collected from brown trout were kept in the refridgerator at 8-10°C in Petri dishes filled with 0.85% NaCl solution. In 1 group male worms became swollen and immobile after 12 days but some females maintained the normal flattened shape and actively everted their proboscides when stimulated with a seeker. In another trial, females were found in the same condition after 35 days.

In similar trials with *Echinorhynchus truttae* from brown trout in 0.9% NaCl solution at 8-10°C, active proboscis movement was seen after 26 days. In this case the worms were kept at a fairly high density and worms were found knotted together on occasion and acanthors were found on the culture dish base. In another trial some female worms were found alive after 55 days. These living, apparently normal worms were found amongst obviously dead, turgid individuals. This could possibly have been the result of differential handling damage.

Clearly there is a great deal of scope for the improvement in culture methods of *Neoechinorhynchus* species. Once a successful method is devised the possibilities for all types of research, particularly, biochemical and physiological, will be much greater.

2.7.5. Response to stimuli

von Brand (1979) reported that both male and female specimens of *Neoechinorhynchus* spp. were positively thermotactic and have been observed to wander towards a heat source.

2.8. SUMMARY

A review of the knowledge of the classification, morphology of developmental stages, life cycle, ecology and distribution and biochemistry and physiology of *Neoechinorhynchus rutili* is presented. Special reference was made to some attempts at *in vitro* maintenance.

CHAPTER THREE

THE DISTRIBUTION OF *NEOECHINORHYNCHUS RUTILI* IN SCOTTISH FRESHWATER FISH

3.1. INTRODUCTION

Worldwide, *Neoechinorhynchus rutili* has been reported from over 110 species of fish from brackish, freshwater and marine environments (Appendix I) and its distribution has been described as being circumpolar throughout the northern holarctic zone (Van Cleave & Lynch, 1950; Petrochenko, 1956). In Scotland, *N.rutili* appears as a common parasite of a limited number of fish species. Of the 37 species of freshwater fish known to inhabit Scotland (Maitland 1972) only 8 (21%) are reported as being infected by *N.rutili*. *Neoechinorhynchus rutili* is 1 of 6 species of Acanthocephala known to infect British freshwater fish (Brown *et al*, 1986). The reference lists of parasites of British and Irish freshwater fish compiled by Chappell & Owen (1969) and Kennedy (1974) report only 2 and 4 references respectively to infection of Scottish fish by *N.rutili*. During the course of the present study further references to *N.rutili* infections of Scottish freshwater fish have been collated from published papers, unpublished reports and p.c. The list of references presented here is obviously not an exhaustive one, there being over 9000 lochs over 0.3 ha in area in Scotland (Campbell, 1971) and numerous river systems which are potential sites for definitive hosts of *N.rutili*. However, preparation of this list has been started with the aim of recording the general distribution of *N.rutili*. Clearly this type of information is lacking and should complement recent work describing the British distribution of *Acanthocephalus anguillae* and *Pomphorhynchus laevis* (Kennedy, Bates & Brown, 1989).

3.2. AN APPROACH TO THE STUDY OF THE DISTRIBUTION OF *NEOECHINORHYNCHUS RUTILI* IN SCOTLAND

Each report for a *Neoechinorhynchus rutili* infection has been recorded in terms of the site name, region, altitude and Ordnance Survey map (1:50,000) grid reference (4 figures for large lochs >50 ha, burns or rivers, or where the site is not sufficiently described in the original reference to permit a 6 figure reference and 6 figures for lochs < 50 ha in area) in alphabetical order of the site names. The date of the observation or reference is given along with the species of fish infected. Multiple dates are given where there are several reports of infection at a site. For Loch Maragan, which is described in detail elsewhere (Chapter 4) only the dates for the first record of *N.rutili* infection for each species are given.

3.3. OBSERVATIONS ON THE DISTRIBUTION OF *NEOECHINORHYNCHUS RUTILI* IN SCOTLAND

The list of sites where fish have been found to be infected with *Neoechinorhynchus rutili* infection is presented in Table 3.1 (p.34). A total of 41 sites of *Neoechinorhynchus rutili* infection are cited involving 8 species of fish. Records of *N.rutili* have been reported from 6 of the 9 mainland Scottish regions (Fig. 3.1). The sites of infection are from a wide spectrum of geographical locations, including small, isolated, acidic hill lochans, large lowland lochs, small burns, reservoirs and fish farms supplied by river water. These sites are at a range of altitudes and include water bodies which freeze over the winter period. The distribution map (Fig. 3.1) shows a concentration of records around the Glasgow and Pitlochry (Department of Agriculture and Fisheries for Scotland) areas and this probably reflects the distribution of fish parasite surveys rather that of a biological phenomenon. During the course of this study fish samples

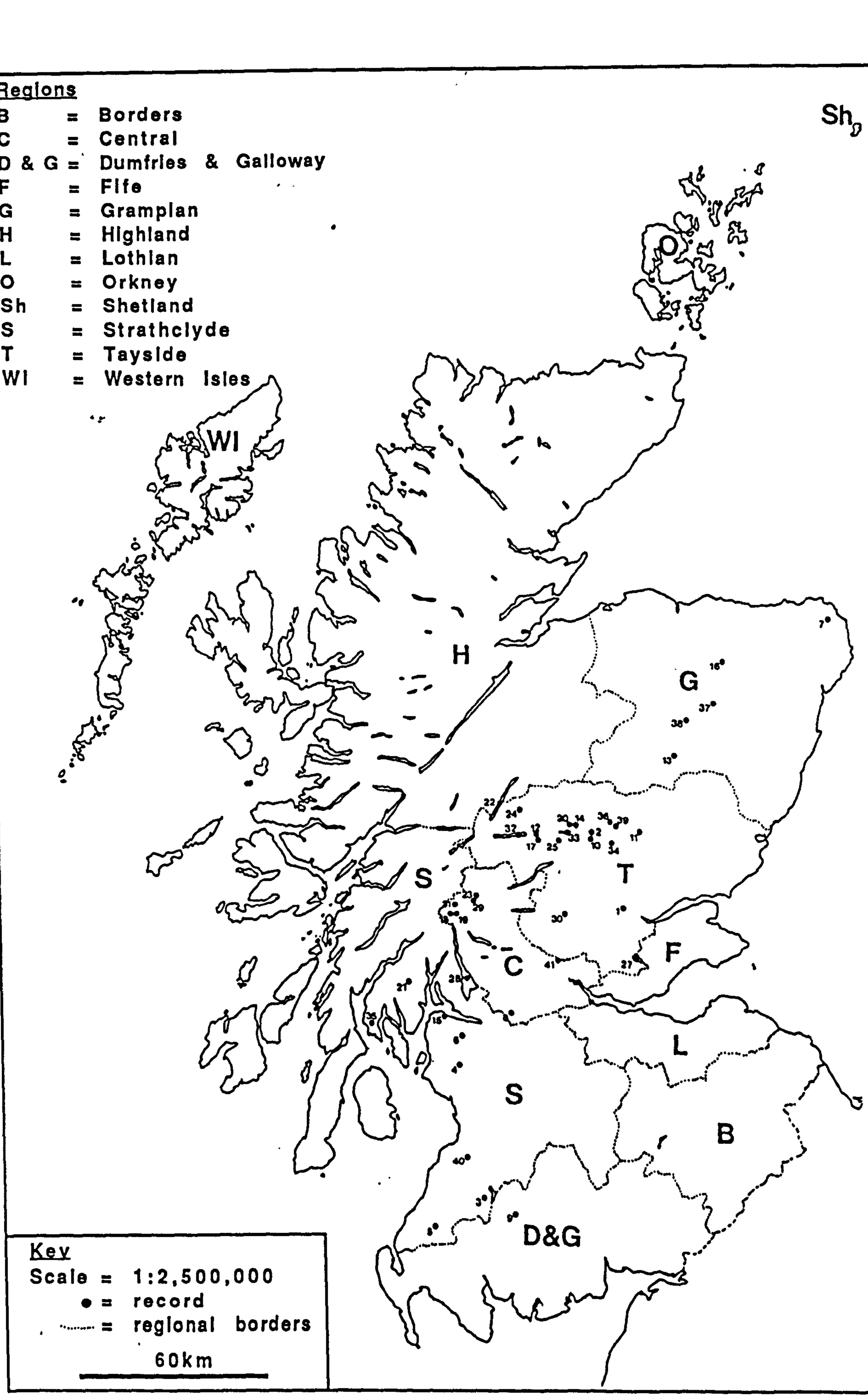


Fig. 3.1 Records of the distribution of *Neoechinorhynchus rutili*
in freshwater fish in Scotland since 1915

from a wide range of sites around Scotland, including the Orkney and Shetland Isles, have been examined for *Neoechinorhynchus rutili* infections to increase the range of potential sites that have been investigated. A total of 1189 fish of 11 species from 51 sites around Scotland have been examined and a summary of these investigations is given on Table 3.2 (p.38).

In an attempt to establish the temporal stability of *Neoechinorhynchus rutili* populations, a number of fish from sites of known *N.rutili* infection were examined during the present study in order to establish if the parasite was still present at these sites. These fish included sticklebacks and minnows from the Beith area (Site 4), brown and rainbow trout and sticklebacks from Bridge of Weir (Site 6) and brown trout from Dunalastair Reservoir (Site 12).

Following up the report of Ritchie (1915) at site 4, all the water bodies in a four mile radius around Beith were marked on the 1:50,000 O.S. map and sites for fish collection were chosen on the basis of their accessibility by car. A total of 4 sites in the area were visited on 24.7.86. and 16 sticklebacks and 5 minnows were collected; 4 minnows were collected at Bamboburn (NS 309487) and 1 minnow and 16 sticklebacks at Dusk Water (NS 340487). Dissections of these did not reveal any infections by *N.rutili*. One of the problems in this area was capture of large numbers of fish. In addition, Ritchie (1915) gave no indication of the exact sites where he captured the infected fish. Therefore a more thorough survey would be necessary to assess the temporal stability of the *Neoechinorhynchus rutili* populations in the Beith area.

At the West of Scotland Trout Farm, Bridge of Weir (Site 6), Turnbull (1958) found *Salmo trutta* to be infected with *Neoechinorhynchus rutili*. She reported that 4/22 (18%) brown trout

that she examined were infected but gave no indication of the intensity of the infection. During the course of this study 3 fish species from this site were examined for *N.rutili* infections on a number of occasions (see Tables 3.2 & 3.3): Of 25 rainbow trout (mean wet weight = 167.3g) obtained on 5.5.87. 10 fish were infected with between 1 and 15 *N.rutili* per host. The population of worms included actively reproducing female worms which contained acanthors; 6 rainbow trout collected on 4.1.88. were found to all be infected with *N.rutili*, with the intensity of infection ranging from 1 to 891 worms per host; 2 further rainbow trout collected on 24.2.88. were also infected and contained 25 and 31 *N.rutili* respectively; a brown trout collected on the same date harboured 6 *N.rutili*; 2 samples of three-spined sticklebacks were also collected from this site on 24.2.88. and 11.3.88 respectively. Of the 29 fish in the February sample, 10 fish were infected with between 1 and 4 *N.rutili* per host and 3 out of 7 fish in the March sample were infected with 1 to 3 worms per host. No gravid worms were recovered from the sticklebacks and this may be indicative of the time of capture rather than of a physiological deficiency which prevents the maturation of *N.rutili* in this species. In conclusion, it appears that at this site *N.rutili* has remained as part of the parasite fauna, at least of *S.trutta*, since 1958. The consecutive years of observed infections of rainbow trout and high intensities of infection of trout indicate that there must be a high rate of transmission of *N.rutili* to the trout and also a good source of infected intermediate hosts. Further to this, worms collected from rainbow trout in May 1987 were seen to be actively reproducing and this, in conjunction with the previous observations indicates that the Bridge of Weir *N.rutili* suprapopulation is still well established and thriving.

The West of Scotland Trout Farm consists of a number of unlined

fish ponds connected by channels and fed by water from the River Gryfe. The flow through of water is fairly slow, permitting the build up of a considerable layer of both allochthonous and autochthonous material on the bottom of the ponds. Consequently the benthic invertebrate fauna is varied and abundant (p.o.) and provides additional food for the farmed trout which are fed on commercial pelleted food. It is known that *Sialis lutaria* larvae act as intermediate hosts for *Neoechinorhynchus rutili* at this site and that the population is well established (Kennedy, p.c.). Thus site 6 appears as a stable site, with a source of potential intermediate and definitive hosts maintained by the methods of husbandary adopted there. It seems fair therefore, to conclude that these factors have contributed to the temporal stability (29 years) of the *N.rutili* suprapopulation.

Gut samples from 31 brown trout from site 12, collected by Gordon Struthers of the Department of Agriculture and Fisheries for Scotland as part of a survey to determine the extent of predation of salmon parr by brown trout, between 16.4.86. and 2.7.86. were examined for gut parasites in an attempt to follow up the finding of *Neoechinorhynchus rutili* by Robertson (1953). However, no infection by *N.rutili* was detected. Robertson (1953) examined 100 brown trout between March and September 1952 and found the highest mean intensity of infection to be 4 worms per fish (in May 1952), the prevalence of infection ranging between 0% in April, July, August and September and 90% in June. Robertson (1953) reported that only 4% of the trout were infected with the parasite over the entire period. The negative results from the examination of the 1986 trout samples may indicate one of two possibilities: either the sample was not large enough to detect any infection by *N.rutili* despite the fact that 74% of the

trout (24) were caught in May and June, the months in which Robertson (1953) reported an over 80% prevalence of infection; alternatively, the *N.rutili* population which Robertson (1953) investigated was declining at that time and consequently there were too few reproducing adults to maintain the population.

In contrast to the situation at Dunalastair Reservoir, sites investigated by Robertson (1953) were subsequently found to have fish harbouring a *Neoechinorhynchus rutili* infection. Campbell (1974 and p.c.) found *N.rutili* in Loch Tummel, Loch Leven and Loch Kinardochy where Robertson (1953) reported no infections. This can most probably be explained by the limited numbers of fish which the latter examined, as opposed to a genuine negative report. The negative reports for perch and pike by Robertson (1953) may also be a consequence of the month of capture. Campbell (1974) reported infections of perch in November, January, February and April and of pike in March and April at Loch Leven and it appears that Robertson (1953) sampled her fish only during the summer months. Interestingly, both Robertson (1953) and Campbell (1974) did not find *N.rutili* in the brown trout at Loch Leven, nor have samples taken during the present study shown any infection of brown trout by *N.rutili*. In addition, Campbell p.c. found *N.rutili* in *Salmo trutta* in Loch Kinardochy but Robertson (1953) did not find it in pike from the same site.

A number of other sites have shown infections of the fish populations by *Neoechinorhynchus rutili* over a number of years. These include: Lochan Creag nan Caorann; Loch Essan; Loch Garry; Loch Leven; Loch Maragan; Loch Rannoch. Conversely, trout from Powder Works Dam Lochan which were found to be heavily infected in June 1986, were no longer harbouring any visible infection in subsequent samples in that year or the following year. This may, in part, be explained by the nature of this site which is an artificial lake whose level shows wide

fluctuations over short periods of time. Kennedy (p.c., 1987) suggests that crashes in parasite populations in fish at such physically unstable sites is as a result of a crash in the potential intermediate host population. It is not known what caused the observed crash at this site.

3.4. OBSERVATIONS ON THE DISTRIBUTION OF *ACANTHOCEPHALUS LUCII* AND *ECHINORHYNCHUS TRUTTAE* IN SCOTLAND

As a result of the examination of 1109 freshwater fish from 50 sites around Scotland two other acanthocephalan species, *Acanthocephalus lucii* and *Echinorhynchus truttae* were recorded at a number of sites and details of the date of collection and site names are given on Table 3.3 (p.39). *Acanthocephalus lucii* was found in 6 fish species at 8 sites and *E.truttae* was found in two species of fish at 11 sites. These records are presented with the aim of extending the current knowledge of the distribution of these parasites in Scotland.

Table 3.1. Sites of *Neoechinorhynchus rutili* infections of freshwater fish in Scotland

Sites of *N.rutili* infection are given in alphabetical order with lochs over 50 hectares in area marked with an asterisk. Site numbers correspond to those on the distribution map (Fig. 3.1). (p.c. = personal communication; - = no data available; Unpub. = Unpublished record)

Site	Grid reference Altitude (m)	Date	Species	Reference
1. Almondbank Tayside	NO0626 46	2.72.	*Salmo gairdneri*	Campbell p.c.
2. Balnakeilly Tayside	NN9459 229	-	*Salmo trutta*	Robertson (1953)
3. Ballochling Loch Strathclyde	NX455946 305	21.7.67.	*Salmo trutta*	Campbell p.c.
4. Beith area Strathclyde	NS3453 76	-	*Salmo trutta*	Ritchie (1915)
		-	*Gasterosteus aculeatus*	
5. Black Clauchrie Burn Barrhill Estate Strathclyde	NX298842 183	25.8.86.	*Salmo trutta*	Lassiere (Unpub.)
6. Bridge of Weir, West of Scotland Trout Farm Strathclyde	NS3965 47	-	*Salmo trutta*	Turnbull (1958)
		24.2.88.	*Salmo trutta*	Lassiere
		5.5.87.	*S.gairdneri*	(Unpub.)
		4.1.88.		
		24.2.88.		
		26.2.88.	*G.aculeatus*	Lassiere
		11.3.88.		(Unpub.)
		-		Tierney (1989)
7. Burn of Savoch Grampian	NK0558 15	-	*Salmo trutta*	Bwathondi (1976)
8. Carbeth Loch Central	NS535793 123	31.5.88.	*Salmo trutta*	Lassiere (Unpub.)

9. Carsfad Loch Dumfries & Galloway	NX608860 107	10.6.67.	*Salmo trutta*	Campbell p.c.
10. The Cuilc & Burn, Pitlochry, Tayside	NN936587 137	-	*G.aculeatus*	Pike & Edwards (1983)
11. Drumore Loch Tayside	NO165608 300	10.87.	*Salmo trutta* *S.gairdneri* *G.aculeatus*	Devine (1988)
		14.10.87.	*G.aculeatus*	Lassiere (1988), Tierney (1989)
		-		
12. Dunalastair* Reservoir Tayside	NN6958 199	-	*Salmo salar* *Salmo salar* *Salmo trutta*	Campbell p.c Robertson (1953)
13. Girnock Burn Grampian	NO3293 255	7.2.77.	*Salmo trutta*	Campbell p.c.
14. Glenfin Castle, Tayside	NN8661 215	3.10.77.	*Esox lucius*	Campbell p.c.
15. Greenock Strathclyde	NN2876 0	-	*Salmo trutta*	Robertson (1953)
16. Huntly, fish farm, Grampian	NJ5240 122	-	*Salmo gairdneri*	Pike p.c.
17. Lochan an Daim, Tayside	NN718574 377	-	*Salmo trutta*	Robertson (1953)
18. Lochan Creag nan Caorann Central	NN302218 487	3.8.86. 9.8.86. 16.8.86. 5.7.87. 3.7.88.	*Salmo trutta*	Lassiere (Unpub.)
19. Lochan Duin Central	NN315218 426	5.7.87.	*Salmo gairdneri*	Lassiere (Unpub.)
20. Loch Bhac Tayside	NN822623 340	3.10.77.	*Salmo trutta*	Campbell p.c.
21. Loch Eck* Strathclyde	NS1391 46	-	*Gasterosteus aculeatus*	Turnbull (1958)
22. Loch Ericht* Highland	NN5675 400	3.8.78.	*Salvelinus alpinus*	Campbell p.c.
23. Loch Essan Central	NN413285 442	31.10.86. 20.6.87.	*Salmo trutta*	Lassiere (Unpub.)

24. Loch Garry* Tayside	NN6270 415	18.6.71. 4.74.	*Salmo trutta*	Campbell p.c.
25. Loch Kinardochy Tayside	NN775552 367	26.4.76.	*Salmo trutta*	Campbell p.c.
26. Loch Linnhe* Crinan	- -	-	*Salmo trutta*	Robertson (1953)
27. Loch Leven* Tayside	NO1401 106	11.69. 2.70. 4.70. 1.71. 3.70. 4.70.	*Perca fluviatilis* *Esox lucius*	Campbell (1974)
28. Loch Lomond* Strathclyde	NN3313 16	-	*Salmo trutta*	Copland (1957)
29. Loch Maragan Central	NN402278 472	12.7.86. 30.5.87.	*Salmo trutta* *Phoxinus phoxinus*	Lassiere (Unpub.)
30. Loch Monzievaird Tayside	NN843233 61	22.3.87. 3.4.87. 3.4.87.	*Salmo trutta* *G.aculeatus*	Lassiere (Unpub.) Lassiere (Unpub.)
31. Loch Oss Central	NN300252 625	5.7.87.	*Salmo trutta*	Lassiere (Unpub.)
32. Loch Rannoch, Tayside	NN5957 213	3.5.77. 15.9.78.	*Salvelinus alpinus*	Campbell p.c.
33. Loch Tummel* Tayside	NN8159 152	3.10.77.	*Esox lucius*	Campbell p.c.
34. Pitcarmick Loch Tayside	NO053563 351	-	*Salmo trutta*	Pike & Edwards (1983)
35. Powder Works Dam Lochan Tighnabruaich Strathclyde	NR952742 137	22.6.86. 26.6.86.	*Salmo trutta*	Lassiere (Unpub.)
36. River Brerachan Tayside	NO047636 250	-	*Phoxinus phoxinus*	Pike & Edwards (1983)
37. River Don at Mossat Grampian	NJ4719 199	-	*Salmo trutta*	Bwathondi (1976)
38. River Don at Strathdon Grampian	NJ3512 290	-	*Salmo trutta*	Bwathondi (1976)

39. Straloch Tayside	NO044638 250	-	*Salmo trutta*	Robertson (1953)
40. River Doon Trout Co. Dalrymple Strathclyde	NS362145 60	8.5.87.	*Salmo gairdneri*	Lassiere (Unpub.)
41. Waltersmuir Reservoir Dunblane Central	NN810005 185	21.10.67.	*Salmo trutta*	Campbell p.c.

Summary of records of *Neoechinorhynchus rutili* distribution in Scottish freshwater fish

Fish species	No. of sites recorded with *N.rutili* infection
Esox lucius	3
Gasterosteus aculeatus	6
Perca fluviatilis	1
Phoxinus phoxinus	2
Salmo gairdneri	6
Salmo salar	1
Salmo trutta	28
Salvelinus alpinus	2

Table 3.2. Records of fish species examined between October 1985 and October 1988 for *Neoechinorhynchus rutili* infections in Scotland*

Fish species	No. of specimens examined/ No. of sites 1985	1986	1987	1988	Total
Anguilla anguilla	0	30/6	1/1	0	31/7
Coregonus lavaretus	25/1	14/1	0	0	39/1
Esox lucius	0	1/1	0	0	1/1
G.aculeatus	-/6	182/7	141/3	36/1	359/13
G.cernua	7/1	9/1	-/1	0	16/1
N.barbulatus	0	1/1	0	0	1/1
Perca fluviatilis	9/1	6/4	2/2	0	17/6
Phoxinus phoxinus	-/2	13/5	194/1	11/1	213/4
Rutilus rutilus	1/1	0	0	1/1	2/2
Salmo gairdneri	0	22/6	63/7	26/3	111/9
Salmo trutta	6/2	162/21	169/23	57/8	394/38
Total	48/7	440/29	570/25	131/12	1189/51

G.aculeatus = *Gasterosteus aculeatus*
G.cernua = *Gymnocephalus cernua*
N.barbulatus = *Noemacheilus barbulatus*

* = One collection of *Gasterosteus aculeatus* in 1986 was from County Durham in England.

Table 3.3. Details of fish collections for parasitic examinations

Fish Species	Date	Site	No. examined	A sp.*
Anguilla anguilla	18.9.86.	River Teviot	4	-
	18.9.86.	River Tweed (Lowood)	5	-
	23.9.86.	River Esk	5	-
	24.9.86.	River Almond	5	-
	24.9.86.	Water of Leith	5	-
	26.9.86.	River Leven	6	-
	6.8.87.	Loch Maragan	1	-
Coregonus lavaretus	25.10.85.	Loch Lomond	10	-
	6.11.85.	"	3	-
	28.11.85.	"	12	-
	21.2.86.	"	1	-
	23.7.86.	"	13	-
Esox lucius	28.4.86.	Loch Awe	1	-
				-
Gasterosteus aculeatus	3.10.85.	Springburn Park Glasgow	-	-
	3.10.85.	River Luggie Kirkintilloch	-	-
	3.10.85.	Victoria Park Glasgow	-	-
	5.10.85.	Blane Water, Dumgoyne	-	-
	9.10.85.	River Kelvin, B.B.C. Glasgow	-	-
	11.10.85.	Barrhead, Glasgow	-	-
	10.1.86.	White Cart Water Pollok Park, Glasgow	9	-
	17.1.86.	River Kelvin B.B.C. Glasgow	20	AL
	24.1 86.	River Kelvin B.B.C. Glasgow	4	-
	6.2.86.	River Luggie, Kirkintilloch	3	AL
	13.2.86.	River Kelvin, B.B.C. Glasgow	4	AL
	6.5.86.	Springburn Park	14	-
	13.5.86.	Springburn Park	17	-
	7.6.86.	Springburn Park	8	-
	23.5.86.	Springburn Park	6	-
	13.6.86.	Monkton Pond, County Durham	80	-
	24.7.86.	Dusk Water, Beith	-	-
	23.9.86.	Gladhouse Reservoir	1	-
	3.4.87.	Loch Monzievaird	1	NR
	28.4.87.	River Kelvin B.B.C. Glasgow	-	AL
	14.10.87.	Drumore Loch	16	NR
	31.10.87.	"	64	NR
	11.11.87.	"	30	NR
	10.12.87.	"	30	NR
	26.2.88.	Bridge of Weir	29	NR
	11.3.88.	"	7	NR

Fish Species	Date	Site	No. examined	A sp.
Gymnocephalus cernua	25.10.85.	Loch Lomond	4	-
	28.11.85.	"	3	AL
	21.2.86.	"	3	AL
	8.5 86.	"	5	AL
	25.9.86.	"	1	AL
	28.4.87.	"	-	AL
Nemacheilus barbulatus	6.2.86.	River Luggie	1	-
Perca fluviatilis	25.10.85.	Loch Lomond	6	AL
	6.11.85.	"	3	AL
	8.5.86.	Dubh Loch	1	-
	20.8.86.	Loch Doon	1	AL
	27.8.86.	Lake of Menteith	1	AL
	3.11.86.	Loch Lomond	3	AL
	17.5.87.	Lake of Menteith	1	AL
	26.7.87.	Lochan Duin	1	AL
Phoxinus phoxinus	3.10.85.	River Luggie	-	-
	9.10.85.	River Kelvin	-	-
	10.1.86.	White Cart Water	2	-
	17.1.86.	River Kelvin	1	-
	6.2.86.	River Luggie	5	-
	24.7.86.	Bamboburn, Beith	4	-
	24.7.86.	Dusk Water, Beith	1	-
	25.4.87.	Loch Maragan	1	-
	30.5.87.	"	3	NR
	16.7.87.	"	1	-
	6.8.87.	"	150	NR
	5.9.87.	"	39	-
	24.7.88.	"	3	NR
	20.8.88.	"	2	-
Rutilus rutilus	25.10.85.	Loch Lomond	1	-
	4.7.88.	Lake of Menteith	1	AL
Salmo gairdneri	17.3.86.	Asgog Loch	1	-
	17.3.86.	Loch Awe	1	-
	28.4.86.	"	3	-
	25.4.86.	Lake of Menteith	2	AL
	1.6.86.	"	3	AL
	22.6.86.	Asgog Loch	1	-
	26.6.86.	Powder Works Dam L.	2	-
	28.6.86.	Asgog Loch	2	-
	19.7.86.	Powder Works Dam L.	1	-
	2.8.86.	"	1	-
	24.8.86.	Loch Fitty	5	ET
	16.3.87.	Linlithgow	7	AL
	30.3.87.	Loch Fitty	1	-
	5.4.87.	Linlithgow	3	-
	4.5.87.	Bridge of Weir	25	NR,ET
	8.5.87.	River Doon Trout Co.	18	NR
	17.5.87.	Lake of Menteith	1	-
	29.5.87.	Powder Works Dam L.	7	-

Fish Species	Date	Site	No. examined	A sp.
Salmo gairdneri cont.	5.7.87.	L. Duin	1	NR
	4.1.88.	Bridge of Weir	6	NR,ET
	8.2.88.	"	1	NR
	9.3.88.	River Doon Trout Co.	18	-
	28.5.88.	Linlithgow	1	AL
Salmo trutta	5.10.85.	Blanewater, Dumgoyne	1	ET
	6.11.85.	Loch Lomond	3	-
	28.11.85.	"	2	-
	21.2.86.	"	23	-
	12.4.86.	River Ba, Rannoch Moor	1	-
	16.4.86.	Dunalastair Reservoir	2	ET
	18.4.86.	"	2	-
	23.4.86.	"	1	ET
	27.4.86.	Jaw Loch	4	-
	28.4.86.	Dunalastair Reservoir	1	-
	2.5.86.	Dunalastair Reservoir	1	-
	16.5.86.	Gladhouse Reservoir	4	ET
	21.5.86.	Dunalastair Reservoir	1	-
	25.5.86.	"	1	ET
	28.5.86.	Loch Awe	1	-
	29.5.86.	Dunalastair Reservoir	1	ET
	30.5.86.	"	2	-
	31.5.86.	"	1	-
	1.6.86.	Dunalastair Reservoir	3	ET
	2.6.86.	"	2	ET
	3.6.86.	"	1	ET
	5.6.86.	"	1	ET
	8.6.86.	"	1	-
	15.6.86.	"	1	-
	17.6.86.	"	1	-
	18.6.86.	"	1	-
	19.6.86.	"	10	-
	22.6.86.	Powder Works Dam L.	4	NR
	25.6.86.	Dunalastair Reservoir	1	-
	26.6.86.	Powder Works Dam L.	4	NR
	28.6.86.	Asgog Loch	7	-
	29.6.86.	Dunalastair Reservoir	3	ET
	30.6.86.	Loch Awe	10	ET
	2.7.86.	Dunalastair Reservoir	2	-
	12.7.86.	Powder Works Dam L.	4	-
	31.7.86.	Dunalastair Resrvoir	1	-
	2.8.86.	"	1	-
	3.8.86.	L. Creag nan Caorann	3	-
	9.8.86.	"	4	NR
	11.8.86	Gladhouse Reservoir	2	ET
	12.8.86.	Loch Leven	1	-
	16.8.86.	L. Creag nan Caorann	2	-
	20.8.86.	Loch Doon	1	-
	24.8.86.	Loch Fitty	2	ET
	24.8.86.	Loch Humphrey	2	ET
	25.8.86.	Black Clauchrie Barrhill	11	NR

Fish Species	Date	Site	No. examined	A sp.
Salmo trutta cont.	8.86.	River Doon Trout Co.	2	-
	7.9.86.	Secret 7	3	-
	18.9.86.	Powder Works Dam L.	50	-
	23.9.86.	Gladhouse Reservoir	6	ET
	9.86.	Secret 7	1	-
	9.86.	Gladhouse Reservoir	1	ET
	17.10.86.	Powder Works Dam L.	18	-
	31.10.86.	Loch Essan	4	NR
	10.86.	Water of Leith	6	-
	10.86.	White Adder	3	ET
	10.86.	Talla Reservoir	3	ET
	14.11.86.	Powder Works Dam L.	26	AL
	20.3.87.	"	6	-
	23.3.87.	Loch Monzievaird	5	NR,ET
	3.4.87.	"	3	NR,ET
	5.4.87.	Linlithgow	1	-
	12.4.87.	Burncrooks Res.	1	-
	18.4.87.	Gladhouse Reservoir	1	ET
	25.4.87.	"	1	ET
	25.4.87.	Loch Awe	3	-
	27.4.87.	Gladhouse Reservoir	2	ET
	28.4.87.	"	2	ET
	28.4.87.	Loch Lomond	-	ET
	29.4.87.	Gladhouse Reservoir	2	ET
	30.4.87.	Gladhouse Reservoir	4	ET
	1.5.87.	Powder Works Dam L.	27	-
	10.5.87.	Loch Humphrey	4	ET
	23.4.87.	Gladhouse Reservoir	3	ET
	25.5.87.	"	2	ET
	26.5.87.	"	2	ET
	27.5.87.	"	2	ET
	28.5.87.	"	4	ET
	29.5.87.	Powder Works Dam L.	17	-
	5.6.87.	Gladhouse Reservoir	1	ET
	18.6.87.	Loch Awe	3	-
	19.6.87.	Gladhouse Reservoir	2	ET
	20.6.87.	"	2	ET
	20.6.87.	Loch Essan	6	NR
	22.6.87.	Gladhouse Reservoir	1	ET
	24.6.87.	"	2	ET
	25.6.87.	"	1	ET
	25.6.87.	Powder Works Dam L.	9	-
	26.6.87.	Gladhouse Reservoir	1	ET
	6.87.	Loch Harray, Orkney	15	-
	6.87.	Stenness, Orkney	1	-
	6.87.	Boardhouse, Orkney	2	-
	5.7.87.	Butterstone, Dunkeld	2	-
	5.7.87.	L. Creag nan Caorann	7	NR
	5.7.87.	Loch Oss	2	NR
	7.87.	Gladhouse Reservoir	5	ET
	16.8.87.	Lochore Meadows	1	-
	17.8.87.	Tighnabruaich Res.	2	-
	21.8.87.	Powder Works Dam L.	11	-
	23.8.87.	Loch Monzievaird	1	-

Fish Species	Date	Site	No. examined	A sp.
Salmo trutta cont.	26.7.87.	Glen Etive	1	-
	9.87.	Gladhouse Reservoir	1	ET
	8.2.88.	Bridge of Weir	2	NR,ET
	8.4.88.	Loch Awe	1	-
	1.5.88.	"	8	-
	8.5.88.	"	6	-
	31.5.88.	Carbeth Loch	2	NR
	5.6.88.	Loch Harray, Orkney	5	-
	7.6.88.	Loch Skail, Orkney	1	-
	8.6.88.	Loch Boardhouse, Orkney	1	-
	3.7.88.	L. Creag nan Caorann	4	NR
	17.7.88.	Foula, Shetland Isles	27	-

* Acanthocephalan species (A sp.) recovered from the fish examinations were as follows:

AL = *Acanthocephalus lucii*

ET = *Echinorhynchus truttae*

NR = *Neoechinorhynchus rutili*

The records are shown in chronological order for each species.

(L = lochan)

3.5. DISCUSSION

The manner by which *Neoechinorhynchus rutili* has become distributed in Scottish freshwater bodies, especially the isolated sites, is intruiging. Van Cleave & Lynch (1950) addressed the problem of explaining the wide distribution of *N.rutili* in the northern hemisphere and concluded that this broad distribution pattern could have been achieved by utilizing a great diversity of definitive hosts, including both freshwater and anadromous (migratory) fish species. On the basis of the known habits of the hosts, Van Cleave & Lynch (1950) proposed that at least 3 types of relationship exist between *N.rutili* and its definitive hosts. In summary, these were as follows:

i) Cumulative hosts

Representatives of this class of host include the sticklebacks which inhabit either freshwater or brackish environments. In these locations the fish populations build up and maintain foci for the infection of suitable arthropod hosts by their close association. Possible examples of this type of host in Scotland include Bridge of Weir (Site 6), Burn of Savoch (Site 7), The Cuilc & Burn, Pitlochry (Site 10) and Loch Eck (Site 21).

ii) Disseminating or dispersion hosts

These include species of fish which migrate from one area to another, especially anadromous species like *Salmo salar*, *Salmo trutta* and *Gasterosteus aculeatus*. These fish are exposed to *Neoechinorhynchus rutili* in the foci of infection established and maintained by the cumulative hosts. Infection is brought about by feeding upon infected intermediate hosts in the habitat dominated by the cumulative hosts or by feeding on the cumlative hosts themselves. Subsequent migrations of these hosts introduces acanthors into new localities where new foci become established, if suitable intermediate

hosts are available, and help to maintain foci already established. In Scotland, examples of these types of host include sites where *Salmo trutta*, *Salmo salar* and *Gasterosteus aculeatus* have free access to the marine environment. Examples of this type of site include Burn of Savoch (Site 7), Loch Eck (Site 21), River Don (Sites 37 and 38) and Loch Lomond (Site 28). (see Table 3.1)

iii) Fortuitous or adventitious hosts

These hosts include species of fish which are not the usual definitive host of *Neoechinorhynchus rutili* in the habitat but as a result of their feeding activities become exposed to the parasite and consequently allow for its full development. Examples of this type of host are piscivorous species like the chub *Leuciscus cephalus* and the Catostomidae. In Scotland, pike (*Esox lucius*) and perch (*Perca fluvatilis*) at Glenfin Castle (Sites 14), Loch Leven (Site 27) and Loch Tummel (Site 33) respectively fall into this category.

Van Cleave & Lynch (1950) accounted for the distribution of *Neoechinorhynchus rutili* only on the basis of the habits of the definitive host species and in this context their description of 3 types of hosts is a valid one. However, in terms of the whole set of dynamic processes likely to be involved in producing the observed geographical distribution of *N.rutili* the role of the natural movements of the definitive host is only part of the explanation. For example, Van Cleave & Lynch (1950) made no attempt to explain how infections at isolated sites may originate. In this context, the role of another group of fish hosts must be considered. These are the eels which not only migrate between marine and freshwater environments (catadromous) but also migrate across land and can therefore reach isolated water bodies and set up new foci for infection. Eels (*Anguilla anguilla*) are known hosts for *N.rutili* (Barysheva & Bauer,

1958; Lindstrom, 1942 (reference given by Walkey, 1963; Markevich, 1963; Meyer, 1932; Valtonen p.c.; Van Cleave & Lynch, 1950) although they have not been reported as such in Scotland, and are therefore candidates for this mode of distribution. My own examinations of eels from various Scottish sites (Table 3.2), including one individual caught at Loch Maragan where it was a potential host and as such could set up new infections at other locations, did not reveal any *N.rutili* infections.

In addition to the role of the natural movements of the definitive hosts in the distribution of *Neoechinorhynchus rutili* a number of other factors may be operating. These include the influence of man, bird carriers and viability of infected intermediate hosts. Firstly the influence of man. For the most part this consists of deliberate movements of fish from one site to another. Most commonly this takes the form of stocking water bodies with fish species suitable for angling and/or food and the normal source of such fish is fish farms. A number of the sites of *N.rutili* infection in Scotland include fish farms and it seems fair to conclude that this may be a route for the distribution of *N.rutili* to new sites. Indeed, during the last century many small lochs in Scotland were stocked with brown trout (Ritchie, 1920) and this may partially account for the observed distribution. For example, Christie (p.c.) reported that Loch Maragan was stocked with brown trout over 80 years ago by his grandfather. It is possible that the trout were already infected with *N.rutili* when they were introduced. The introduction of farmed trout, especially *Salmo gairdneri* is common practice for small angling clubs around Scotland and it seems likely that *N.rutili* is being inadvertently distributed over wide areas in this manner. More often than not fish farmers appear unaware of the nature of the parasitic infections of

their fish and therefore the dissemination of *N.rutili* continues unchecked (p.o.). Indeed, Mills (1989) described Bridge of Weir fish farm as one of the longest established trout farms in Scotland and bearing the apparent temporal stability of *N.rutili* infection in mind there, it is possible that stocking in the past from this farm could explain many cases of infected fish around the country.

Secondly, birds may act as important carriers of *N.rutili* developmental stages between water bodies. Two possible routes can be envisaged here; either simply via acanthors trapped in detritus from the water body being carried externally on the birds from one site to another or by the birds feeding on infected fish (e.g. common gulls *Larus canus* and black-headed gulls *Larus ridibundus* feeding on infected minnows at Loch Maragan; grey herons *Ardea cinerea* and red throated divers *Gavia stellata* at Powder Works Dam Lochan feeding on brown trout) or on weed harbouring infected arthropods and subsequently releasing the various developmental stages of *N.rutili* in their faeces at another site. The latter route would only be a possibility if the digestive system of the bird did not affect the viability of the *N.rutili* developmental stages.

Thirdly, infected *Sialis lutaria* larvae, which are known intermediate hosts of *N.rutili* at a number of Scottish sites may provide a transmission route assuming that they successfully pupate and the adults fly between one site and another and become prey to the fish at another site.

Some of the ways by which *Neoechinorhynchus rutili* may become distributed to various geographical locations have been discussed and their likelihood commented upon. In all cases however, *N.rutili* can only become established where suitable definitive and intermediate host species are present. Once established the temporal stability of a focus of infection will be influenced by the dynamic relationship

between the physical, chemical and biological characteristics of the site. Since *N.rutili* matures in a fish host it would be classified as an autogenic parasite species by Esch, Kennedy, Bush & Aho (1988). Esch *et al.* (1988) stated that such species can 'only colonize new aquatic localities by the natural migration, or human assisted movements of fish and/or invertebrate intermediate hosts habouring intact parasites into the new localities'. The general view is that *N.rutili* has been distributed by such means in Scotland although the movement of acanthors by piscivorous birds merits some investigation.

3.6. SUMMARY

3.6.1. *Neoechinorhynchus rutili* has been found infecting 8 species of freshwater fish at 41 sites in 6 regions of Scotland.

3.6.2. The sites of infection include representatives from a wide spectrum of aquatic environments in terms of size, altitude, water quality and faunal community structure.

3.6.3. Circumstantial evidence from some sites of known infection implies that there is a spectrum in the temporal stability of *Neoechinorhynchus rutili* suprapopulations ranging from well established sites of infection to those which appear to 'die out' over a short period.

3.6.4. The manner by which *Neoechinorhynchus rutili* has achieved worldwide and local distribution can in part be explained by the differing habits of the definitive host species.

3.6.5. Man, bird carriers and invertebrate intermediate hosts may have a role in the dissemation of this parasite, especially to geographically isolated sites.

3.6.6. Once established the temporal stability of a focus of infection will be influenced by the dynamic relationship between the physical, chemical and biological characteristics of the site.

CHAPTER FOUR

LOCH MARAGAN

4.1. GENERAL INTRODUCTION

Loch Maragan, near Crianlarich, Central Region, Scotland was the main field site for the investigation of the ecology of *Neoechinorhynchus rutili* in brown trout. The initial approach was to compare the population dynamics of *N.rutili* at 2 Scottish sites, Loch Maragan and Powder Works Dam Lochan, these having been identified as suitable sites during the summer of 1986. Bwathondi (1976) compared the dynamics of *N.rutili* in brown trout at a lentic and a lotic site in North East Scotland. It was hoped that my study would elucidate how much variability there can be in the same host-parasite system, in the same climatic zone but in geographically isolated lentic locations. Both sites are relatively small lochans, less than 10 ha in area, and support natural populations of brown trout (*Salmo trutta*). Loch Maragan was considered to be a fairly undisturbed site lying at an altitude of 472m with the nearest road access 1.2km away and known to freeze over winter. By contrast Powder Works Dam Lochan is a low lying lochan, 137m above sea level, which is managed by the Kyles of Bute Angling Club, does not freeze over in winter for long periods and is stocked annually with rainbow trout. However, after only 3 months sampling at Powder Works Dam Lochan the prevalence of *N.rutili* dropped significantly and then fell to zero (see Table 4.1) and so it was decided to abandon this site and concentrate on Loch Maragan. A full description of the physical characteristics of Loch Maragan is given with a description of the population of brown trout inhabiting it. This information would aid future studies on similar systems.

Table 4.1. Collections of brown trout from Powder Works Dam Lochan

Date	No.of fish caught	No. infected with *N.rutili*	% prevalence of infection	Mean intensity of infection
22.6.86.	4	4	100	99.75
26.6.86.	4	1	25	0.75
12.7.86.	4	0	0	0
2.8.86.	1	0	0	0
10.8.86.	1	0	0	0
18.9.86.	50	0	0	0
17.10.86.	18	0	0	0
14.11.86.	26	0	0	0
20.3.87.	6	0	0	0
30.4.87.	27	0	0	0
29.5.87.	17	0	0	0
25.6.87.	9	0	0	0
21.8.87.	11	0	0	0

4.2. SITE DESCRIPTION

4.2.1. Location, dimensions and topography

The loch (Grid Ref. NN 402278) lies 3.2 km north east of Crianlarich which is 82 km north of Glasgow (by road) (see aerial photograph Fig 4.1). Access is via an unsealed Forestry Commission track, which ends 1.2 km west of the loch, through a Sitka spruce plantation and along a Loch Tay Limestone ridge leading to the loch basin. Transfer between the end of the road and the loch is a 40 min walk, on average. The track was constructed during the last 10 years; before this time access to Loch Maragan was limited to people prepared to walk a considerable distance on foot and so the loch has remained an undisturbed site since its initial stocking with brown trout at the end of the last century. The loch lies at an altitude of 472 m, has a surface area of 7.3 ha and an irregular perimeter of 1689 m. The loch encloses 3 islands (total area = 0.94 ha) and so it has a relatively high value for shoreline development (D_L) of 1.658. The maximum length of the loch is 535 m and the maximum recorded depth is 10.2 m. Along the north western shore, the water is shallow with depths of no more

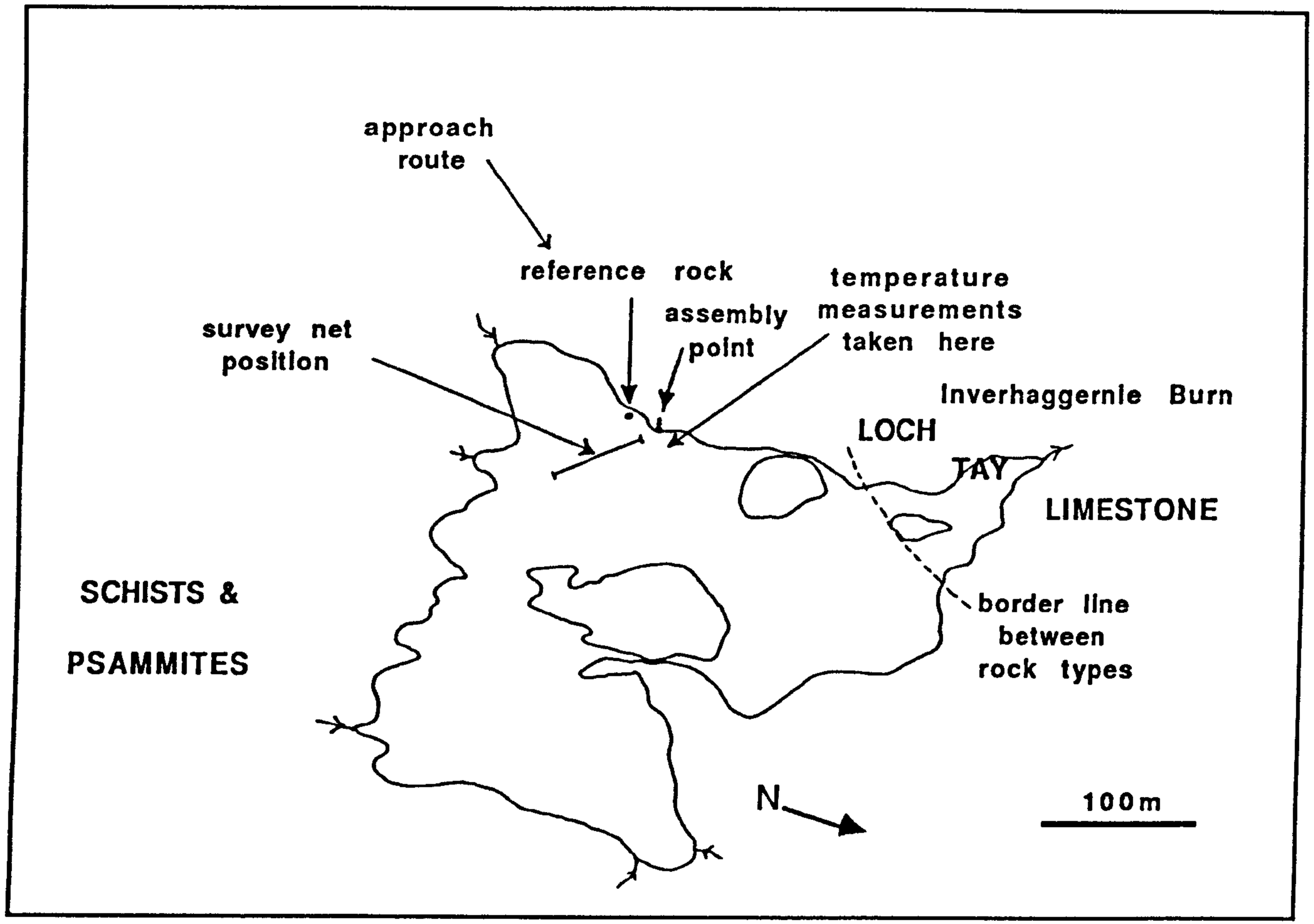
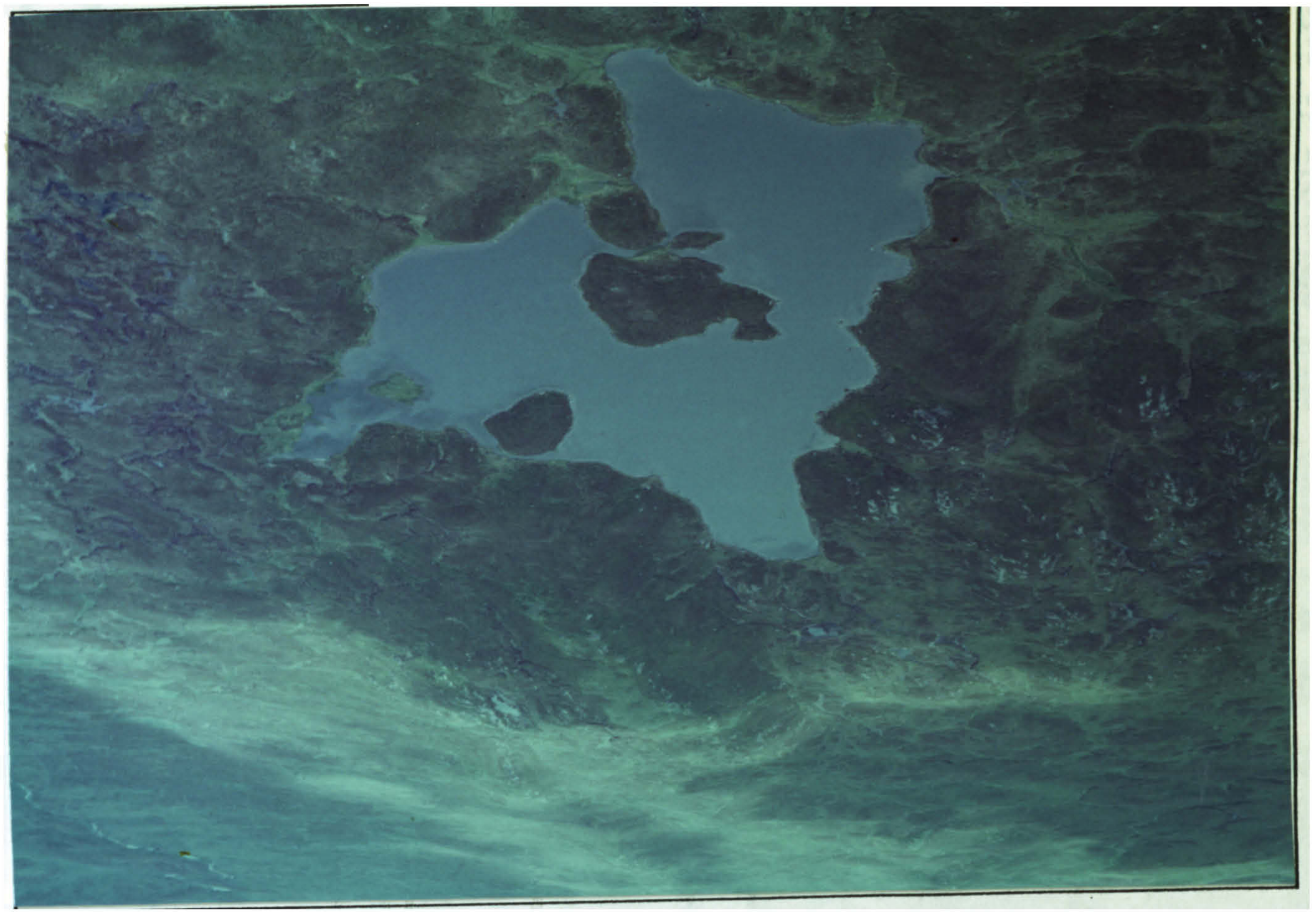


Fig. 4.1 Aerial view of Loch Maragan, Central Region, Scotland

(Photograph taken July 1987 by Roddy MacDonald)



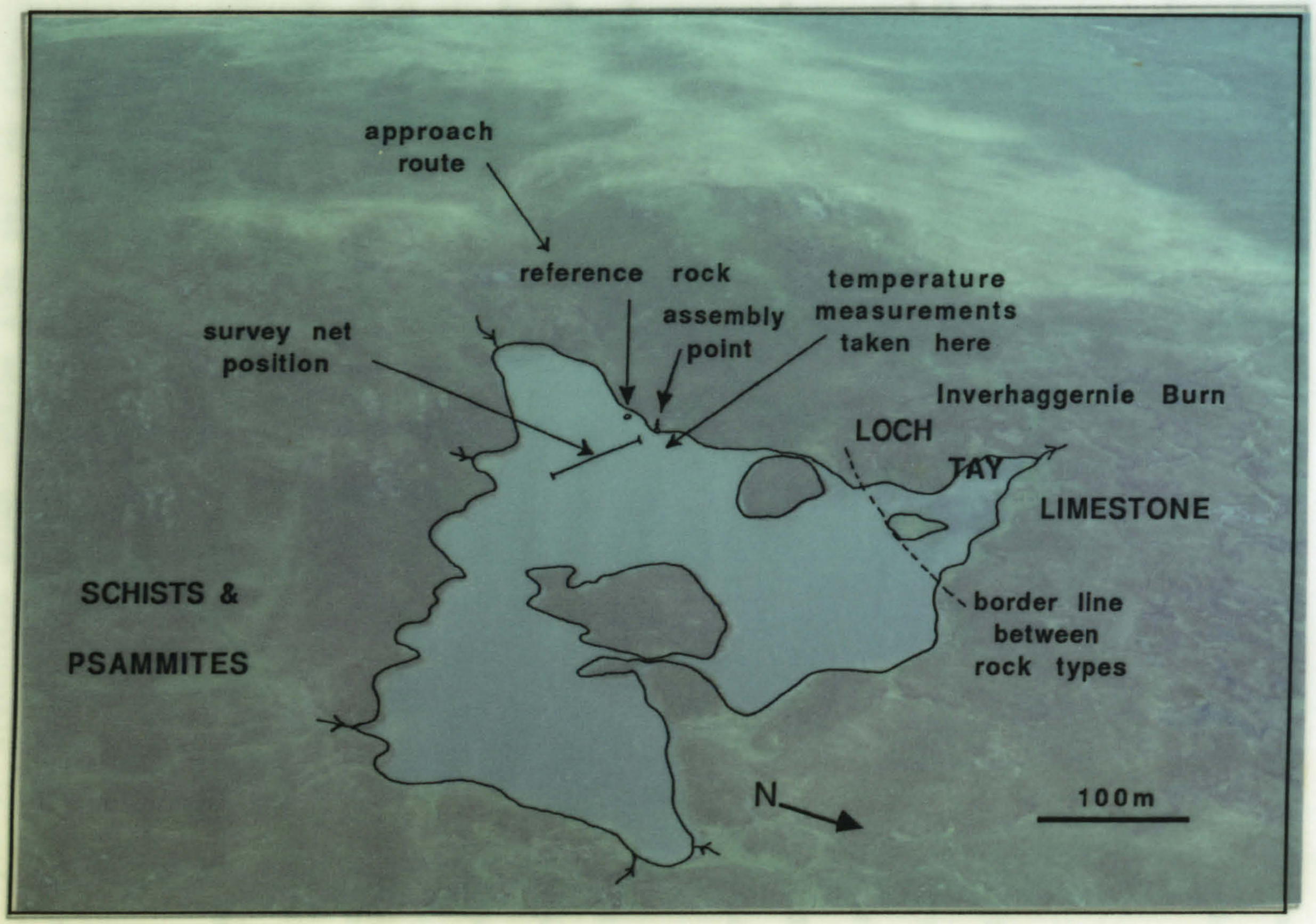


Fig. 4.1 Aerial view of Loch Maragan, Central Region, Scotland

(Photograph taken July 1987 by Roddy MacDonald)

than 1 m. Along the southern shore, the sides are fairly steep dropping down to 10 m depth at the centre of the loch west of the largest island and between the 2 larger islands (see contour map Fig. 4.2). Details of the depth survey and the production of a contour map of the loch are given in section 4.3.3.1.6. Five burns flow into the loch and there is a single outflowing burn at the northern end of the loch called the Inverhaggernie burn which carries the water downstream to the River Fillan in the glen below.

4.2.2. Geology and water chemistry

The majority of the loch is underlain by mica schist and psammite, rocks resistant to weathering and providing little nutrition in the form of dissolved chemicals. The northern tip of the loch is underlain by Loch Tay limestone which is more susceptible to weathering and is likely to influence the p.H. and calcium content of the water in that region. This influence will be of limited significance to the overall water quality because the limestone only underlies the outward flowing Inverhaggernie burn (Leake p.c.). In November 1986, the water p.H. was 6.44 (at 17^{o}C), the conductivity was 242 uS/cm and the alkalinity was 0.12 meq/1. More generally the water appears as a clear peaty brown colour. The deeper areas are covered with a thick peaty substrate which probably influences water quality. Most of this substrate is likely to be allochthonous in origin.

4.2.3. Name and recent history

Maragan has a number of meanings: one explanation from the Gaelic dictionary is 'fat ugly person' which presumably is refers to the irregular outline of the loch. Another derivation states that Maragan means 'mother loch' and is the source of the Inverhaggernie burn (Gilles, 1980).

Loch Maragan was originally used as a source of water to power a crofters flour mill in the last century (Christie, p.c.) and a derilict mill stone lies at the source of the Inverhaggenie Burn. The loch is known to have been stocked with brown trout by the grandfather of the present owner John Christie over 80 years ago and since has been unmanaged. Consequently this loch now has a wild population of brown trout.

4.2.4. Fauna

Three species of fish have been found in the loch: brown trout (*Salmo trutta*), minnow (*Phoxinus phoxinus*) and eel (*Anguilla anguilla*). The eels may have reached the loch via land or by the Inverhaggernie burn from the River Fillan. The origin of the minnows is unknown, but might have occurred when the brown trout were stocked. Campbell (1971) suggested that the occurrence of minnows in Scottish trout lochs can be the result of their introduction as trout food or with the release of unused live-bait by anglers. Other vertebrate species observed at the loch included newts (*Triturus* sp.), common frog (*Rana temporaria*) adults and tadpoles and common gulls (*Larus canus*).

4.2.5. Flora

The surrounding vegetation is typical of a Scottish peat bog, with mosses, bilberries, cow berries and heather being prominent. Within the loch there are a number of emergent plants including horsetails and *Pomatogeton* sp. On the small island, stunted trees (silver birch and alder) and reeds are established.

4.3. FIELD METHODS

4.3.1. Faunal Collections

4.3.1.1. Trout

Monthly collections of trout were initiated in October 1986 and

continued for 22 months until August 1988. Samples were taken in every month between these dates when the loch was unfrozen and during the close season, when permission to fish was obtained from the Department of Agriculture and Fisheries for Scotland. In Scotland, among other protection laws (described by Mills, 1989) Acts of Parliament [Trout (Scotland) Act, 1937; Salmon and freshwater fisheries (Protection)(Scotland) Act, 1951] state that no brown trout may be taken during the close season between 7th October and 14th March, not even for scientific purposes. They also state that the purpose of the law is to improve stocks and knowledge of trout. Thus fish taken during the close season were collected under the supervision of a member of staff from the Department of Agriculture and Fisheries and Food for Scotland who represents the Secretary of State for Scotland. This legal restriction resulted in only a limited number of samples being taken during the close season. A request for a special dispensation from this law to carry out this study was turned down by the Secretary of State for Scotland, despite the efforts of the M.P. for Glasgow (Hillhead).

Trout were caught in pelagic gill nets laid overnight using a small inflatable tender. For the samples up to April 1987, fish were caught in cotton gill nets of various mesh sizes including 1.0", 1.5" and 2.0". Thereafter a Swedish monofilament survey net was also used. This net (Lundgrens Fisheredskaps-Fabrik A.-B.) has 12 panels of monofilament mesh as follows: 3/4", 4 3/4", 2 3/8", 3 3/8", 1 3/4", 4", 2 1/2", 1", 2", 3", 6" and 1 1/4" (Measurements were given in Imperial units by the company). Each section of net is 10ft x 5ft and the overall length of the net is 108ft. A multimeshed net was used to increase the size range of fish caught during each sampling session. For each fishing the net was laid in the same position in the water as

shown in Fig. 4.1. If more nets were used these were placed in other positions in the loch. Nets were retrieved after remaining in the loch overnight and the hours of set were recorded for each fishing trip. The captured trout were removed from the nets at the loch side and the size of mesh in which each fish was caught was recorded. The trout were transported whole back to the laboratory in an ice box to be dissected.

A small number of trout were also caught by fly fishing and 1 by seine netting. A total of 214 trout were caught by gill netting and 11 by fly fishing during the study (Table 4.2a). A summary is given in Table 4.2b.

4.3.1.2. *Minnows*

A total of 205 minnows were caught in gill, trawl, hand and seine nets on a number of occasions during the course of this present study as follows: 25.4.87. 3; 30.5.87. 1; 16.7.87. 1; 6.8.87. 150; 5.9.87. 40; 23.11.87. 3; 14.5.88. 3; 24.7.88. 3; 20.8.88. 2. In addition 2 whole minnows which had been consumed by trout in May 1988 were also examined.

4.3.1.3. *Sialis lutaria* adults and larvae

Adults were collected in June 1987 from around the shores of the loch by hand and larvae were collected in hand nets from benthic material obtained to the west of the middle sized island in May, July and August 1988. The results of these collections are considered in sections 7.2.4 and 7.3.2 of Chapter 7.

4.3.1.4. *Benthic faunal surveys*

Two benthic faunal surveys were carried out in November 1986 and May 1987 respectively. A diving team collected samples from the lake substrate using a technique similar to that of Holt (1984) and

described by Lassiere (1987). The samples were examined to identify potential and actual intermediate hosts of *Neoechinorhynchus rutili* at Loch Maragan (Table 4.3). Ostracods were restricted in number and no signs of *N.rutili* developmental stages were found in any of the faunal groups. No *Sialis lutaria* larvae were found in the samples and this may be due to the burrowing habit of the insects.

Table 4.3. Faunal counts for the sediment samples from Loch Maragan

Faunal element	Winter sample	Summer sample
Oligochaeta	20	p
Pisidium sp.	20	p
Hydracarina	9	p
Cyclopoidea	6	p
Cladocera (adults)	7	p
(ephippia)	many	p
Ostracoda	14	0
Ephemeroptera (nymphs)	2	0
Trichoptera (unident.)	1	p
Hydroptilidae (larvae)	5	p
Chironomidae	31(larvae)	p(pupae)
Dytiscidae	1	0
Nematoda	0	p

(Note: the counts for the benthic faunal samples are for 5 separate sample bags in the winter and p indicates presence in the summer samples).

Relatively few animals were collected in each sample. By comparison Macan (1949) found up to 1196 individual organisms in one Petersen Grab sample at Three Dubs Tarn, Cumbria. The range of faunal elements found in the benthic material was similar to those found by Macan (1949) and Walkey (1967) in the Lake District and County Durham respectively. The fact that the benthic fauna appeared rather 'sparse' may partially be linked to the p.H. of the loch water. Morgan (1966) showed that in Scottish lochs the biomass of bottom fauna per unit area increased with increasing alkalinity and this may be the case at Loch Maragan.

4.3.2. Trout population estimate

4.3.2.1. *Introduction*

One problem associated with field investigations of the population dynamics of parasites is the need to acquire knowledge of the structure and size of the host population as well as that of the parasite population. There are many references in the fish parasitology literature which describe prevalence and intensity values at a site only in terms of a sample of fish (e.g. Chappell, 1969c; Tedla & Fernando, 1969; Bwathondi, 1976; Kennedy & Burrough, 1978; Amin, 1985, 1986a). Thus if 5 out of 10 fish are infected with a parasite, the prevalence of infection is described as 50%. In this case the extent to which this is representative of the host population as a whole is unknown. Ossiander & Wedermeyer (1973) and later Simon & Schill (1984) used computer programs to generate tables of sample size requirements for detection of fish infected by pathogens. The main purpose of this work was aimed at the assessment of disease levels in commercial systems. Simplistically these programs generated tables from which the prevalence of infection in the whole population could be estimated with a certain degree of statistical confidence given the size of the host population and the prevalence of infection in a sample of specified size. For example, if a population is known to be 1000, detection of a 10% prevalence of infection with 95% confidence would require a sample size of 28 fish of which at least 1 must be found to be infected with the parasite concerned (Simon & Schill, 1984).

If the host population size is known then the appropriate size of sample can be taken to give the maximum amount of information about the prevalence of the parasite in the whole population at that point in time. The advantages of this approach are two-fold; not only is

there an increase in the amount of reliable information obtained but also the minimum size of sample required to obtain such information is known and controlled.

The practical extension of this theory was to make a population estimate for *Salmo trutta* at Loch Maragan. The dimensions of this relatively small loch (section 4.2.1) make such an estimate reasonalby feasible. The methods for estimating fish population sizes vary in complexity and in amount of experimental manipulation (e.g. Dahl, 1919; Schnabel, 1938; Cooper, 1952, Ricker, 1975). As described by Youngs & Robson (1978): "The method chosen for a given study will in the large part be determined by consideration of the biology of the species, objective of the study, characteristics of the habitat and the resources available". Considering each of these points in turn for Loch Maragan:

i) Biology of the species

Trout are territorial in habit, although the results of some studies on this feature have been ambiguous (e.g. see Jensen, 1977 for discussion). Also, the activity of the trout is directly related to the ambient temperature, being greater the higher the temperature. Consequently, following the advice of Dr J.E. Thorpe, Department of Agriculture and Fisheries for Scotland, Pitlochry, it was decided to carry out the population estimate in August 1987.

ii) Objective of the study

The objective was to obtain an estimate of the order of magnitude of the trout population and therefore did not need to be too accurate in terms of the actual numbers of individuals present.

iii) Characteristics of the habitat

Details of the location, dimensions and topography of Loch

Maragan are given in section 4.2.1. The loch is of manageable proportions for an attempt at a population estimate.

iv) Resources available for the study

The main limitations in terms of resources for work at Loch Maragan were manpower and the type of equipment, which needed to be light enough to be carried up to the site from the road.

Bearing the previous factors in mind Loch Maragan provided a site at which it was relatively feasible to make an attempt at population estimation, despite some restrictions.

Fish populations can be estimated in 3 ways: using ratio methods, catch-effort methods and direct enumeration (Youngs & Robson, 1978). Direct enumeration is obviously not suitable for a standing lake and catch-effort methods require a series of samples to be taken where the decline in catch size per unit effort should decrease with each successive fishing. The catch-effort method is therefore rather 'expensive' in terms of numbers of fish that must be caught in order to make the estimate and also in actual fishing effort. This method also assumes that the probability of capture of all fish must be equal.

Consequently a ratio method was considered to be the most appropriate for the Loch Maragan brown trout population. These vary from a simple single-mark and recapture operation (Petersen method) to multiple mark and recapture operations. Clearly, the most simplistic methods are likely to be the least accurate but in this case only a rough estimate was required and also the resources in terms of time, manpower and equipment were limited. Therefore a single mark-recapture operation was considered to be the most suitable in this case.

The Petersen method assumes that if a sample of fish is caught, marked and released, the marked fish will mix randomly with the whole

population so that, in a second sample, the proportion of marked fish will be representative of the relative number of marked fish in the entire population. This can be expressed algebraically:

$$N = \frac{mc}{r}$$

where N = number of fish in population
m = number of marked fish in the population
c = number fish in sample
r = number of marked fish in c

There are a number of assumptions which are made in this method of population estimation.

i) The population is stable and closed to recruitment and immigration

ii) Marked fish are in every way the same as unmarked fish.

iii) Marked fish do not lose their mark.

iv) All fish are reported upon recapture.

v) Either the marking or recapture sample is random or there is random mixing of marked and unmarked fish.

Estimates of trout populations in small lake have been made in the past, for example the classic experiment by Dahl (1919) in a Norwegian tarn. In Scotland no such estimates have been published. In the 1970's the International Biological Programme Project at Loch Leven made an estimate of the brown trout population size. This was a large project which utilized the manpower of the sport fishery to aid in the collection of data. The population estimate was made on the basis of 4036 fish marked over 3 years (Thorpe, 1974). This type of strategy would obviously not be suitable for a small lake like Loch Maragan where the sport fishing is quite limited, unmanaged and sporadic.

As this was a first attempt at such an estimate in Scotland, the

simplest Petersen method was used as this best fulfilled the requirements of the resources available. In terms of the assumptions of this method the loch was:

i) Closed to recruitment and or immigration over a short period of time especially in the summer months since the trout breed in winter.

ii/iii) The fish were batch marked with alcian blue dye administered with a Panjet inoculator between the pectoral fins over the heart, which is a long lasting mark and should not have any effect on the fishes behaviour or susceptibility to gear.

iv) This assumption would obviously be met since all the recaptured trout were recaptured by the investigator.

v) The initial sample was taken with a fine-meshed seine net. This is the least selective netting procedure available and thus makes the capture method as random as possible. Capture of trout with electrofishing gear is one of the least selective of all fishing methods (Lagler, 1978), but the combination of low efficiency of this type of equipment in low conductivity water as in Loch Maragan, the importable nature of the equipment and unsuitabity for use from small inflatable boats made the use of this method at Loch Maragan impractical. The recapture was with gill nets and as pointed out by Thorpe (1974) the utilization of different methods for the capture and recapture of fish helps to overcome the types of bias introduced into estimates based on fishing with the same gear.

4.3.2.2. *Materials and Method*

4.3.2.2.1. *Equipment List*

-1 Campari inflatable boat (6 adult size) + oars, pump and baseboard

-1 Seine net with the following dimensions plus ropes. The whole net was 35 m long and 2.2 m deep. The net consisted of 5 separate

sections of mesh as follows: 7 m of 35 mm mesh; 7 m of 20 mm mesh; 9 m of 10 mm mesh; 6 m of 20 mm mesh and 6 m of 35 mm mesh. All mesh sizes are knot to knot.

-1 Hand net

-1 Set of 1:10,000 O.S. maps plus enlarged detailed maps of the site with details extrapolated from an aerial photograph (Fig. 4.1).

-2 x 5 l plastic bowls

-1 Inflatable paddling pool (holding tank)

-1 fish measuring board

-1 Panjetting device + alcian blue dye

- Benzocaine in alcohol solution (0.01%) for anaethetising fish

-1 Household weighing scale, accurate to within 5 g

- Data recording slates and pencils

-1 Wet sponge

4.3.2.2.2. *Method*

4.3.2.2.2.1. Capture

Trout were captured using the seine net which was shot in a semicircular path and then drawn towards the shore. Each seine netting point and the numbers of fish captured were recorded. Fish were captured on 2 dates, 1.8.87. and 6.8.87. At each seine netting point a 'marking station' was set up, consisting of a holding tank (inflatable paddling pool) containing loch water for holding fish before marking, a 5l bowl containing a 1:20,000 solution of benzocaine in loch water for inducing anaesthesia, a measuring board and weighing scale, a marking area (wet sponge) and a recovery tank (5 l bowl). As the fish were brought to the marking station they were placed in the holding tank and treated as follows:

1. Two to 3 fish at a time were taken from the holding tank with a hand net and were placed in the benzocaine solution for 1 to 2

min.

2. The fork length and wet weight of each fish were measured and recorded on a data slate.
3. Each fish was marked between the pectoral fins over the heart with a single shot from a Panjetting device containing alcian blue dye. During this process each fish was supported on a wet sponge to reduce mechanical damage and keep the body moist.
4. Each marked fish was placed in the recovery tank and was released once 'normal' swimming behaviour had been observed for 5 min.
5. When all the fish had been measured and marked they were returned to the lake at the same capture point.

Throughout the entire marking procedure, the handling of the trout was kept to a minimum to ensure that they were as unstressed as possible. At each fishing site a new marking station was set up to this aim. Scale samples were not taken from the trout because the removal of scales may have caused considerable stress to the trout which in turn would alter their mortality rate and susceptibilty to gear etc. Instead ages were estimated on the basis of the known lengths of aged trout in the recapture sample. Seventy four trout were caught and marked in this way of which 73 were returned to the loch. A total of 9 seine nettings were made on 1.8.87. and 16 on 6.8.87. One eel and several hundred minnows were also captured during the seine netting procedure. These were killed by a long exposure to benzocaine and examined for gut parasites.

4.3.2.2.2.2. Recapture

Five gill nets were used to recapture trout on 15.8.87, 9 days after the second marking session. These nets were of varying dimensions and mesh sizes as follows (Table 4.4).

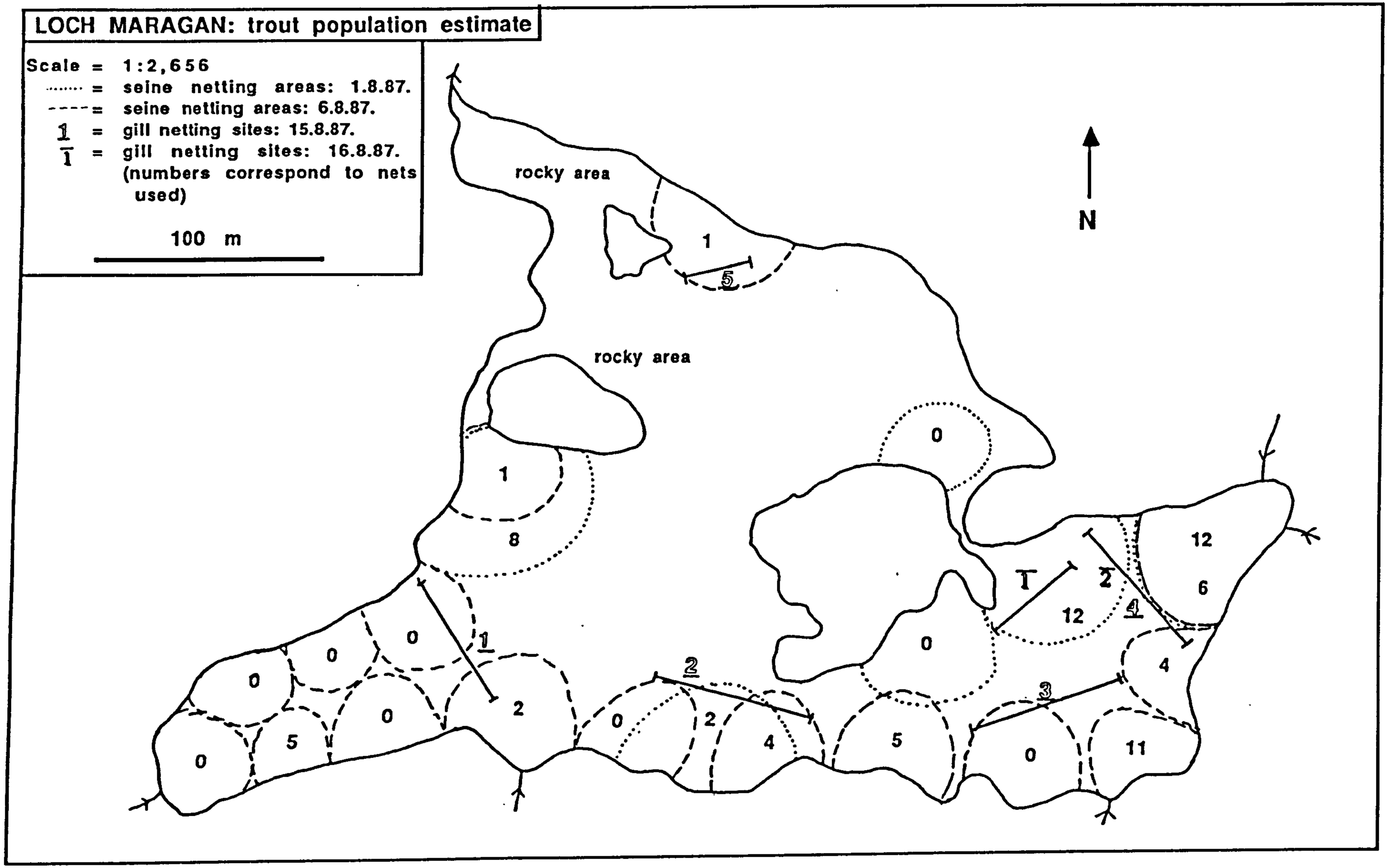


Fig. 4.3 Map of Loch Maragan showing the seine netting areas and numbers of trout caught and the positions of gill nets for their recapture

Table 4.4. Gill nets used for recapturing trout

Net No. Day 1	Net No. Day 2	Length m	Depth m	Mesh mm	Comments
1	1	21.0	1.2	40	
2		22.5	1.5	55	
3		23.0	1.6	50	
4	2			12 meshes	Survey net
5		6.0	1.4	52	Damaged

Two further nets (Nos. 1 and 4) were set on 16.8.87. to capture more trout. The nets were set around the loch at points corresponding to where the trout had been originally marked (Fig. 4.3).

The age and status of the *Neoechinorhynchus rutili* infection of each recaptured trout were determined by examination of scale samples and by dissection respectively. On the basis of the known ages of the recaptured trout and their dimensions, the marked trout were assigned to various age classes on the basis of their length measurements. Examination of the frequency distribution of lengths of gill netted fish indicated a cut off between 1+ and 2+ fish at about 158 mm and of 215 mm between 2+ and 3+ fish. These values were used to assign an age class to each of the marked trout. All trout ‹158 mm fork length were assigned as 1+, all › 158 mm and ‹215 mm assigned as 2+ and all ›215 mm assigned as 3+ fish.

With information on the lengths (and age class) of the marked trout and the recaptured trout, the selectivity of the recapture gear could be assessed. On the basis of this a very rough estimate was made of the size of the subpopulation of this age class. By means of the Robson & Chapman (1961) method as described by Youngs & Robson (1978), the survival rate of each year class was calculated and a rough estimate of the whole population size was made.

The original capture method only permitted the capture of fish in

the shallower areas of the loch. Accordingly, in an attempt to deal with the number of trout in the entire loch, the volume of the loch was estimated (see section 4.3.3.4) and the percentage of the loch volume that had been fished calculated.

4.3.2.3. *Results and Discussion*

A total of 73 trout were marked and released into Loch Maragan on 1.8.87. and 6.8.87., ranging from 10 to 260 g in wet weight and 88 to 277 mm in fork length. These fish were caught during 25 seine nettings around the loch as shown in the diagram (Fig. 4.3), 9 on 1.8.87. and 16 on 6.8.87. After 9 days, on 15.8.87. five gill nets of various mesh sizes were set around the loch as shown in the diagram Fig. 4.3 and 2 nets were also set on 16.8.87. which are also shown in the same figure. A total of 32 trout, ranging from 158 to 260 mm fork length, 166 to 275 mm total length and 44.82 to 175.43 g wet weight, of which 5 were marked, were caught. The fish age distribution was 1x 1+, 22x 2+ and 9x 3+. All of the marked fish were in the 2+ age class which suggested a severe gear bias.

By dividing the loch water up by depth contour lines into areas fished and not fished during the seining procedure the volume of the loch water fished was calculated to be 43,263 m^3 which represents 28.10% of the total loch volume. This volume can be used to make a more realistic estimate of the total loch trout population. The recaptured trout were all from the 2+ age class. Therefore, the recapture method was highly selective. However an estimate of the size of this cohort in the population can be made.

The number of 2+ trout marked and released = 32 = m

The number of 2+ marked trout recaptured = 5 = r

The number of 2+ trout in the recapture sample = 22 = c

Therefore an estimate of N = 2+ trout population size is:

$$N = \frac{mc}{r} = \frac{32 \times 22}{5} = 140.8$$

Strictly an estimate of the variance of this value should be calculated but the available data is so limited that improbable values are obtained by any of the methods suggested by Bailey (1952) or Youngs & Robson (1978). Obviously when accuracy is the main objective of the estimate then the variance should be considered.

On the basis that only 28.10% of the loch water was fished this value for the 2+ trout cohort should be multiplied as follows to obtain an estimate for the whole loch population:

$$140.8 \times \frac{100}{28.1} = 501.06 \text{ 2+ trout in the entire population}$$

The survival rate of each year class was calculated using the seine netted trout data, assigning the 1+ age class as Code 0, the 2+ age class as Code 1 etc. as described by Youngs & Robson (1978). Chi squared tests of the agreement of the data with the model showed that the 3+ age class and older age classes were not fully recruited to the gear, which could have been due to behavioural differences. The Chi squared results for the 1+ and 2+ trout indicated that the numbers of fish in the sample were not due to sampling error. Thus the estimates of the cohort sizes in the population were made on the basis of the estimate 2+ cohort size and the survival rate of 1+ and 2+ trout. Ninety-five percent confidence limits for these survival rates were also calculated. The survival rates were as follows: 1+ trout = 0.33945 +/- 2 x 0.04557; 2+ trout = 0.00309 +/- 2 x 0.0556. Thus the estimation of the numbers of trout of age classes 1, 2 and 3 in Loch Maragan are as follows:

Table 4.5. Estimate of Loch Maragan trout population size

Age class	Population size		
	Minimum estimate	Middle estimate	Maximum estimate
1	1,163.7	1,476.1	2,017.9
2	501.1	501.1	501.1
3	10.2	65.9	121.7
All	1,675.0	2,043.1	2,640.7

It is not clear exactly whether the 1+ age class is the youngest age class represented in the loch because no scale samples were taken during the seine netting procedure. A single trout, 88 mm total length and weighing 10 g, was caught during the seining and this might be a representative of the 0+ cohort on the basis of the back calculation results (section 4.4.3.). These dimensions are similar to values given by Campbell (1971) for the 0+ trout in other Scottish lochs. Interestingly, minnows with total lengths as small as 38 mm were caught in the seine net suggesting that the majority of the 0+ trout were not in the main loch and possibly still inhabiting the feeder streams. With reference to the paper of Simon & Schill (1984), their tables indicated that for a population of this estimated size, 28 fish would have to be sampled to detect a 10% prevalence of infection with 95% confidence.

The estimate is a crude one and the values must be treated with caution. However the overall impression is of a population of around 2000 individuals of which the first 3 age classes represent the greatest proportion. It is possible that the larger individuals were in the deeper, unfished sections of the loch so it is not feasible to predict the maximum age of the trout from the netting procedure although individuals aged up to 5+ have been caught during the gill

net sampling. The fact that older individuals were poorly represented in the seine netting samples may also be a true reflection of the trout population itself. Campbell (1971) described mass mortality of trout post-spawning so a reduction in cohort size from year to year is inevitable. This mortality may be as a result of increased susceptibility to parasitism or starvation effects post-spawning (Frost & Brown, 1967) or predation by bird or mammal predators while in the spawning burns (Campbell, 1971).

Subsequent to the initial recaptures of marked fish in August 1987, a further 6 marked fish were recaptured in the samples taken up to 20.8.88. with clearly visible blue marks, over a year after the marking procedure. This substantiates the observations of Hart & Pitcher (1969) who found good retention of similar marks on chub (*Leuciscus cephalus*) and dace (*Leuciscus leuciscus*) in the field and also indicates that marking does not have an adverse effect upon trout survival.

4.3.3. Physical and Chemical data

4.3.3.1. *Water*

4.3.3.1.1. *Temperature*

Temperature was measured monthly, while the loch was unfrozen, with a maximum and minimum thermometer placed at a depth of 300 mm in a shaded area close to the assembly point (see Fig. 4.1). This system was used by Eure (1976) in a heated reservoir in South Carolina, U.S.A. The maximum and minimum temperatures during the period of gill netting were recorded to the nearest 0.5 ^{o}C (Table 4.2.).

Table 4.2a: Loch Maragan Field data

Date	No. of hours gill net was set (h)	No. of fish caught	Temperature (^{o}C)	Depth (mm)
12.7.86.	-	(3)	-	-
20.7.86.	-	(4)	-	-
4.10.86.	16	14	-	-
31.10.86.	19.25	4	5.5	300
30.11.86.	22.50	4	4.0-4.5	450
10.1.87.	a	2	Frozen	-
9.4.87.	-	10	-	-
25.4.87.	15.50	6	9.5-10.5	-
25.4.87.[b]	3 months	36	-	-
30.5.87.	21.0	7(1)	16.0	-
20.6.87.	18.0	4(2)	14.0-15.0	310
17.7.87.	26.0	5(1)	14.0-15.0	405
1.8.87.	-	1 s	-	-
15.8.87.	16.0	20	13.0	280
16.8.87.	21.5	12	-	480
5.9.87.	-	3	-	330
6.9.87.	-	-	-	430
13.10.87.	26.0	15	5.0-6.0	315
21.11.87.	-	5	4.0	420
18.3.88.	-	9	0.0-1.0	310
19.3.88.	-	-	0.0-2.0	390
15.4.88.	19.25	16	7.0	370
13.5.88.	19.00	18	11.0	260
25.6.88.	-	5	15.0	230
24.7.88.	25.00	7	14.0	365
20.8.88.	18.50	16	11.0-16.0	430

a = Net was set on 29.12.86. and was frozen into the loch until 10.1.87. when it was partially removed from the ice.

b = These are fish which were removed from the ice trapped net which was in the loch between 29.12.86. and 25.4.87.

s = Seine netted trout. () = fly-caught trout.

Table 4.2b: Summary of Loch Maragan field data collection

Year	Visits	Number of Months	Trout caught
1986	5	3	29
1987	11	11	126
1988	6	6	71
Total	22	20	226

4.3.3.1.2. *p.H.*

A sample of loch water was collected in November 1986 and was kept frozen in a polythene bottle prior to analysis as recommended by Golterman, Clyco & Ohnstad (1978). The p.H. was measured as 6.44 with a laboratory based Whatman p.H. electrode at a water temperature of 17°C.

4.3.3.1.3. *Conductivity*

The conductivity of the November 1986 water sample, measured with a conductivity meter at 17°C was found to be 0.242 mS/cm.

4.3.3.1.4. *Alkalinity*

For the alkalinity measurement a volume of the November 1986 sample was filtered through a GF/A 90 mm filter in a Buchner Filter funnel. A 100 ml aliquot of water was titrated against N/100 hydrochloric acid using B.D.H. p.H. 4.5 indicator. Alkalinity was calculated using the following formula:

$$\text{Alkalinity (meq/l)} = (n.1000/V).v$$

where, n = acid normality
V = sample volume
v = volume of acid added (ml) and had a value of 0.12 meq/l.

4.3.3.1.5. *Depth fluctuations*

Water depth was measured monthly at the Reference rock (see Fig. 4.1) and the results are given in Table 4.2. Measurements taken on 2 consecutive days gave an indication of the rate of variation of depth with time. The depth readings had a range of 250 mm with depths fluctuating up to 200 mm in 1 day.

4.3.2.1.6. *Depth Survey*

A depth survey was carried out to map the topography of the loch and so calculate the water volume. A knowledge of the loch's volume

was required to increase the accuracy of the estimate of the trout population. A large number of the freshwater lochs of Scotland were surveyed by Murray & Pullar (1910) including Loch Essan, which is the nearest loch to Loch Maragan. A contour map of this loch, which is larger than Loch Maragan, was drawn on the basis of 40 soundings taken while rowing a boat at an estimated pace between recognisable points on the loch shore.

The present depth survey of Loch Maragan was carried out as follows. A 350 m transect line, marked every metre, was stretched across the loch between easily identifiable shore points, chosen using the 1:10,000 O.S. map and aerial photograph (Fig. 4.1). Reference points used included burns, peninsulas, large rocks and islands. For each transect, the line was stretched between the 2 predetermined points and the depth, every 5 m along the line, was measured using a weighted plumbline marked out every 100 mm. A series of 26 transect lines and 407 soundings were taken on 3 different dates. Comparison between soundings on different dates was made possible on the basis of the known depth at the reference rock recorded on each date.

Depth contours were drawn on the map every 1.0 m, on the basis of the depth soundings. The volume of the loch was calculated by estimating the area enclosed by each contour line with a computer linked to a digitising pad. For each measurement, 10 estimations were made and the mean value was used in the calculations. The total volume was calculated by used the following formula:

$$V = 0.5 \quad z \ [A_o + 2(A_1 + A_2 + \ldots\ldots A_{(zmax - z)} + A_{zmax}]$$

where z = increments between contours

A = area enclosed by each contour line

The depth at the reference rock on each day was recorded and the depth soundings were corrected to the first date (Dates = 31.10.87.,

20.11.87. and 27.9.88.) from which a contour map was drawn (Fig. 4.2). The areas enclosed by each contour are shown in Table 4.5. The volume of the loch was calculated to be 153,943 m^3. Overall the loch is fairly shallow with only the cental area reaching any great depth.

Table 4.6. Depth survey measurements

Feature	Area m^3	Perimeter m
Loch Maragan total area (inc. islands)	82,534	1,689
Island 1 (smallest)	469	104
Island 2	2,296	192
Island 3 (largest)	6,647	403
Loch Maragan water area (A_o)	73,122	
0-1 m deep (A_1)	47,849	1,761
1-2 m deep (A_2)	24,298	1,145
2-3 m deep (A_3)	15,327	749
3-4 m deep (A_4)	10,912	615
4-5 m deep (A_5)	7,501	485
5-6 m deep (A_6)	5,600	358
6-7 m deep (A_7)	3,493	244
7-8 m deep (A_8)	1,731	169
8-9 m deep (A_9)	647	111
> 9 m deep (A_{10})	48	28

4.4. THE BROWN TROUT POPULATION OF LOCH MARAGAN

4.4.1. Body measurements

For each trout the following measurements were made:

a) Fork length mm

b) Total length mm

c) Wet weight g (measured to the nearest 0.01 g)

d) Wet weight of gonad g (measured to the nearest 0.01 g)

e) Wet weight of body - guts (including liver) and gonads

The guts were removed by making an incision along the mid-ventral

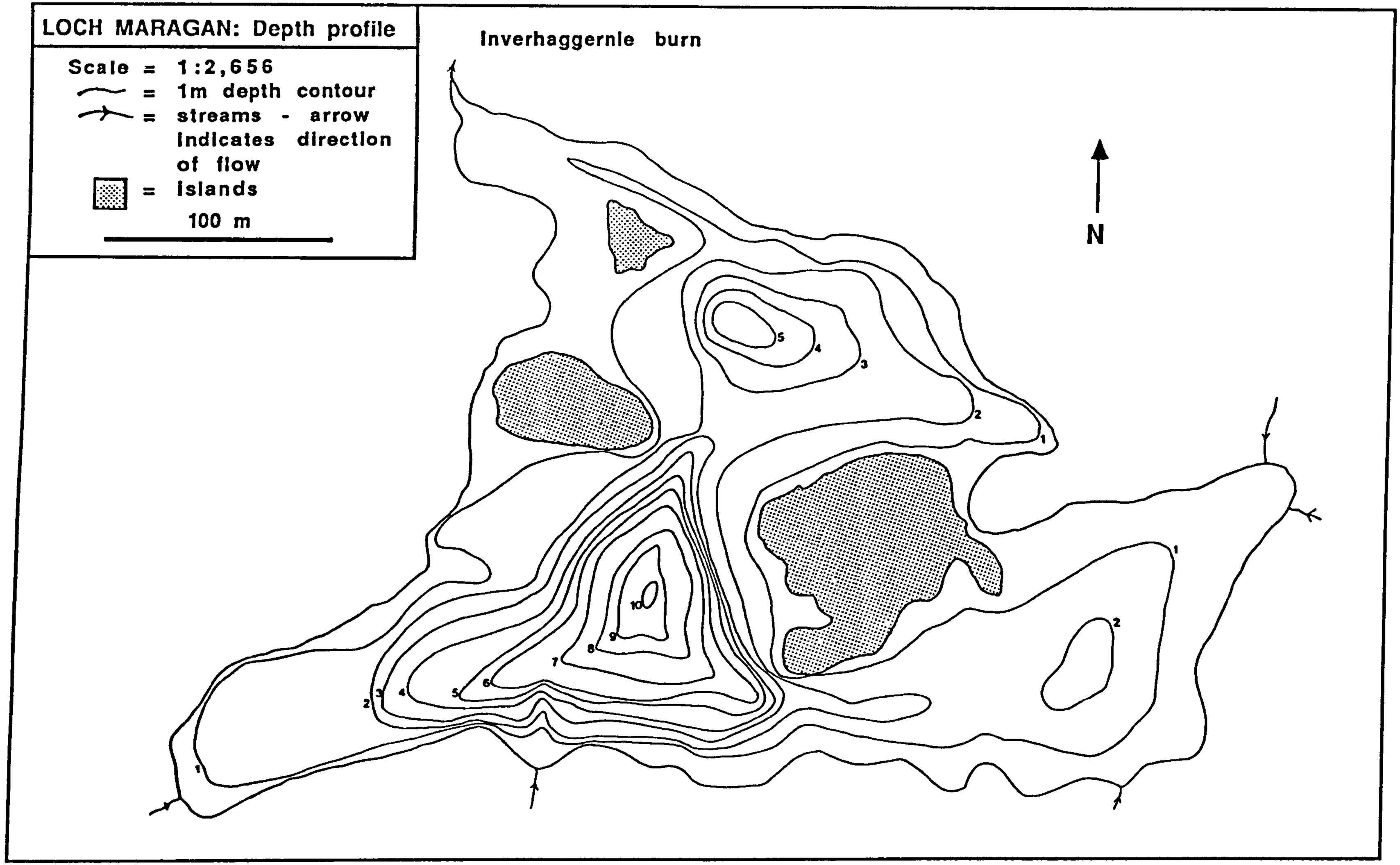


Fig. 4.2 Depth profile of Loch Maragan, Central Region, Scotland

line of the fish from the region just over the heart to the anus. The gut was cut as far forward as possible on the stomach at the anterior end and around the anus at the posterior end. The gonads were removed whole.

4.4.2. Determination of sex and state of maturity

The sex organs were examined and the sex and state of maturity of the trout was assigned according to the description given by Frost & Brown (1967). Males were described as either immature (gonads appear as small, pale pink, fine threads), mature (gonads appear as large white organs in the body cavity) or spent. Females were assigned to 6 stages according to the size and form of the ovaries. When mature eggs were found (Stage V) in the body cavity these were counted in order to assess the potential fecundity of the individual fish. An examination of the age of mature fish and their temporal occurrence in the samples gave an impression of the reproductive biology of the trout in the loch.

4.4.2.1. *Fecundity of Loch Maragan trout*

Nine stage V fish ranging from 241 to 330 mm in total length with gonads weighing up to 21.12 g were caught. The egg numbers in these individuals ranged from 291 to 451. Five fish were of the 3+ age class and 1 individual was a 2+ fish.

Twenty-nine mature or maturing male trout were caught. These ranged in total length between 131 and 337 mm and included fish from the 1+ to 5+ age classes. The maximum testes weight (wet) was 9.82 g in a 5+ trout weighing 338.91 g. Campbell (1971) reported that female trout in Scotland reach maturity between the ages 2+ and 4+ with 3+ being the 'norm'. He also stated that a few 1+ males in the population reach maturity. In the case of Loch Maragan, this early maturation of males at a small size, may facilitate spawning in the restricted

stream areas or be evidence for a sneaking form of reproductive strategy (see Gross, 1985).

4.4.2.2. *Seasonality of trout maturity*

An examination of the numbers of trout at various stages of maturity gives a confusing picture of the time of spawning at Loch Maragan. In 1986, mature stage V females were observed in July and mature males in early October. Spent females (Stage VI) were caught at the end of October indicating that spawning had taken place. In 1987 spent male and female trout were recovered as late as May which appears to indicate that spawning can occur throughout the winter and spring period. This prolonged period possibly indicates a dearth of suitable spawning sites for all fish at one time, that is stream areas, although it is possible that some spawning occurs on the rocky shore areas of the loch itself. From June to October mature and maturing male and female trout were caught with the first evidence of female spawning seen in late November. Evidence of spawning was seen again in the March 1988 sample. In April, May and June of 1988 no spawning evidence was observed and in July and August both maturing and mature trout were caught. Thus the general picture is one of spawning beginning in late October with spawning runs lasting up to May in the following year, followed by development of gonad tissue in the summer months in preparation for the next spawning season.

4.4.3. Age and growth determination

At least 20 scales were removed from each fish in the area between the dorsal and adipose fin above the lateral line (Dannevig & Host, 1931) for age assessment. Scale samples were kept in individually labelled scale packets before microscopic examination. For each trout, 5 non-replacement scales were examined and

measurements were made in arbitrary units with 1 unit equivalent to 82 um. When the annual growth ring did not occur at the border of the scale the fish was ascribed as the year class with a +. One hundred and eighty-seven scale samples were examined (total = 935 scales) and the regression line of scale length on total length was calculated by the least squares method for all trout and female and male trout separately (Figs. 4.4a, b, c). For estimates of growth rate, 60 trout were selected and a back calculation on 5 scales was carried out and the scale length at each age estimated. Least squares regression lines, as recommended by Bagenal & Tesch (1978) and used by Hesthagen (1985) for female and male fish, were plotted separately (Fig. 4.5) and the back calculated lengths estimated on the basis of the regression equations. The scale samples came from trout from 4 cohorts: 1983, 1984, 1985 and 1986 respetively. For each year cohort, the mean length of all trout and for female and male trout was calculated and the annual specific growth rate was estimated using the following formula:

$$G_L = \frac{\ln L_2 - \ln L_1}{T_2 - T_1}$$

where G_L = specific growth rate

L_1 and L_2 are natural logs of mean length of populations at times T_1 and T_2 respectively. This formula was also used by Bwathondi (1976) for his analysis of Scottish trout populations. The growth rate for all cohorts together was also calculated and there was no evidence of significant differences between cohorts (Figs. 4.6a, b, c). Values of 69.14, 40.96 and 25.45 were determined for the second, third and fourth years of life, respectively. The maximum aged trout caught in the loch was a 5+ male trout, 337 mm total length, in July 1988. This agrees with Campbell's (1971) view that relatively few trout survive more than 5 years in Scottish lochs.

a.

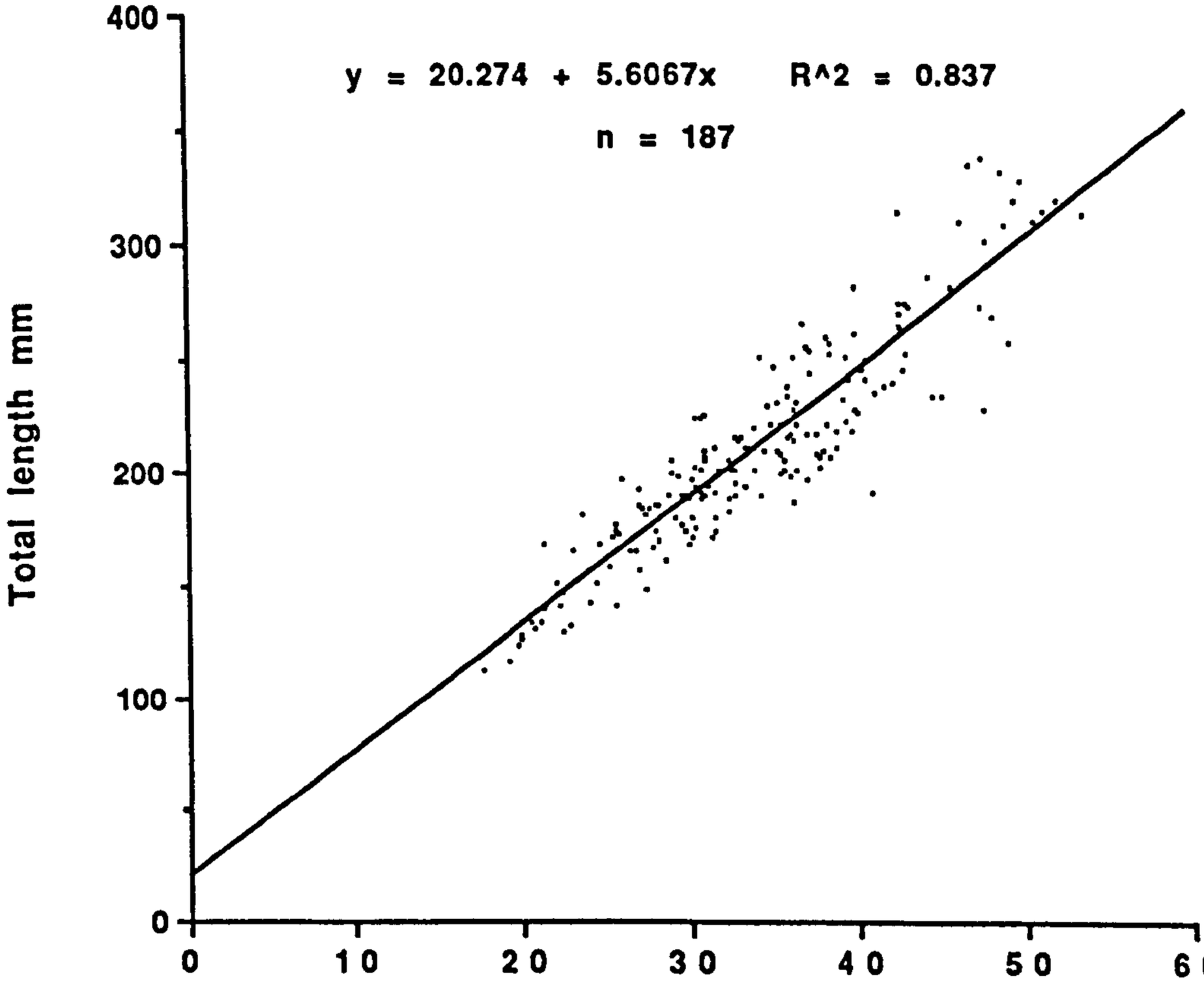
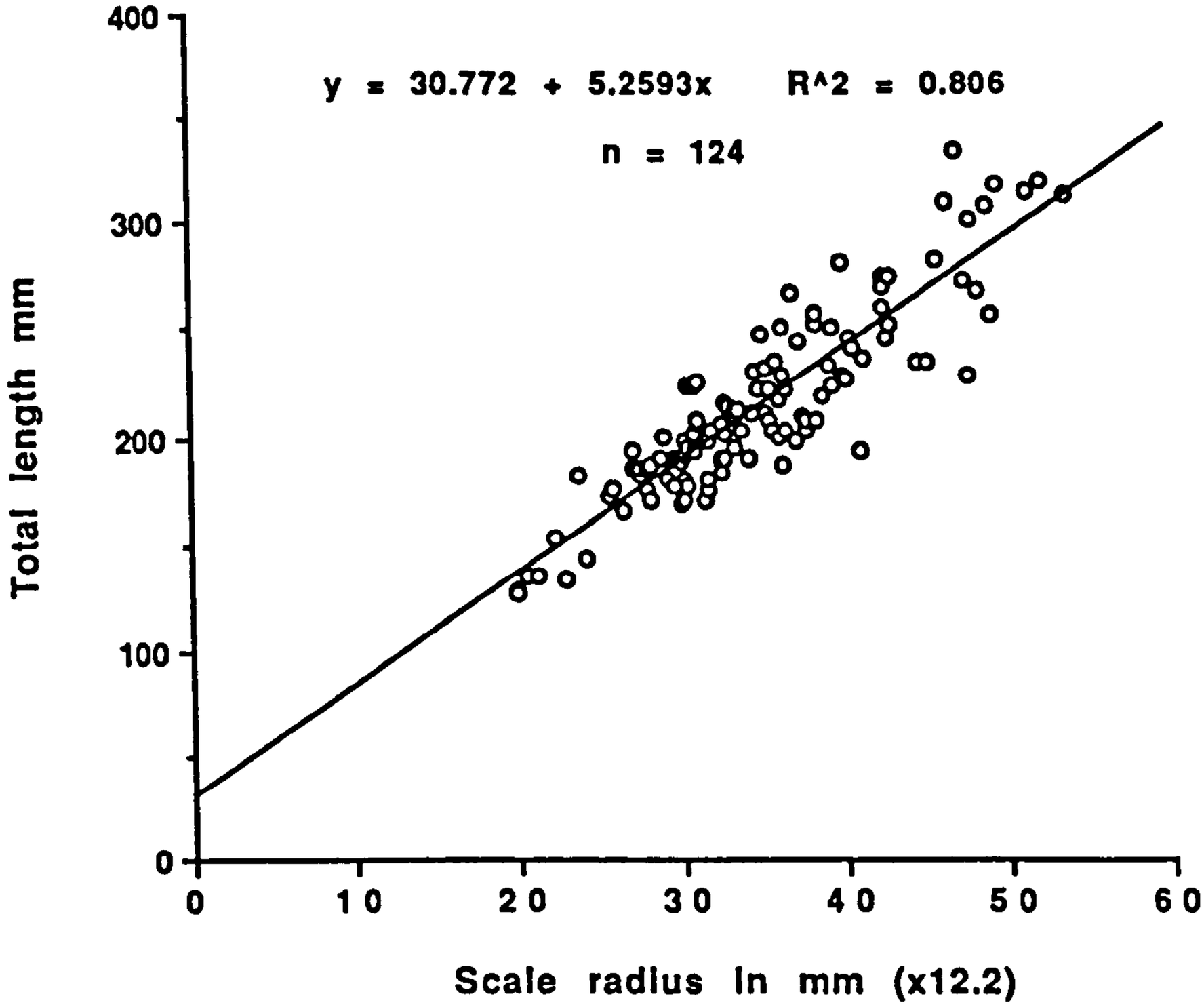
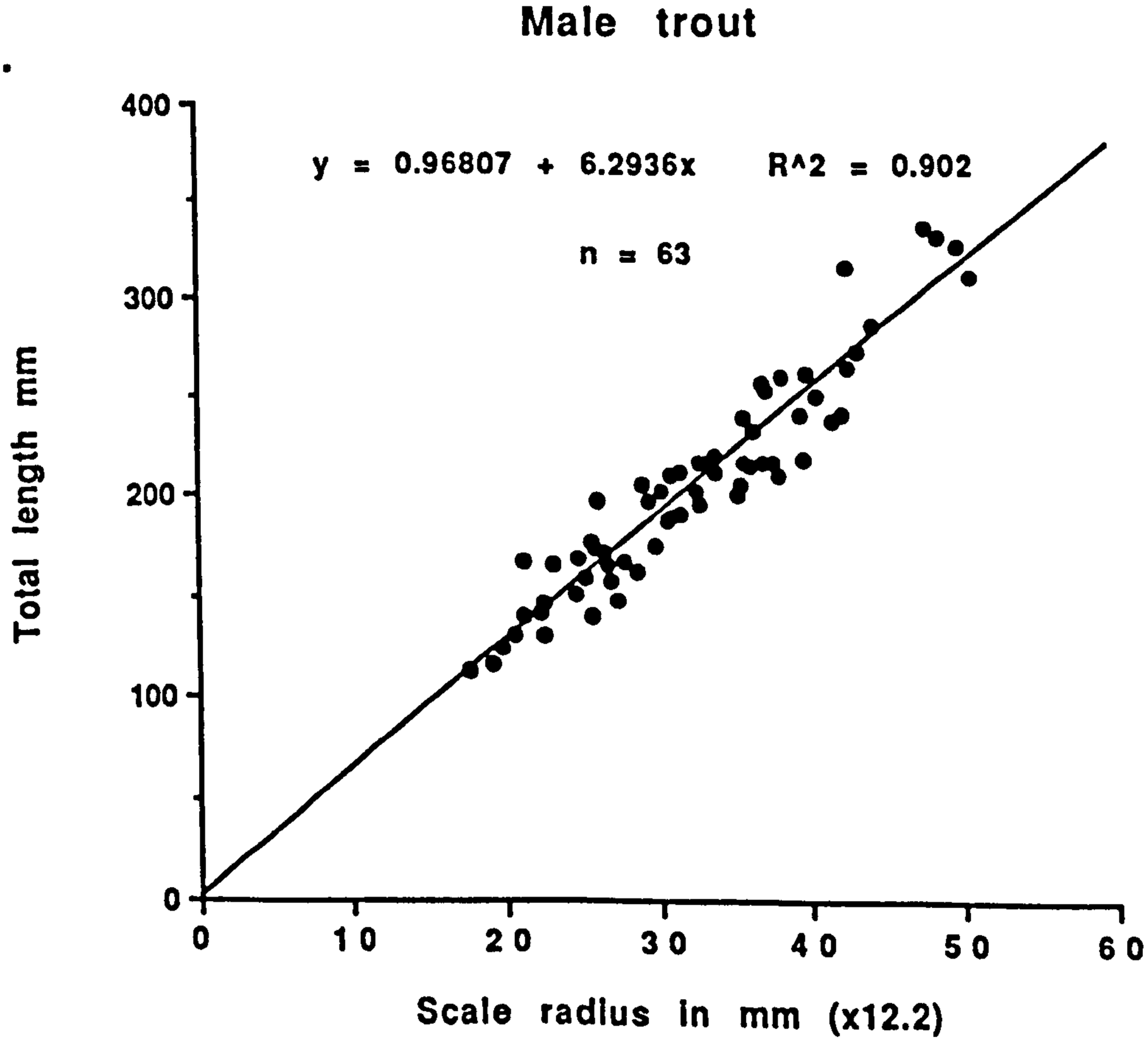


Fig 4.4 Body:scale relationship for Loch Maragan brown trout
a. all trout, b. female trout, c. male trout

b.


Female trout
y = 30.772 + 5.2593x R^2 = 0.806
n = 124
Total length mm
400
300
200
100
0
0
10
20
30
40
50
60
Scale radius in mm (x12.2)

c.


Male trout
y = 0.96807 + 6.2936x R^2 = 0.902
n = 63
Total length mm
400
300
200
100
0
0
10
20
30
40
50
60
Scale radius in mm (x12.2)

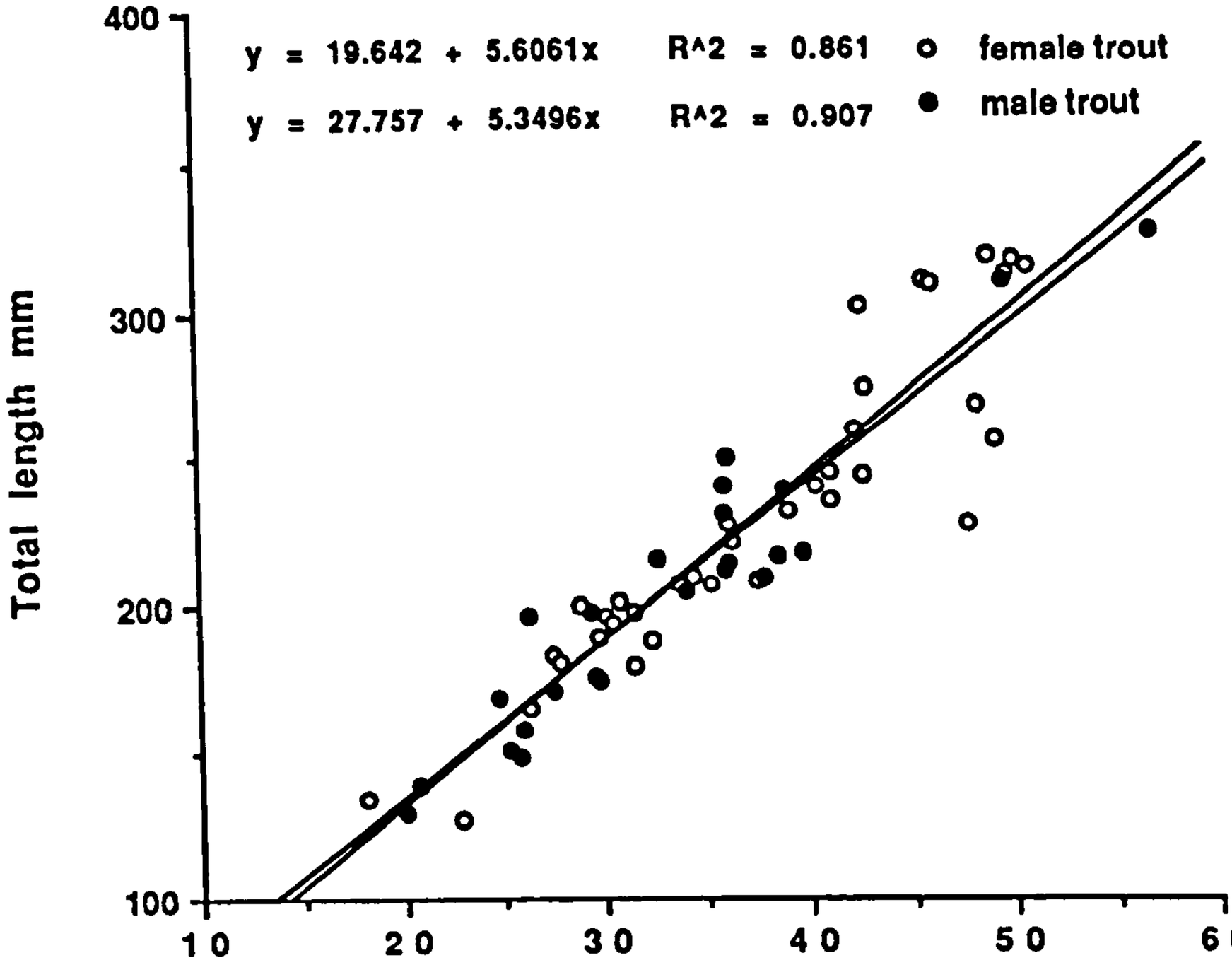
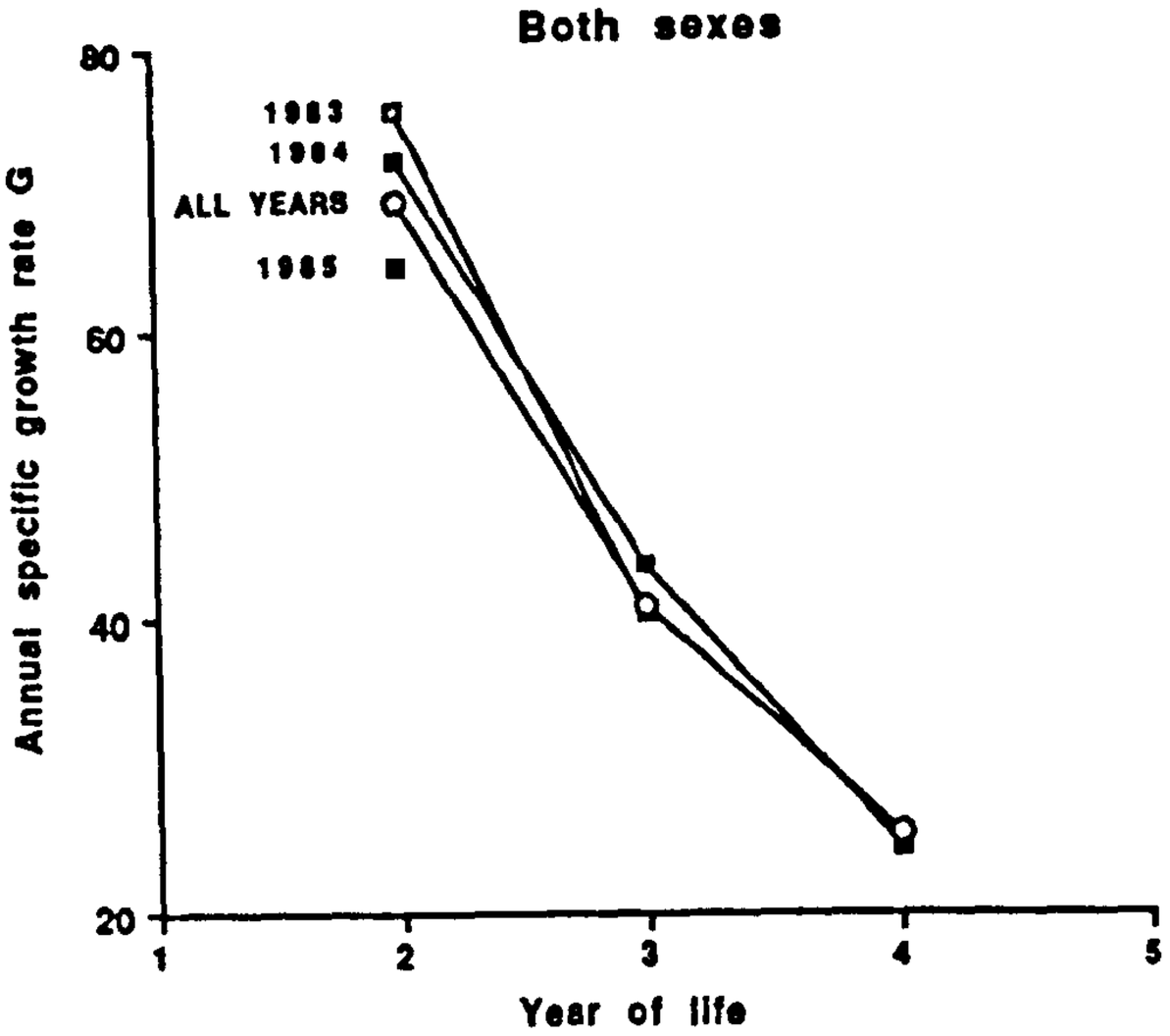
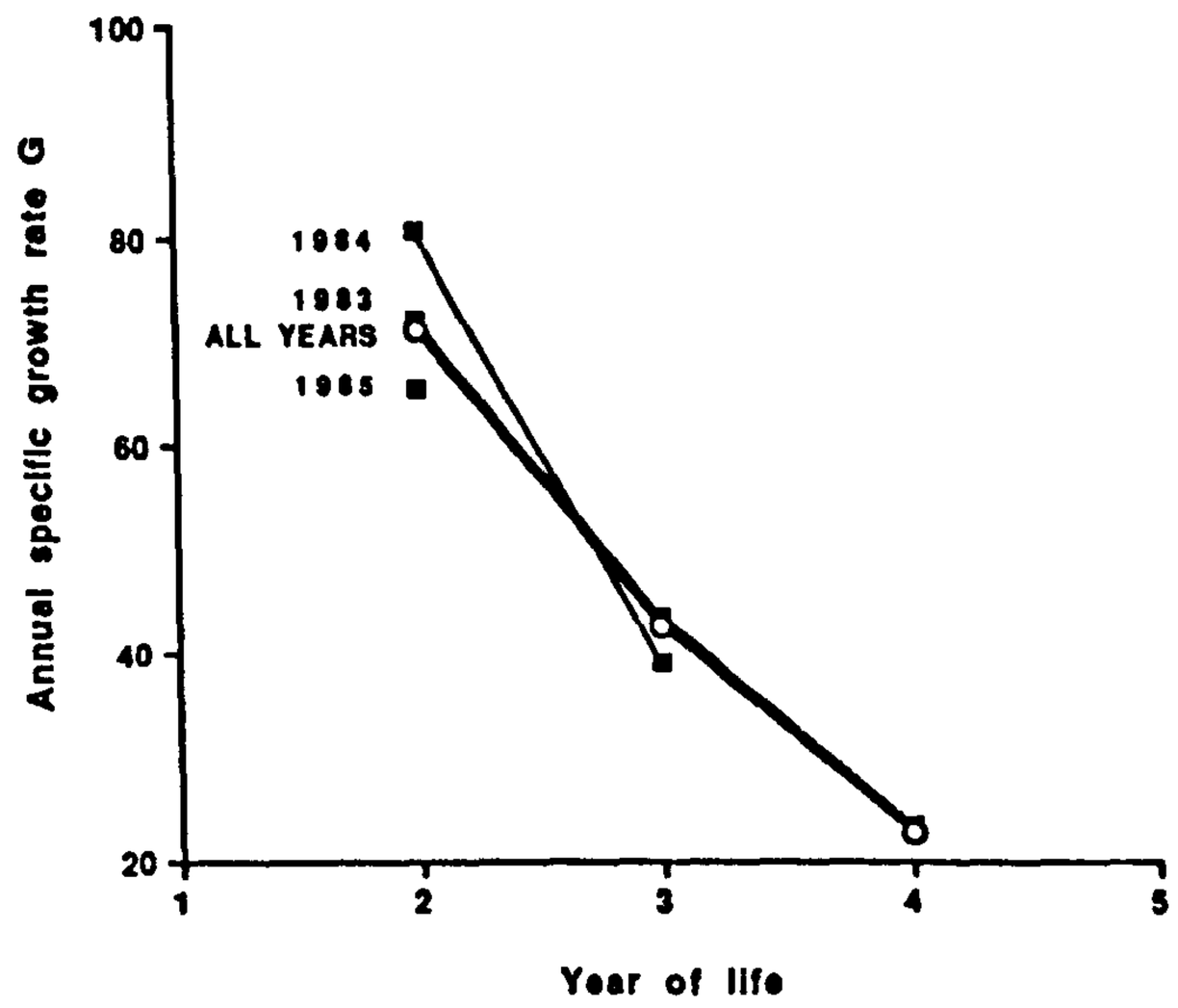


Fig. 4.5 Body : scale relationship for Loch Maragan brown trout used for back calculations

a.



b.



c.

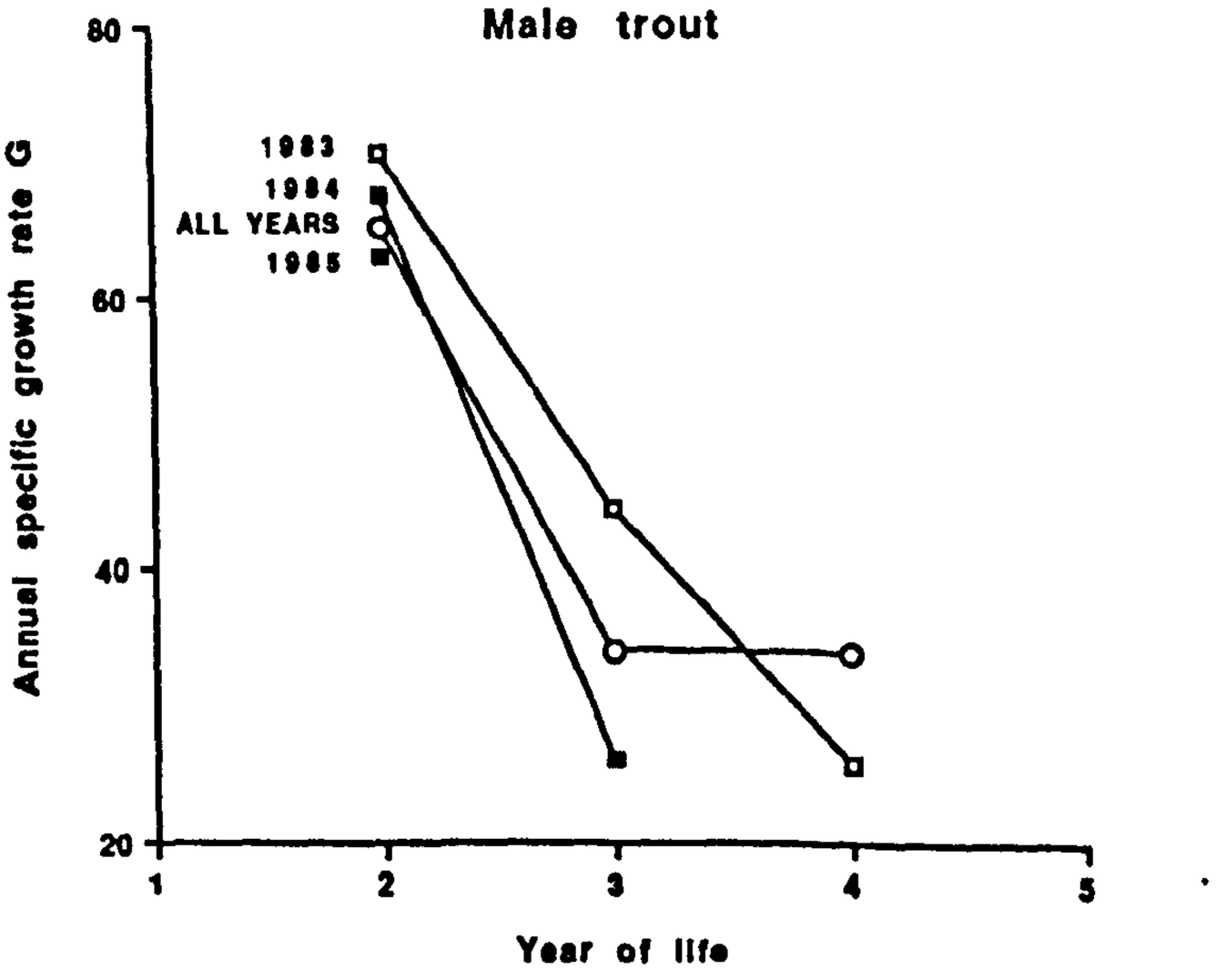


Fig. 4.6 Annual specific growth rates of Loch Maragan brown trout a. both sexes, b. female trout, c. male trout

The mean lengths of all trout in each age class and the 95% confidence limits (Fig. 4.7) were calculated, the former being 75.80, 151.33, 227.94 and 293.99 mm for 1-, 2-, 3- and 4-year-old trout respectively. These values for length are similar to those found in Lochs 8, 11 and 12 in the survey of growth of brown trout growth in 24 Scottish lochs by Campbell (1971). The trout from these lochs were in the middle of the growth rate range of the 24 lochs he considered. These 3 sites ranged from 7.7 to 526 ha in area, 15 to 305 m above sea level, 5.6 to 9.04 p.H. values and 1.1 to 87.6 ppm alkalinity values. Campbell concluded that there is no direct relationship between the growth rate of trout and the environment. He emphasized that there is a direct relationship between the productivity of the spawning ground, loch population density and the ultimate size of the adult trout with food availability also playing an important role.

4.4.4. Condition factor

Fulton's (1911) condition factor can be used to compare the 'condition' or 'well being' of a fish (Bagenal & Tesch, 1978; Bolger & Connolly, 1989). Since the range of lengths of trout in the Loch Maragan samples was quite large, the value b in the weight-length equation (weight = al^b) had to be calculated. A least squares regression line, as recommended by Bagenal & Tesch (1978) of $\log_{10}$ total length on $\log_{10}$ total weight (see Fig. 4.8) had the formula y = -4.9809 + 2.9662x as the value for b approximated to 3 this value was substituted into the following formula for the calculation of condition factors:

$$\text{Condition Factor} = K = \frac{100w}{l^b}$$

where w = weight in g
l = total length in cm
b = slope of the regression line of $\log_{10}$ length on $\log_{10}$ weight

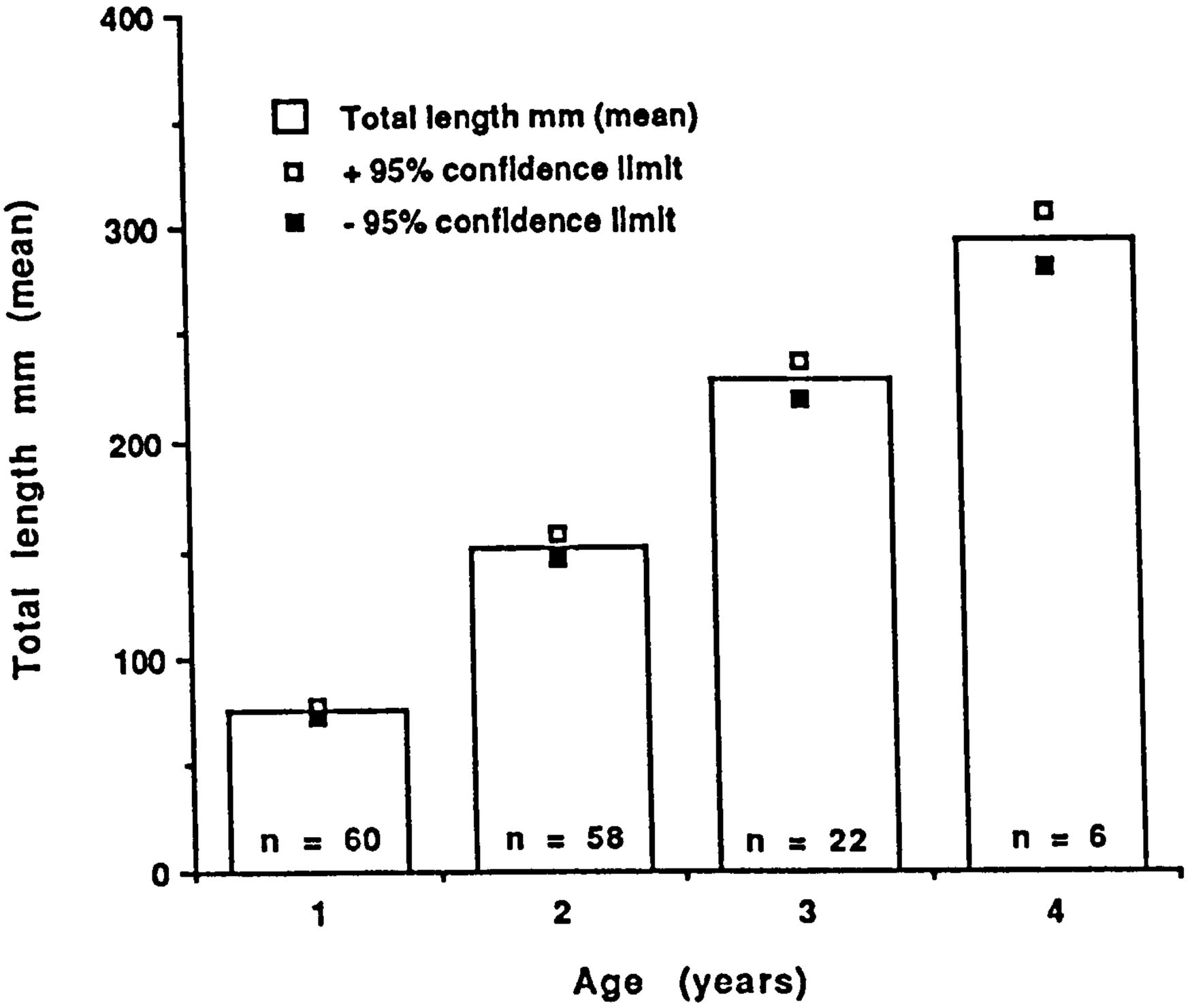


Fig. 4.7 Mean total lengths of Loch Maragan brown trout

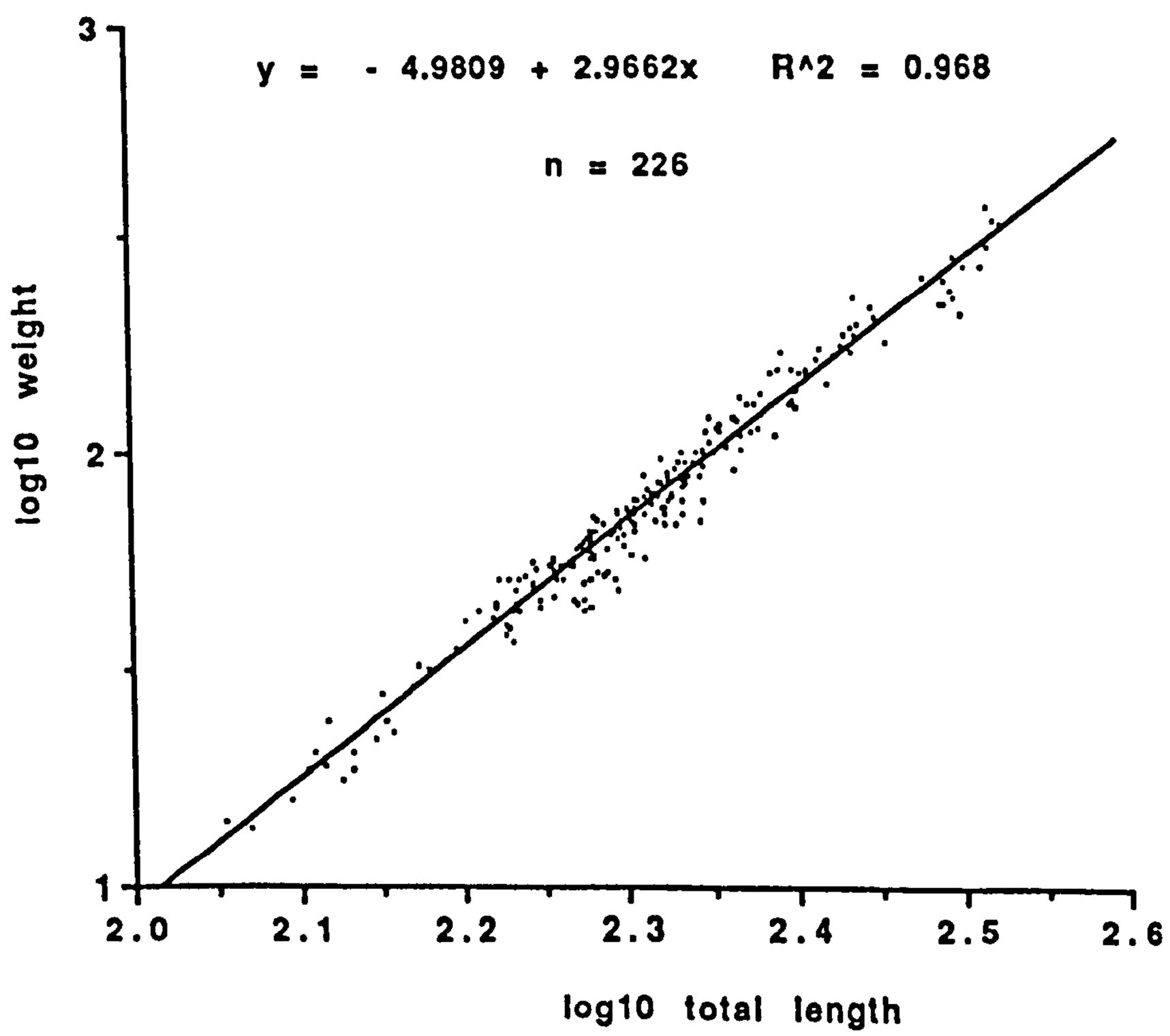


Fig. 4.8 Relationship between log10 total length and log10 weight of Loch Maragan brown trout

In the case of Fulton's condition factor b = 3 implying isometric growth.

The range of condition factors of fish samples from each month was calculated (Fig. 4.9). A pattern emerged of high condition factor values in the summer months, dropping off to lower levels over winter, reflecting food availability and time of spawning. The mean condition factor values indicated that overall, the Loch Maragan trout are 'thin' for a given length (condition factors < 1). Seventeen trout had condition factor values above unity, but the majority of these were mature individuals.

4.4.5. Gut measurements

For some trout, the gut was laid out and the lengths of the stomach, comprising the cardiac and pyloric limbs, the duodenum, ileum and rectum were measured in mm (Burnstock, 1959). In some cases, the numbers of pyloric caeca were counted and the widths of the gut sections were measured. These measurements gave an impression of the variability in gut area available for the establishment of *Neoechinorhynchus rutili* and other gut parasites.

Each section of gut was then examined separately as follows:

a) Stomach: see section 4.5.3 on dietary analysis for details.

b) Duodenum, ileum and rectum: each section was split longitudinally and the width measured. The tissue was examined microscopically while immersed in 0.9% NaCl solution and any parasites or dietary items of interest were retrieved. Each pyloric caecum was opened and examined.

The cardiac limb of the stomach ranged from 20 to 85 mm in length and the pyloric limb from 10 to 56 mm. The total post-pylorus length of the intestine measured from 68 to 185 mm. The individual sections

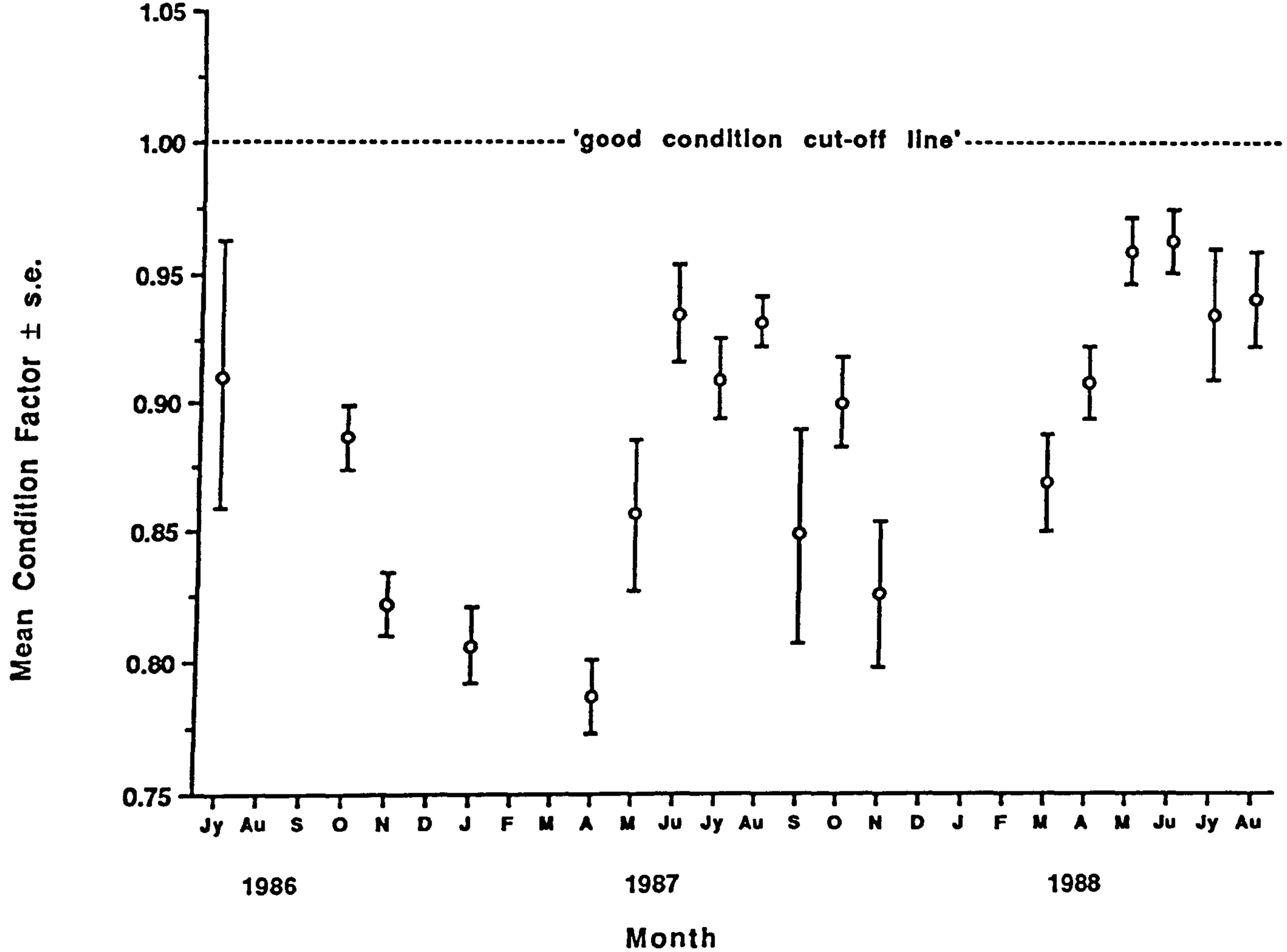


Fig 4.9 Mean condition factors of Loch Maragan trout during the sampling period

of the intestine ranged in length as follows: 10 to 47 mm duodenum; 22 to 85 mm ileum; 23 to 73 mm rectum. The range of widths of the ileum and rectum were 6 to 19 mm and 7 to 21 mm respectively. The Loch Maragan trout had between 22 and 45 pyloric caeca but these values only fall partially into the range described by Burnstock (1959).

4.5. THE DIET OF BROWN TROUT AT LOCH MARAGAN: IMPLICATIONS FOR THE TRANSMISSION OF *NEOECHINORHYNCHUS RUTILI*

4.5.1. Introduction

The diet of brown trout in lakes and rivers has been well studied both in Britain and abroad (Pentelow, 1932; Slack, 1934; Frost, 1939; Swynnerton & Worthington, 1940; Hartley, 1948; Frost & Smyly, 1952; Ball, 1961; Hunt & Jones, 1972; Stenson, 1979; Crozier, 1985). Some studies also include detailed analyses of the available fauna in the water body in relation to the diet of the trout (Frost & Smyly, 1952). Since it has been found that the brown trout at Loch Maragan are definitive hosts of *Neoechinorhynchus rutili*, the study of the trout diet at Loch Maragan aimed to identify the intermediate hosts for *N.rutili* and the relative importance that these animals might play in the diet of trout both on an individual and seasonal level. When the work began in October 1985, a number of species of ostracod, a species of leech and the megalopteran *Sialis niger* had been cited as the intermediate hosts of *N.rutili* at a number of locations (Levander, 1905; Robin, 1871; Villot, 1885; Brady, 1910; Jarecka, 1956; Styczynska, 1958; Merritt & Pratt, 1964; Golvan, 1959; Valtonen, 1979; Walkey, 1967; see Table 4.7). Consequently, an analysis of the seasonal dietary spectrum of Loch Maragan trout was initiated to provide information about the potential and actual intermediate hosts of *N.rutili*. The main objective was a qualitative analysis of the diet. Ninety eight percent of the trout in the survey were caught in

gill nets laid overnight in the loch for between 15.5 and 26.0 h which removed the opportunity to analyse differences in diurnal feeding patterns. Fish caught in gill nets may regurgitate their food and the prolonged periods in the nets results in further digestion of food items in the gut subsequent to the capture time (Windell & Bowen, 1978). Despite these limitations, Crozier (1985) used stomach samples from trout caught in gill nets to assess their diet in Lough Neagh, Ireland. These 2 factors cause inaccuracies in terms of the true stomach contents at the time of capture and make any thorough quantitative analysis of the diet unfeasible although general trends in the proportion of various elements in the diet will still be evident.

Table 4.7: Species considered to be intermediate hosts of *Neoechinorhynchus rutili*

Intermediate host species	Locality	Reference
Erpobdella octoculata	Paris, France	Robin (1871)
Sialis niger = S.lutaria	Grenoble, France	Villot (1885)
Ostracod	Helsinki	Levander (1905)
Candona angulata	Northumberland, England.	Brady (1910)
Cyclocypris laevis	Poland	Jarecka (1956) Styczynska (1967)
Cypria turneri	Suttle Lake, Oregon, U.S.A.	Merritt & Pratt (1964)
Cypria ophthalmica	Monkton Pond,	Walkey (1967)
Candona candida	County Durham, England.	
Candona neglecta	Bay of Bothnia, Finland.	Valtonen (1979)

As part of an analysis of the diet of brown trout in Llyn Tegid, Ball (1961) divided the list of dietary elements into 'bottom fauna' and 'surface-midwater' foods. Use of this classification of the food items of Loch Maragan trout allowed an assessment to be made of the proportion of benthic fauna in the diet during the year and provided information about the time of potential infection with

Neoechinorhynchus rutili. In order to maximise the chances of infection with *N.rutili* by feeding on shelled acanthors in the loch substratum, the intermediate hosts of *N.rutili* will be expected to be benthic in habit.

4.5.2. Materials

One hundred and sixty one non-empty stomachs were examined from 183 brown trout caught between July 1986 and August 1988 (Table 4.8). One hundred and seventy eight of these were caught overnight in gill nets (see Field Methods section 4.3.1.1), 1 in a seine net and 4 were fly caught in May, June and July 1987. In addition, the contents of the entire guts of 7 fly-caught trout in July 1986 were also analysed. All samples were examined fresh apart from 29 samples collected between April and June 1988 which had been frozen.

Table 4.8: Details of stomach samples from brown trout, Loch Maragan

Month	No. trout collected	No. of non-empty stomachs examined	% full stomachs	Mean stomach fullness
Jul 1986	7	whole guts examined	-	-
Oct 1986	18	14	0.0	0.57
Nov 1986	4	4	0.0	0.31
Jan 1987	2	2	0.0	0.50
Apr 1987	16	16	0.0	0.36
May 1987	7 (1)	6	0.0	0.29
Jun 1987	4 (2)	4	50.0	0.81
Jul 1987	5 (1)	4	0.0	0.35
Aug 1987	33	27	3.0	0.36
Sep 1987	3	3	0.0	0.33
Oct 1987	15	10	6.7	0.32
Nov 1987	5	3	0.0	0.15
Mar 1988	9	9	0.0	0.50
Apr 1988	16	16	62.5	0.86
May 1988	18	18	50.0	0.72
Jun 1988	5	5	40.0	0.70
Jul 1988	7	6	42.9	0.68
Aug 1988	16	14	25.0	0.52
Total	190	161		

The numbers in parentheses represent fish that were fly caught.

4.5.3. Procedure

The stomach was dissected away from the rest of the gut, its dimensions were measured and then it was split longitudinally and the relative fullness was assessed by eye. Values for fullness were recorded on a scale from 0 (empty) to 1 (full) in increments of 1/4. The contents of each stomach were examined under a binocular microscope and the dietary elements were identified down to order or family according to Fitter & Manuel (1986), Macan (1959) and Elliot (1977a). The overall classification of the faunal groups follows Fitter & Manuel (1986). For each faunal group, the number of individuals was counted; where digestive processes had resulted in the specimens breaking up the numbers present were calculated by counting the representative 'hard' parts e.g. heads for larval *Sialis lutaria*. In the samples taken after January 1987, the widths of unbroken heads of all *S.lutaria* larvae were measured. For those faunal groups which were found in large numbers in the stomach samples, e.g. cladocerans, the specimens were spread out evenly in a Petri dish, 25 individuals were counted and then the total number present was estimated using the original count as a basic scale. In all cases, the dietary items were dissected and examined for the presence of any developmental stages of *Neoechinorhynchus rutili* or other acanthocephalan species.

Each dietary element was classified as either benthic or surface/ midwater fauna (Ball, 1961) to reveal gross seasonal dietary changes (see Table 4.9). This resulted in some taxonomic groups being divided between these classifications e.g. dipteran adults and pupae were classified as surface-midwater fauna and dipteran larvae as benthic fauna. Data for each month was collated and expressed as the number of faunal elements present in each of the 2 faunal classifications. Within each faunal group, the numbers of individuals consumed by both

individual and by all trout in a monthly sample were calculated. For some dietary faunal groups, the degree of feeding by trout greater or less than 200 mm in total length was also calculated to assess any size related differences in the diet.

Subsequent to the discovery in April 1987 that *Sialis lutaria* larvae were habouring *Neoechinorhynchus rutili* at Loch Monzievaird, all *S.lutaria* larvae from the entire gut were measured and dissected. The relative size of larvae was determined by measurement of the width of the head capsule. The identification of instars was based on the cephalic width measurements given by Giani & Laville (1973) for a French population of *S.lutaria* larvae, values being rounded up to the nearest unit. The goodness of fit of the distribution of the *N.rutili* infection in the larvae with the negative binomial distribution was compared by means of a Chi squared test (Elliot, 1977b). The distribution of infection in the whole size range and within instars was also considered. Worms recovered from these larvae were measured fresh and some specimens were also prepared as whole mounts. A number of *S.lutaria* larvae were found alive in the stomachs of some trout and these were used for histological investigation (see Chapter 7).

Any whole fish consumed were measured and dissected. Evidence of prey fish from any part of the gut was also recorded.

4.5.4. Results

4.5.4.1. *The diet of Loch Maragan trout*

A total of 19 dietary elements from 16 faunal groups were recovered from the 161 stomach samples. Of these 16 faunal groups, 11 were classified as benthic fauna and 5 as surface-midwater fauna after Ball (1961). The faunal groups and faunal elements were as follows:

Table 4.9. List of dietary components of Loch Maragan Brown Trout

a) Bottom fauna

Faunal group	Faunal element
ANNELIDA	
Hirudinea	Leech
MOLLUSCA	
Bivalvia	*Pisidium* sp.
CRUSTACEA	
Cladocera	*Eurycercus* sp.
INSECTA	
Ephemeroptera nymphs	Ephemeroptera nymphs
Plectoptera nymphs	Plecoptera nymphs
Hemiptera	*Corixa* sp. adults & nymphs
Trichoptera	Phryganeidae
	Limnephilidae
	Hydroptilidae
Megaloptera	*Sialis lutaria* larvae
Diptera	Dipteran larvae
Coleoptera	Coleopteran larvae
CHORDATA	
Amphibia, Urodela	*Triturus* sp. larva

b) Surface/ midwater fauna

CRUSTACEA	
Cladocera	*Leptodora* sp.
Copepoda	Harpacticoidea
INSECTA	
Diptera	Pupae and adults
Coleoptera	Adults
CHORDATA	
Pisces	*Phoxinus phoxinus*

The occurrence of these faunal groups during each monthly sample is summarised in Table 4.10. The details and significance of each dietary element are considered separately. Since the nature of the sampling method did not permit a qualitative analysis of the dietary elements, the numbers of each element counted in individual stomachs are not be given in full. However, details of the general trends in

terms of numbers of each dietary element consumed are given in the appropriate results sections which follow. Where appropriate, comments on the diet in relation to the benthic faunal surveys are made.

Table 4.10: Monthly occurrence of dietary elements in the stomach samples of Loch Maragan brown trout

Dietary elements	Faunal group	1986 Ju	O	N	1987 J	A	M	J	J	A	S	O	N	1988 M	A	M	J	J	A
Leech:a	B										x								
Pisidium sp.	B		x						x		x			x					
Cladocera:E	B		x	x			x	x	x	x	x	x					x	x	x	x
:ep	B		x						x		x								
Ephemeroptera:n	B					x	x		x					x	x	x			
Plecoptera:n	B					x		x	x					x	x			x	
Corixa sp.	B					x			x					x					
Trichoptera:l	B		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
Sialis lutaria:l	B			x	x		x	x	x	x	x		x	x	x	x	x		
Diptera:l	B					x	x	x		x		x		x	x	x	x	x	
Coleoptera:l	B					x	x		x	x		x	x		x		x		x
Triturus sp.:l	B																x		
Cladocera:L	S								x		x						x		x	x
Harpacticoidea	S													x					
Diptera:a	S		x	x	x			x	x	x	x		x				x	x	x	x
:p	S							x		x	x	x					x	x		x
Coleoptera:a	S			x	x			x		x	x	x	x				x	x	x	x
Phoxinus:a *phoxinus*	S					x	x			x				x		x			x	x

a= adult p= pupa l= larva L= *Leptodora* sp. E= *Eurycercus* sp.
ep= Cladoceran ephippia
B = bottom fauna; S = surface/mid-water fauna (after Ball, 1961)

4.5.4.1.1 *Invertebrate diet*

4.5.4.1.1.1. Hirudinea

A single leech (unidentified) was found in a trout stomach in September 1987. This is not an important element of the diet although it is interesting to note that leeches, particularly *Erpobdella* sp. have been cited as potential intermediate hosts of *Neoechinorhynchus rutili* by Robin (1871). However, developmental stages of *N.rutili* were not found in this individual.

4.5.4.1.1.2. Mollusca

Five specimens of *Pisidium* were recovered from the guts of 4 trout during April, August and October none of which were found to be infected with developmental stages of *Neoechinorhynchus rutili*. The benthic faunal surveys showed this to be a fairly common in the substrate. Nevertheless, this species appears not to be an important element of the trout diet.

4.5.4.1.1.3. Cladocera

Cladocerans were only found as part of the diet in 12 of the 18 sampling months between May and October. This corresponds to life cycle involving a 'spring' hatch of cladocerans as the water temperature rises. The majority of the cladocerans consumed by the Loch Maragan brown trout were *Leptodora* sp. and *Eurycercus* sp. As many as 500 specimens of either species were found in an individual trout stomach. None of the specimens was found to be infected with developmental stages of *Neoechinorhynchus rutili*. Ephippia, the resting stages of some cladocerans, were also found in some of the trout stomachs.

4.5.4.1.1.4. Copepoda

A single harpacticoid copepod was retreived from a trout stomach

in March 1988. These are small copepods which would probably be eaten accidentally by trout. Their 'absence' from the diet might reflect the rapidity of digestion of such small items in the stomach.

4.5.4.1.1.5. Ephemeroptera and Plecoptera nymphs

These nymphs were only recovered from the guts of trout during the spring and summer months from March to August. The majority of the ephemeropteran specimens were *Paraleptophlebia* sp. with large numbers being consumed in the months of March, April and May. As many as 118 individuals were recovered from a single trout stomach (May 1988). The X^2 value of 168.0 ($P < 0.001$) indicated that larger fish (>200 mm long) fed more on these nymphs than the smaller size class. None of the specimens was found to be infected with developmental stages of *Neoechinorhynchus rutili*.

4.5.4.1.1.6. Hemiptera

Two corixid adults and 1 nymph were recovered from the stomachs of Loch Maragan trout in March, April and July and none were found to be infected with developmental stages of *Neoechinorhynchus rutili*.

4.5.4.1.1.7. Trichoptera

These were considered to form an important element of the trout diet and were found in every month of stomach analysis. A number of families of trichopterans were identified in the diet including Phryganeidae, Limnephilidae and Hydroptilidae. As many as 76 phryganeids and 23 hydroptilids were found in individual trout stomachs in June 1987 and March 1988 respectively, the largest numbers of trichopterans being consumed in March, April, May and June. None of the routine dissections of these specimens revealed any infection by *Neoechinorhynchus rutili*.

4.5.4.1.1.8. Megaloptera

The Megaloptera were represented by *Sialis lutaria* larvae in the diet. Overall 379 *S.lutaria* larvae were recovered from 95 trout in 13 of the 18 sampling months. Of these 328 were measured and dissected and 56 were found to be infected with *Neoechinorhynchus rutili*. The size of larvae consumed and the distribution of the *N.rutili* infection is summarised on Table 4.11 and the details are shown and discussed in Chapter 7. Between 1 and 21 *S.lutaria* larvae were found in the gut of any individual trout during the study period. The trout found to be feeding on *S.lutaria* larvae ranged from 127 mm to 315 mm fork length. Of these 95 trout, 85 were also infected with *N.rutili*. Those fish with the highest intensities of *N.rutili* infection recorded at Loch Maragan were among those found feeding on *S.lutaria* larvae. Fifty nine specimens of *N.rutili* from *S.lutaria* larvae recovered from the guts of Loch Maragan brown trout were measured fresh and these results are considered in Chapter 7.

Table 4.11: Size range (head width) of *Sialis lutaria* larvae consumed by Loch Maragan brown trout between October 1986 and June 1988 and the distribution of *Neoechinorhynchus rutili* infection

Head[a] width	No. of *S.lutaria* larvae Uninf.	Inf.	Total	Int.	Instar	Number	Percentage infected
12	1	0	1				
13	0	1	1	0.33	VI	4	25.0
14	2	0	2				
15	1	2	3				
16	3	0	3	0.33	VII	8	25.0
17	2	0	2				
18	3	1	4				
19	3	1	4				
20	3	1	4	1.19	VIII	26	38.5
21	3	2	5				
22	4	5	9				
23	11	5	16				
24	20	1	21				
25	30	11	41	0.36	IX	150	18.0
26	30	3	33				
27	32	7	39				
28	19	2	21				
29	6	3	9				
30	23	3	26				
31	18	2	20				
32	18	2	20	0.20	X	140	11.4
33	17	3	20				
34	11	1	12				
35	8	0	8				
36	1	0	1				
37	3	0	3				

a. 1 head width unit = 80 um

Cephalic head widths of instars were as follows:
VI = 0.85-1.10 mm; VII = 1.10-1.36 mm; VIII = 1.36-1.72 mm;
IX = 1.72-2.12 mm; X = 2.12 mm+ (After Giani & Laville, 1973).

b. Total number of *Sialis* larvae measured = 328
Total number infected = 56
Number of unmeasured heads = 54
Total number of *Sialis* larvae consumed = 382

Sialis lutaria larvae formed a considerable element of the diet of Loch Maragan trout during all but the late summer months (July, August and September) (See Table 4.12). The highest numbers of *S.lutaria* larvae consumed by trout were in March, April, May and June. 45 trout ‹200 mm long ate 177/359 larvae and 50 trout ›200 mm long ate 182/359 larvae. The X^2 value of 0.57 indicates that there was no significant difference between the consumption rate of *S.lutaria* by the 2 size classes of trout. The mean number of *S.lutaria* larvae per trout during the months when they were found in the diet ranged from 1.00 to 6.78; the highest numbers were found in the spring months, falling during July and August and rising again in October and November. No data were available on the significance of *S.lutaria* larvae in the diet between December and February due to the restrictions imposed on brown trout fishing as discussed in section 4.3.1.1.

Table 4.12: Details of brown trout feeding on *Sialis lutaria* larvae in Loch Maragan between October 1986 and June 1988

Month	No. of trout examined	Trout feeding on *S.lutaria* larvae Total	%	No. of *S.lutaria* larvae Total	Inf.	Mean No. *S.lutaria* larvae/fish[a]
Oct 1986	14	4	28.6	7	0	1.75
Nov 1986	4	1	25.0	1	0	0.25
Apr 1987[b]	42	21	50.0	41	2	1.95
Apr 1987	16	9	56.3	61	4	6.78
May 1987	7	2	28.6	2	2	1.00
Jun 1987	4	3	75.0	14	10	4.67
Jul 1987	5	2	40.0	4	2	2.00
Aug 1987	32	2	6.3	3	1	1.50
Oct 1987	15	9	60.0	30	0	3.33
Nov 1987	5	1	20.0	2	0	2.00
Mar 1988	9	9	100.0	36	0	9.00
Apr 1988	16	13	81.3	69	14	5.31
May 1988	18	17	94.4	107	18	6.29
Jun 1988	5	2	40.0	2	0	1.00

a = Values are calculated on the basis of numbers of trout actually feeding on *S.lutaria* larvae in each month.

The values for April 1987 are from the results of two sampling sessions combined.

b = These are the combined results from trout caught between 10.1.87. and 25.4.87.

4.5.4.1.1.9. Diptera

Dipterans were present in 17 out of 18 months for which gut and stomach contents were analysed. The adults were restricted to the 'summer' months which corresponds to their period of emergence and the consequent availability to trout as a food source. Quite often it was not possible to estimate the numbers of dipterans present in the sample because they had fragmented. As many as 189 individual pupae (April 1988) and 120 adults (July 1988) were found in an individual stomach. Overall the largest numbers of dipterans were recovered from

trout collected between April and September in each year. None of the routine dissections of these specimens revealed any evidence of infection by *Neoechinorhynchus rutili*.

4.5.4.1.1.10. Coleoptera

Adult and larval Coleopterans were found in the diet, occurring in 14 out of 18 sampling months. Up to 3 individuals were found in any 1 stomach (Mean = 62/48 = 1.29) indicating the lack of importance in the diet. None of the routine dissections of these specimens revealed infection by *Neoechinorhynchus rutili*.

4.5.4.1.2. *Vertebrate diet*

Phoxinus phoxinus (minnow) and *Triturus* sp. (newt) were found in the stomachs of the trout. One newt was found in June 1988 in a trout 188 mm (fork length) long. The partially digested specimen was 20 mm long and was probably a young larval stage. This species probably does not form an important part of the trout diet at Loch Maragan. Evidence of minnows was found in 7 of the monthly samples in 9 individual trout. Between 1 and 4 minnows were consumed by any 1 trout (Table 4.13).

Table 4.13. Details of the piscivorous brown trout captured in Loch Maragan

Date of capture	Trout No.	Fork length mm	Total length mm	Wet weight g	No. of minnows in stomach/ gut	Infected by *Ligula intestinalis*
9.4.87.	1	300	310	247.65	Present	-
30.5.87.	4	207	220	100.40	Present	-
15.8.87.	3	230	241	120.06	Present	-
18.3.88.	6	208	222	102.14	Present	-
14.5.88.	6	233	247	156.08	1:84mm,4.68g	Yes
14.5.88.	18	300	315	285.65	1:98mm,11.37g	Yes
24.7.88.	1	318	334	348.41	1: p.d.	-
20.8.88.	2	315	331	372.33	4: p.d.	Yes
20.8.88.	13	252	262	163.27	1: p.d.	-

p.d.= partially digested -= no data

The minnows were found to be partially digested in the stomach so it was not possible to measure all of the specimens. However, minnows up to 98 mm in length and weighing up to 11.37 g (wet weight) had been eaten by trout. Dissection of the whole minnows consumed showed that they were all infected with plerocercoids of *Ligula intestinalis*. No evidence of a *Neoechinorhynchus rutili* infection was found in any of the minnows.

4.5.4.1.3. *Vegetable matter and stones*

These occurred infrequently in the diet and presumably were eaten accidentally when the trout was attempting to capture an item of live food (Ball, 1961).

4.5.4.1.4. *Stomach fullness*

Despite technical limitations, only 22/183 (12%) of the stomachs examined were found to be empty. The percentage of full stomachs found in each monthly sample is presented in Table 4.8 with calculated values for mean stomach fullness.

4.5.4.1.5. *Proportion of benthic and surface-midwater fauna in the diet*

The number and percentage of faunal elements in the diet in each sampling month is shown in Table 4.14. In general, the proportion of benthic faunal elements in the diet was found to be greatest during the winter months representing up to 100% of the diet in January and November 1987 repectively. The highest percentage of surface-midwater faunal elements in the diet occurred in July 1986 and 1988 (57 to 58%) and higher proportions were recorded in the spring and summer months. In terms of numbers, the summer diet was dominated by the surface-midwater elements, especially the adult dipterans and cladocerans.

Table 4.14: Monthly composition of the diet of brown trout from Loch Maragan

Month	No. of dietary elements			% of dietary elements	
	B	S	Total	B	S
Jul 1986	2	2	4	50.0	58.0
Oct 1986	4	2	6	60.0	40.0
Nov 1986	2	1	3	66.7	33.3
Jan 1987	1	0	1	100.0	0.0
Apr 1987	7	1	8	87.5	12.5
May 1987	6	4	10	60.0	40.0
Jun 1987	5	1	6	83.3	16.6
Jul 1987	6	3	9	66.6	33.4
Aug 1987	6	5	11	54.5	45.5
Sep 1987	3	2	5	60.0	40.0
Oct 1987	5	2	7	71.4	28.6
Nov 1987	4	0	4	100.0	0.0
Mar 1988	6	2	8	75.0	25.0
Apr 1988	7	3	10	70.0	30.0
May 1988	5	4	9	55.6	46.4
Jun 1988	6	2	8	75.0	25.0
Jul 1988	3	4	7	42.9	57.1
Aug 1988	6	1	7	85.7	14.3

All records are from stomach contents except from occurrences of *Phoxinus phoxinus* and *Sialis lutaria* in the intestines of fish which are also included. July 1986 data are for the entire gut contents.

4.5.4.2. *Comparison with diets of trout from other sites of* Neoechinorhynchus rutili *infection*

Details of the dietary elements recovered from 385 trout caught at 9 known sites of *Neoechinorhynchus rutili* infection around Scotland have been collated and are presented in Table 4.15. The greatest similarities in the dietary elements are seen in trout caught in small, highland lochans where the dietary range is fairly limited. Trout caught in larger lochs showed a wider dietary composition which included a number of species of molluscs and crustaceans (e.g. Loch Monzievaird). At 7 sites, *Sialis lutaria* larvae formed part of the diet and at 4 of these the larvae were also found to be infected with

N.rutili. At 3 sites ostracods were present in the stomach samples, but none was found to be infected with *N.rutili*. The maximum numbers of *S.lutaria* larvae observed in trout gut samples from these sites of *N.rutili* infection are shown in Table 4.16, with most being consumed in the summer months. At Powder Works Dam Lochan (Site 9), the importance of *S.lutaria* larvae in the diet is worth commenting upon in detail. *S.lutaria* larvae were found as part of the diet of brown trout in 5 of the 10 sampling months at this site. As many as 25 individuals were recovered from a single gut (June 1986) but subsequent to this date only 1 or 2 specimens were recovered from individual trout. This paucity of larvae in the diet subsequent to this time may be indicative of a population crash and hence may explain the observed crash in the *N.rutili* population in the trout. At 3 sites ostracods were present in the stomach samples but none of these were found to be infected with developmental stages of *N.rutili* (Table 4.16).

Table 4.15: Dietary elements of trout from Scottish sites of *Neoechinorhynchus rutili* infection

Dietary elements	Site No. 1	2	3	4	5	6	7	8	9
P: Tricladida:a									x
A: Leech:a						x			x
M: Gastropoda		x					x		x
M: *Pisidium* sp.		x		x	x	x			x
Ch:Araneae:a									x
Ch:Hydracarina:a									x
C: Cladocera:a		x				x	x		x
C: Ostracoda	x						x		x
C: Copepoda						x	x		x
C: *Gammarus pulex*							x		
C: *Asellus* sp.	x								
U: Ephemeroptera:n		x				x			x
U: Odonata:n	x				x		x		
U: Plecoptera:n		x			x	x			x
U: *Corixa* sp.					x	x	x		x
U: *Sialis lutaria*:l +	x	x			x	+	+		+
U: Diptera:l,p,a.	x	x	x		x	x	x	x	x
U: Coleoptera:l,a.		x			x	x	x		x
U: Hymenoptera:a									x
Pi:*P.phoxinus*:a						x			
A: *Triturus* sp.:l						x			
Total no. faunal elements	2	7	8	3	8	13	11	1	17

Sites, numbers of fish examined and dates of collection:
(All fish were brown trout apart from site 4 which was a rainbow)

1 = Bridge of Weir: 2 fish; Feb 1988 (incomplete dietary analysis)
2 = Carbeth: 2 fish; May 1988
3 = Lochan Creag nan Caorann: 20 fish; 11 Aug 1986; 7 Jul 1987; 4 Jul 1988
4 = Lochan Duin: 1 fish; Jul 1987
5 = Loch Essan: 10 fish; 4 Oct 1986; 6 Jun 1987
6 = Loch Maragan: 161 fish; see Table 2 for details of fish
7 = Loch Monzievaird: 9 fish; 5 Mar 1987; 3 Apr 1987; 1 Aug 1987
8 = Loch Oss: 2 fish ; Jul 1987
9 = Powder Works Dam lochan: 178 fish ; 8 Jun 1986; 4 Jul 1986; 2 Aug 1986; 50 Sept 1986; 18 Oct 1986; 26 Nov 1986; 6 Mar 1987; 9 Jun 1987; 11 Aug 1987

Unless specifically named, only the major faunal groups are listed here and different stages of the life cycle are included in a single group.

P = Platyhelminthes; A = Annelida; M = Mollusca; Ch = Chelicerata; C = Crustacea; U = Uniramia; Pi = Pisces; A = Amphibia.
x = present in the gut of trout samples
+ = dietary item infected with developmental stages of *N.rutili*.

Table 4.16: Numbers of *Sialis lutaria* larvae consumed by trout at sites of *Neoechinorhynchus rutili* infection in Scotland

Site	Date	Numbers of *S.lutaria* recovered	Gut region
Bridge of Weir	Feb 1988	23	whole gut
Carbeth Loch	May 1988	3	stomach
Lochan Creag nan Caorann	Aug 1986	57	whole gut[a]
	Jul 1988	10	whole gut
Loch Essan	Oct 1986	n.d.	whole gut
Loch Maragan	Apr 1988	21	whole gut
Loch Monzievaird	Apr 1987	4	stomach
Powder Works[b] Dam Lochan	Jun 1986	25	whole gut

a = This trout was 482.6 mm long and weighed 1276 g.

b = Subsequent to this date only 1-2 larvae were found per individual gut in 170 trout examined between July 1986 and August 1987.

The values shown are the maximum recorded in an individual for all the trout examined at the particular site.

4.5.5. Discussion

The main reason for investigating the diet of brown trout in Loch Maragan was to identify potential and actual intermediate hosts of *Neoechinorhynchus rutili* and to establish the relative importance of these items in the overall diet of the trout, both on an individual and seasonal basis. During the period of the stomach collections, benthic faunal surveys were carried out in November 1986 and May 1987 to collect organisms expected to be available in the diet of the trout. The approach was to identify intermediate hosts of *N.rutili* in

the benthic fauna and then to predict their significance in the diet of the trout. However, neither of the benthic faunal surveys provided information about the invertebrate intermediate hosts of *N.rutili* at Loch Maragan. It was as a result of dissections of food items of trout caught at Loch Monzievaird (April 1987) that the discovery of larval *Sialis lutaria* acting as hosts of *N.rutili* was made (Lassiere, 1988a). After this finding, *S.lutaria* larvae eaten by Loch Maragan trout were also found to be infected and from this point onwards the occurrence of *S.lutaria* larvae in the trout diet was examined in more detail. A further aim for the dietary survey was to investigate the possibility of postcyclic transmission of *N.rutili* through trout feeding on other vertebrates harbouring mature *N.rutili* infections (see Chapter 8).

From the results it is possible to give a general overview of the diet of brown trout in Loch Maragan in terms of the benthic fauna available and also to make comparisons with the diets of trout from other locations in Britain and abroad. The diet of Loch Maragan trout will first be discussed in terms of the findings at other localities in Britain and abroad and where appropriate, in terms of the benthic faunal surveys carried out at Loch Maragan and at some of these localities. Then a comparison between diets of trout infected with *N.rutili* in Scotland and Loch Maragan will be made.

4.5.5.1. *Dietary elements*

The dietary elements are discussed in the same order as they appear in the results section.

4.5.5.1.1. *Invertebrates*

4.5.5.1.1.1. Hirudinea

Leeches were considered to be an unimportant dietary element for brown trout at Loch Maragan. Similar findings have been made at other

sites. Macan (1949) observed Hirudinea in the weeds of Three Dubs Tarn, but Frost & Smyly (1952) did not find trout feeding upon them during their survey of the trout diet there. This paucity in the diet may in part be explained by the low densities in the Three Dubs Tarn fauna of up to only 44 m^{-2} in summer (Macan, 1949). Ball (1961) found *Erpobdella octoculata* in part the diet of Llyn Tegid brown trout and reported that they only occurred sporadically, in small numbers and were of no importance in the diet.

4.5.5.1.1.2. Mollusca

Pisidium sp. was found in fairly large numbers in the benthic fauna of Loch Maragan, but was poorly represented in the trout diet. This observation agrees with those of Ball (1961) and Hunt & Jones (1972), but not with those of Allen (1938), Frost & Smyly (1952) and Swynnerton & Worthington (1940) working in the Lake District. Ball (1961) stated that trout do not feed on stationary objects even if they are suitable as food, although Frost & Smyly (1952) reported that *Pisidium* often lies on the surface of the mud and is easy prey for trout. At Loch Maragan, the benthic substrate is fine and light and any movements of the water above it causes dense localized turbidity which might impair a predator's vision. At depth, stationary *Pisidium* will be difficult to locate and are not likely to be typical food items of the trout. It is possible that the observed occurrences of *Pisidium* in the gut may reflect a fortuitous find for a trout while searching for other food items. Similarly, at Three Dubs Tarn, Lake District, Macan (1949) reported 1376 m^{-2} *Pisidium* in the benthic fauna in spring but Frost & Smyly (1952) found that only 312 were eaten by trout (n = 74). Frost & Smyly (1952) explained that this limited predation pressure was due to the mud-dwelling habit of the mollusc.

4.5.5.1.1.3. Cladocera

Dead adults and ephippia mostly occurred in the samples of the benthic substrate from Loch Maragan, the latter being represented in the trout diet during 3 of the survey months. These were only recovered in small numbers and most probably represent fortuitous feeding by the trout. The trout fed on adult cladocerans, notably *Leptodora* sp., and large numbers were consumed by individual trout, especially during the warmer months. The fish probably feed selectively on these animals whose population densities are known to incrase during the summer. Stenson (1979) described these planktonic crustaceans as being of importance in the diet of salmonids in several Swedish forest lakes only between mid-June and mid-September. He described the genera *Leptodora* and *Eurycercus* as 'larger' cladocerans and found that the trout fed selectively upon them. Frost (1950) reported that the cladoceran *Eurycercus lamellatus* (Muller) occurred in 4.7% of the brown trout (n=57) and represented 15.6% of the number of organisms which she examined in July from the River Forss, Caithness. At Powder Works Dam Lochan, Cladocera appeared in 8 of the 10 sampling months (p.o.). As many as 500 individuals were found in the gut of a single trout in June 1986 and at Gladhouse Reservoir, up to 103 *Leptodora* sp. were found per stomach in brown trout collected in June, July and September 1987 (Lassiere, 1988b). In contrast, Ball (1961) described the cladocerans *Eurycercus* sp., *Sida crystallina* and *Bythotrephes longimanus* as occurring sporadically and in small numbers which did not form important elements of the diet of brown trout at Llyn Tegid, Wales. In general, when Cladocera occur in the diet of brown trout they appear to be a 'summer' phenomenon. However, since none of the specimens was found to be harbouring developmental stages of *N.rutili* they are not likely to be important in the transmission of

the parasite to trout at Loch Maragan.

4.5.5.1.1.4. Copepoda

Copepods were found in small numbers in the benthic substrate of Loch Maragan and it seems most likely that these were picked up from midwater and were not true benthic faunal elements. Only a single harpacticoid copepod was recovered from the Loch Maragan stomach samples. Similarly, Swynnerton & Worthington (1940) did not find any of 101 brown trout, which they caught in July 1938 at Haweswater, Westmorland, to be feeding on copepods, although char (*Salvelinus alpinus lonsdalii*), Skelly (*Coregonus clupeoides stigmatus*) and perch (*Perca fluviatilis*) caught at the same time were feeding on these organisms. Stenson (1979) found that roach (*Rutilus rutilus*) and perch from some small Swedish lakes fed on copepods, but the salmonid fish, including brown trout, did not. He accounted for these observed differences in terms of the relative densities of the fish populations under study. The roach and perch formed very dense populations in their lakes resulting in intense feeding competition. In the salmonid waters, the populations were smaller and the dietary choices were wider. Copepods appear not to be an ideal food source for trout when other food items are available. At other sites in Scotland, a similar situation seems to occur. At Powder Works Dam Lochan, a single harpacticoid copepod occurred in only 1 of the 10 sampling months (June 1987). At Gladhouse Reservoir, a single copepod was found in a brown trout stomach from May 1987. In general, trout do not appear to feed extensively on copepods at any site in Scotland.

4.5.5.1.1.5. Ephemeroptera and Plecoptera nymphs

Few ephemeropteran nymphs were found in the benthic sediment samples collected in November 1986, although these insects were found as an important element of the trout diet at Loch Maragan, during the

spring and summer months. The quick-moving nymphs might have escaped from the path of the sampling gear and there were greater numbers in the benthic fauna. The large numbers found in some trout guts, may indicate that some form of selection is taking place by the trout. The largest numbers of individuals recorded from stomachs were in the spring, a time that may correspond to the migration of the nymphs before emergence. Ball (1961) found that trout had a spring preference for *Leptophlebia marginata* at Llyn Tegid and attributed this to the movement of nymphs towards the shore as a prelude to emergence. Elsewhere, similar patterns are evident with their greatest importance in the trout diet being in spring and summer. Frost (1950) found unidentified ephemeropteran nymphs representing 18% of the frequency of occurrence and 3.1% of the total number of organisms eaten by young brown trout in the River Forss, Caithness in July. Here, plecopterans represented 5% of the frequency of occurrence and 0.2% of the number in the same month. In the River Liffey, Ireland, Frost (1939) observed ephemeropteran nymphs occurring in the diet of brown trout at 100% in February and dropping off in August to around 30%. A similar pattern was seen for plecopteran nymphs which represented up to 100% frequency of occurrence in January, February and March. Pentelow (1932) also found ephemeropteran and plecopteran nymphs as part of the spring and summer diet of River Tees trout. Crozier (1985) noted unidentified plecopterans in the diet of Lough Neagh brown trout. At Gladhouse Reservoir, the highest number of ephemeropterans occurred in a stomach in July 1987 (p.o.). Stenson (1979) described a similar pattern in the feeding of trout in some Swedish forest lakes. Frost & Smyly (1952) found *Leptophlebia* sp. nymphs to be a common element of the trout diet at Three Dubs Tarn during the spring months. Overall the pattern of occurrence of these dietary items at different localities appears as a

spring/summer phenomenon. At Loch Maragan during the spring months, ephemeropterans formed quite an important element of the diet and are fed on more predominantly by the large trout (>200 mm total length). Conversely, Frost & Smyly (1952) found that fish <20o mm long fed more percentage wise on these than larger trout which is contrary to the results from Loch Maragan. Perhaps the paucity of dietary elements results in less severe dietary divisions between size classes of trout at Loch Maragan.

4.5.5.1.1.6. Hemiptera

No evidence of any hemipteran species was found in the benthic faunal surveys of Loch Maragan and corixids formed a very insignificant part of the Loch Maragan trout diet. This scarcity in the diet may be due to the distasteful substances secreted by the animals (Frost & Macan, 1948). Despite this known 'dislike' for these insects as food, there are reports of corixids forming a significant proportion of the diet of brown trout. Swynnerton & Worthington (1940) found that *Micronecta* sp. formed up to 8% of the diet of brown trout 100 to 150 mm long at Haweswater; Frost & Smyly (1952) stated that although corixids were consumed by up to 7% of the trout at Three Dubs Tarn when comparing the numbers in the diet with the numbers available in the fauna (data form Macan, 1949), only very few were taken by trout. For example, in spring when 271 m^{-2} were available in the benthic fauna, only 19 specimens were found in the 74 stomach samples examined. Stenson (1979) reported the salmonids in the Swedish forest lakes to be feeding on Hemipterans throughout his sampling period between March and October. However of the 211 brown trout stomachs analysed, only a total of 116 various hemipterans were recovered. At Powder Works Dam Lochan, shed 'skins' of *Corixa* sp. were present in the December 1986 benthic faunal survey and evidence of corixids was

found in the diet in 9 out of 10 sampling months. The numbers of individuals per stomach rarely exceeded 1, although 10 adults and 3 nymphs were found in the entire gut contents of a trout caught in August 1986. At Gladhouse Reservoir, only a few of the trout were found to have been feeding on *Corixa* sp. although 1 fish caught in May 1987 had 18 adults in its stomach. Generally, hemipterans appear to be an insignificant element of the trout diet at Loch Maragan and elsewhere.

4.5.5.1.1.7. Trichoptera

Large numbers of phryganaeid and hydroptilid trichopteran larval cases were recovered from the benthic substrate samples from Loch Maragan as well as a few live individuals. Trichopterans appeared to be fairly important element of the diet of Loch Maragan trout and occurred in every month of stomach sampling. Ball (1961) recognised the importance of trichopteran larvae in the diet of Llyn Tegid brown trout, which formed 51% by occurrence during his 10 month survey. Ball (1961) described caddis larvae as an important food especially between December and April. In his examination of the species eaten by trout he concluded that larger specimens, for example *Limnephilus lunatus* which move freely amongst plants and stones, are preferred prey of trout. These species do not 'root' about in the substratum (Neill, 1938) when searching for food. Presumably it is only the more active obvious species which are taken by the trout at Loch Maragan. Interestingly, the hydroptilid Trichoptera, which are small cased larvae (fifth instar), described as herbivores and detritivores (Marshall, 1978) and are likely to be fairly inconspicuous, were a significant part of the Loch Maragan trout diet.

Stenson (1979) observed that trout in a number of Swedish lakes were feeding on trichopteran larvae especially the 'house building'

species. Slack (1934) viewed Trichoptera as important dietary elements of brown trout caught during the winter in the River Test, Hampshire. Pentelow (1932) found cased Trichoptera in 26.0% and 34.5% of the brown trout stomachs examined from the River Tees and River Itchen, respectively. Swynnerton & Worthington (1940) found trichopterans making up to 12% of the diet of brown trout at Haweswater. Frost (1950) found trichopterans forming up to 23% of the frequency of occurrence in young brown trout which she examined form the River Forss, Caithness. Again, Frost (1939) found trichopteran larvae represented 100% occurrence in the diet of brown trout from Straffan on the River Liffey, Ireland, in February and March. Overall it appeared that smaller trout fed more upon these larvae than larger trout. At Three Dubs Tarn in the Lake District, Frost & Smyly (1952) found unidentified trichopteran larvae in 17% of the stomachs containing food in March, with trout selecting *Leptocercus* larvae from the fauna. In spring, 374 larvae were recovered from 74 fish and only 40 m^{-2} were found in the fauna (data from Macan, 1949). At Powder Works Dam Lochan, trichopterans were found in small numbers in the benthos and in every month of stomach analysis: 302 hydroptilids were found in one stomach in October 1986 and 13 phryganaeidae in June 1987. At Gladhouse Reservoir, as many as 29 limnephilids were found in an individual stomach in April 1987. Trichopterans form an important element of trout diets in many locations including Loch Maragan. It is not yet known if there is selection of particular species by the trout at Loch Maragan. Trichopterans are known intermediate hosts of several parasite species, but none of those fed upon by Loch Maragan trout contained *Neoechinorhynchus rutili* developmental stages.

4.5.5.1.1.8 *Sialis lutaria* larvae

4.5.5.1.1.8.1. Population densities of *S.lutaria* larvae in benthic faunal substrates

No *Sialis lutaria* larvae were recovered from either of the benthic faunal surveys carried out at Loch Maragan. The most likely explanation for this absence being a combination of inefficiency of the sampling method and a low density of the populations in the sampling areas. The latter seems probable since hand collections of *S.lutaria* larvae from the loch shore were time consuming and involved the search of large volumes of substrate to find few larvae. For example, in August 1988, 1 h was spent in finding 10 larvae in as many hauls of substrate with a hand net. Although no quantitative data are available for the population density of *S.lutaria* larvae in Loch Maragan, collection time data from other sites suggest that the population densities range from 'high to low' values. At Three Dubs Tarn, Lake District, Macan (1949) found 42.7 larvae m^{-2} in the benthic substrates in spring samples. During the thaw (July to October) at Lac Port Beihl, Central Pyrenees (altitude 2,285 m), Giani & Laville (1973) examined the population densities of 1 to 3 year old *S.lutaria* larvae at a range of depths. The densities of all age classes of larvae at 0-6.5 m, 6.5-10 m and 10-15 m depth were found to be 277, 270 and 35 m^{-2} respectively, with a mean value of 194 m^{-2} between 0-15 m depth. Mossberg & Nyberg (1979) found 200 *S.lutaria* larvae m^{-2} at 1 m depth in Lake Trollkarlen, Sweden in December 1977 decreasing to only 20 m^{-2} at 10 m depth. Numbers were considerably smaller in April 1978 at the same site (120 m^{-2} at 1m depth and 0 at 10 m depth). At Loch Monzievaird, large numbers were found in the decaying leaf material found at 1 m depth along the southern shore of the loch in October 1987 when up to 100 larvae were recovered per man/woman hour

of searching. At Drumore Loch, the population at the loch edge appeared sparse and only 25 individuals were collected per hour. At the West of Scotland Trout Farm, Bridge of Weir, 299 larvae were collected in 2 h in March 1988 indicating a fairly dense population at this site. At Powder Works Dam Lochan, a single larva was recovered from the benthic faunal surveys carried out in December 1986 (14.6 m^{-2}).

4.5.5.1.1.8.2. Significance of *Sialis lutaria* larvae in the diet of brown trout

Despite its apparent low population density at Loch Maragan, *Sialis lutaria* larvae formed a common element in the diet of brown trout during 13 of 18 sampling months. The highest numbers were recovered in March, April, May and June, with up to 6.29 (mean value) per fish in May 1988 with general peaks in mean numbers in spring and autumn (see Table 4.12). Similar feeding patterns are evident at other sites. Frost & Smyly (1952) described an 'unexpected and unexplicable preponderance' of these larvae in the diet of brown trout at Three Dubs Tarn in the Lake District in March, when *S.lutaria* larvae were found in 77% of the food-containing stomachs examined; the mean number of larvae per fish was 4.6. In April, these values were 37% and 1.0 respectively. Number per fish and occurrence in the diet fell between May and July, but had increased to 21%, 0.3 (prevalence and intensity) in August and 31%, 0.5 in September. Here mean values per stomach were calculated for Balls' (1961) 'standard stomach' produced by cutting the stomach across from the distal end of the pyloric stomach through the adjacent limb of the cardiac stomach. Thus the size of the stomach sample examined was smaller than in the current study and this may in part explain why the numbers of larvae recovered were smaller than at Loch Maragan. Comparison of the diet of brown trout with the available

fauna at Three Dubs Tarn showed that in spring 42.6 *S.lutaria* larvae m^{-2} were available in the fauna (Macan, 1949) and 177 were fed on by trout (n=74) (Frost & Smyly, 1952), implying selective feeding on larvae but this was not the case in summer. Ball (1961) found larvae formed 4.9%, 0.5% and 2.8% composition of the diet by volume of Llyn Tegid brown trout in February, April and May respectively, corresponding to an average of 0.1, 1.03 and 1.54 individuals per stomach, in the same months. Overall, these values were smaller than those for Loch Maragan. He concluded that this restricted period of feeding on these insect larvae coincided with their migration to the shore to pupate in the soil. He attributed their absence from the diet in other months to their habit of burrowing and also to their distribution, being away from the littoral areas of the lake, where he sampled his trout. Interestingly, he also reported that all the larvae consumed by the trout were 'full-grown', apart from one. This is a different situation from that at Loch Maragan, where larvae of a wide range of sizes were taken by trout (see Table 4.11). Similar to Ball's (1961) finding, Giani & Laville (1973) found that 7 trout caught in July in Lac Port-Beihl, Central Pyrenees, were only feeding on the last 2 larval stages of *S.lutaria* larvae and that many larvae were consumed by the trout. For example, a 310 mm long trout had 118 larvae in its stomach. The Loch Maragan data indicate that, within the range of larvae consumed, the larger larvae form the greatest proportion of the group. The higher proportion of *S.lutaria* larvae in the diet of Loch Maragan trout may, in part, be explained by the dearth of other suitable food items in the loch. Elsewhere, Stenson (1979) found *S.lutaria* as part of the diet of salmonids including brown trout in some Swedish forest lakes during his study period from March To October. The 211 brown trout stomachs which he examined contained 116 *S.lutaria* larvae (0.55 per stomach) and the highest

frequency of occurrence was in the spring samples (over 30%) although larvae did appear to a lesser extent in the summer and autumn samples which is similar to the Loch Maragan observations.

At sites where the fish were known to be infected with *Neoechinorhynchus rutili*, the occurrence of *Sialis lutaria* larvae in the diet has been observed to be variable. Chappell (1969b) stated that sticklebacks from a Baildon Moor pond ate various larval insects but did not mention *Sialis*. Robertson (1953) observed that *Sialis* larvae occurred in 4.4% of the 387 trout stomachs she examined from Dunalastair Reservoir although usually only as single specimens, particularly during the first half of the fishing season. She also described finding *N.rutili* in 1 of 23 *Sialis lutaria* larvae that she examined from Lochan An Daim, a water body close to Dunalastair Reservoir, but made no comment on this as a potential route for the transmission of *N.rutili* to brown trout in Dunalastair Reservoir. Walkey (1967) found *S.lutaria* larvae as part of the diet of sticklebacks from Monkton Pond, County Durham, in February, March and April and suggested that the seasonal nature of the occurrence of *Sialis*, coupled with the observation of continuous new infections of the fish with *N.rutili* throughout the year, made it possible to discount *S.lutaria* as a possible intermediate host at this site. Walkey (1963) found no infection of 30 *Sialis* which he dissected and concluded that 2 ostracod species, *Candona candida* and *Cypria ophthalmica* were intermediate hosts for *N.rutili* at Monkton Pond. His unsuccessful attempt to infect *S.lutaria* with *N.rutili* by offering them shelled acanthors supported his view. He also suggested that Villot's (1885) report of *N.rutili* in *S.lutaria* may have been as a result of predation by *S.lutaria* on infected ostracods. In the light of these studies, it appears that the role of *S.lutaria*, in the

transmission of *N.rutili* is important and there is direct evidence to support the proposition that trout become infected as a result of feeding on infected *Sialis lutaria* larvae in the spring, summer and autumn months.

Unavoidable lack of data for the Loch Maragan trout diets between December and February makes it impossible to comment on the importance of *Sialis lutaria* larvae during this period, but evidence from other sites indicates that the larvae form part of the winter diet of trout. Slack (1934) found 7% (n=100) of the winter (November to March) caught trout from the River Test, Hampshire to be feeding on *S.lutaria* larvae. At the West of Scotland Trout Farm, Bridge of Weir, trout collected in February 1988 were feeding on *S.lutaria* larvae. If winter feeding on *S.lutaria* larvae occurs in Loch Maragan it will have significant effects on the transmission of *N.rutili* and hence on the population observed in the trout in the spring months (see Chapter 5).

This evidence supports the view that *Sialis lutaria* larvae are quite common elements of trout diets in a number of localities, particularly lentic environments and all appear most frequently as dietary elements during the spring and summer months. At Loch Maragan the larvae form a significant part of the diet and this may reflect not only positive selection but also a paucity of other suitable alternative dietary elements in the loch fauna. Trout of a wide range of lengths/age classes were found feeding on *S.lutaria* larvae at Loch Maragan. These included 1 year old specimens (127 mm fork length) and consequently this evidence is important in interpreting the prevalence data for *N.rutili* infections. Feeding by small trout on *S.lutaria* larvae has been found elsewhere: Frost (1950) found 7% frequency of occurrence and a mean number of 0.3 individuals per stomach in small trout (Mean length = 131 mm; n = 57) caught during July in the River Forss, Caithness. From the available data it appears that there is

selective feeding of *S.lutaria* larvae of the larger instars as described by Giani & Laville (1973) in the French Pyrenees. Examination of the range of sizes consumed indicates that larvae with head widths from 24 to 34 arbitrary units (Instars 9 & 10) are most frequently eaten over the year (Fig. 7.3c). If the *S.lutaria* at Loch Maragan population is such that it consists of large numbers of smaller larvae and decreasing numbers of larger individuals then it would appear that selection by the trout was taking place. The extensive study of an *S.lutaria* population at Lac Port-Beihl, Central Pyrenees, by Giani & Laville (1973) found 3 age classes in the population: 68% one-year-olds, 26% two-year-olds and 6% three-year-olds and it is likely that the Loch Maragan population structure is similar but with only 2 age classes. However, further information on the foraging habits of trout in the loch and of the structure and distribution of the *S.lutaria* larval population would be necessary to draw any firm conclusions at Loch Maragan. In terms of foraging, the infrequency of smaller larvae in the diet can in part be explained by the difficulty in seeing and capturing smaller items which would bury themselves in the substrate. Infrequency of the larger larvae may be simply explained by the lower frequency of this size of larvae in the population (Giani & Laville, 1973). The relative numbers of various instars of *Sialis lutaria* larvae changes seasonally (Elliot, 1977a). Examination of the size range of larvae consumed by trout did not reflect these changes at Loch Maragan however.

4.5.5.1.1.8.3. The role of *Sialis lutaria* larvae in the transmission of *Neoechinorhynchus rutili* to trout

Part of the understanding of the dynamics of helminth transmission involving trophic relationships is knowledge of the

prevalence and intensity of the helminth in the predatory host population and in the prey in their diet. It is difficult to draw any firm conclusions on the relative proportion of larvae harbouring a *N.rutili* infection found to have been consumed by Loch Maragan trout. Overall 17.1% (56/328) of the larvae consumed by the Loch Maragan trout between April 1987 to June 1988 were found to be infected with *N.rutili* and by comparison 13.3% (6/45) of larvae collected from the benthic substrate collected in July and August 1988 were found to be infected. These values imply that trout are not selecting for infected *S.lutaria*. It is necessary to account for any possible seasonal variations in the percentage prevalence of *N.rutili* infections of the *Sialis lutaria* larval population when comparing feeding data. If the dietary data for July and August 1987 are examined, 7 larvae were consumed of which 3 were infected (42.9%), as compared to the benthic population data available for *S.lutaria* larvae where the prevalence was 13.3%. Thus the picture appears somewhat different, as if selection in favour of infected individuals were taking place. Obviously the amount of data available in this study is insufficient to draw firm conclusions and further analysis of the percentage prevalence of *N.rutili* in the loch population and the dietary population is required. Detailed experiments examining any changes, either physiological, morphological or behavioural, of the infected larvae, which alters their susceptibility to predation would compliment these studies. Experiments similar to those of Moore (1983) on the predation of the isopod *Armadillidium vulgare* infected with the acanthocephalan *Plagiorhynchus cylindraceus* by starlings *Sturnus vulgaris* and of the altered behaviour of the infected isopods would be appropriate.

The size of infected *Sialis lutaria* larvae consumed by trout was

nearly as wide as the entire range of sizes consumed, suggesting that trout are likely to be exposed to *N.rutili* by feeding on any size of larva. The 8th instar larvae showed the highest prevalence of infection (38.5%) and intensity (1.19) with smaller and larger larvae exhibiting lower values for percentage prevalence. One explanation for this observed distribution may be similar to that proposed by Kennedy (1985a) who cites Muzzall (1978), where a decline in infection level in the largest or oldest hosts is explained by increased mortality and reduced growth rates of infected individuals. No observations of increased intensity of infection with instar was observed which may indicate that larvae are susceptible to infection at a particular stage in their development. This phenomenon could have a physiological and/or behavioural basis. In terms of diet, it appears that Loch Maragan trout, of a wide size range themselves, can potentially be exposed to an *N.rutili* infection by feeding on *S.lutaria* larvae of several instars. Mixed sex infections were observed in individual larvae and this must be directly advantageous to the *N.rutili* if the larva is consumed by a trout and the worms become established. In these cases, suitable mates will be available immediately and copulation may take place. The distribution of *N.rutili* in the *S.lutaria* larvae consumed by Loch Maragan trout was overdispersed (s^2/x = 1.96 worms per host) and this will influence the dynamics of the transmission of *N.rutili* to trout. Insufficient numbers are available to draw conclusions about seasonal differences in the sex ratio of the *N.rutili* infrapopulation in the *S.lutaria* consumed by Loch Maragan trout. Further information on this may shed light on the observed structure of Loch Maragan *N.rutili* infrapopulations in trout during the year in terms of the sex ratio.

Only *Sialis lutaria* larvae have been found to be invertebrate hosts of *Neoechinorhynchus rutili* at Loch Maragan. Ostracods, which

have been cited as the intermediate hosts for *N.rutili* elsewhere in Britain (Brady, 1910; Walkey, 1967) appear in the benthic fauna of Loch Maragan in very small numbers, but none were found to be infected with developmental stages of *N.rutili*. Walkey (1967) suggested that Villot's (1885) observations of *N.rutili* in *S.lutaria* larvae occurred as a result of the carnivorous habits of the insect larvae which resulted in exposure to parasitic infection via feeding on infected ostracods. It is not known whether *Sialis* larvae at Loch Maragan become infected in this way.

4.5.5.1.1.9. Diptera

Dipteran larvae were found in small numbers in the benthic substrate samples of Loch Maragan in November 1986 and these formed part of the trout diet during the spring and early summer months. By contrast, Ball (1961) considered larval dipterans as unimportant in the diet of Llyn Tegid trout and Frost & Smyly (1952) found fewer chironomid larvae in the diet of trout than were available in the fauna at Three Dubs Tarn. Dipteran pupae and adults formed part of the diet during the warmer months of the year at Loch Maragan. This pattern is similar to that described for the diets of brown trout reported by Frost & Smyly (1952) at Three Dubs Tarn, Frost (1940) in the River Liffey, Ireland, Pentelow (1932) in the Rivers Tees and Itchen, Slack (1934) in the River Test, Hampshire and in Gladhouse Reservoir p.o. and Ball (1961) at Llyn Tegid, Wales. At Powder Works Dam Lochan, ceratopogonid larvae were recovered in the December 1986 benthic faunal survey and also formed part of the diet in June 1986 and May, June and August 1987. In general, the pupae and adults form an important summer element of the brown trout diet both at Loch Maragan and elsewhere.

4.5.5.1.1.10. Coleoptera

Some coleopterans were found in both the November 1986 and May 1987 benthic faunal samples from Loch Maragan. Similarly few coleopterans were present in the diet of the Loch Maragan brown trout and may reflect their scarcity in the loch fauna. Elsewhere, Frost & Smyly (1952) found small numbers of coleopterans in the diets of trout from Three Dubs Tarn, although up to 10% of the trout examined in any one month had been eating these faunal elements. Generally numbers of coleopteran imagines eaten by the trout in spring reflected the numbers available in the fauna at this site. At Llyn Tegid, Wales, Ball (1961) described Coleoptera as an insignificant part of the diet of brown trout. At Powder Works Dam Lochan, coleopterans were not found in the November 1986 benthic faunal survey but they did occur in 8 out of 10 sampling months, with up to 2 individuals per stomach (p.o.). At Gladhouse Reservoir coleopterans occurred in every sampling month and as many as 7 adults and 1 larva were found in an individual stomach (Lassiere, 1988b). They represented 28.2% by occurrence but only 1.0% by number. At Loch Maragan and elsewhere, coleopterans do not form an important element of the diet of brown trout.

4.5.5.1.2. *Vertebrates*

4.5.5.1.2.1. Urodeles

One newt was found in 1 trout collected in June 1988 and is obviously not an important dietary element. Reports of trout feeding on urodeles are not uncommon, however, and Frost & Smyly (1952) found a single newt in the 182 brown trout stomachs which they examined from Three Dubs Tarn in the Lake District. Also, Stenson (1979) found the remains of the smooth newt (*Triturus vulgaris*) in the stomachs of salmonids from some forest lakes in Sweden.

4.5.5.1.2.2. *Fish*

4.5.5.1.2.2.1. Seasonality of feeding on fish

Fish appear as a summer element in the diet of brown trout from Loch Maragan. Similarly, Frost & Smyly (1952) found fish in the diet of Three Dubs Tarn brown trout in July and Stenson (1979) found fish remains in the stomachs of brown trout from some Swedish lakes in spring, summer and autumn, the percentage occurrence being highest in the summer at around 20%. By contrast, Frost (1939) observed fish in the diets of trout from the River Liffey, Ireland, in February, March, April, May, June, September and October. Also, Slack (1934) noted trout from the River Test, Hampshire, to be feeding on *Gasterosteus* sp. and *Gobio gobio* in the winter months (November to March; n=100), but none were found in his summer-caught trout (May; n=6). These reports suggest that the piscivorous habit of trout occurs in all seasons. The rate of feeding in trout is influenced by ambient temperature and daylength, mediated via the endocrine system such that the activity is greatest during the warmer, longer days of the summer months (Ball, 1961). This would suggest that the finding of Loch Maragan trout feeding more predominantly on minnows during the summer months is genuine, and that during the winter months the lower temperatures and shorter daylength curtails significant predation on fish.

4.5.5.1.2.2.2. Size of fish eaten by trout

The finding that Loch Maragan trout feed on fish is by no means unusual, especially for larger individuals. For example, Ball (1961) observed brown trout ranging from 150 to 310 mm in length feeding on bullhead (*Cottus gobio*) in Llyn Tegid; Crozier (1985) found trout in Lough Neagh ranging from 285 to 662 mm in length to be feeding on a number of fish species, including *Rutilus rutilus*, *Perca fluvialtilis*,

Coregonus pollan and *Noemacheilus barbulatus* and suggested that there was a trend towards a piscivorous diet in larger fish. Swynnerton & Worthington (1940) examined 101 brown trout from Haweswater, Westmorland, and found sticklebacks, and 'probably' minnows, to be an important element of the diet in their largest size classes (fork length 200 to 300 mm). Nilsson (1955) found the larger brown trout from Lake Blasjon, Sweden, to be cannibalistic, especially during the summer months. Pentelow (1932) found trout feeding on *Phoxinus phoxinus*, *Gobio gobio* and *Leuciscus leuciscus*, although he gave no indication of any size restriction of trout for this dietary element in the River Tees and Itchen. By contrast, Frost (1939) observed brown trout of a wide size range feeding on fish at Ballysmuttan and Straffan on the River Liffey in Ireland. At Straffan, the piscivorous trout weighed between 44 and 630 g and were feeding predominantly on minnows although gudgeon, loach and salmon fry were also found in the stomach samples. Twenty two of the 228 stomach examined from this site (9.6%) were found to contain fish. At Ballysmuttan 3 out of 349 (0.8%) of the trout examined contained fish remains. From these reports, fish, including minnows, appear as a common element of the diet of large trout and as regards the postcyclic transmission of helminths, the larger trout are likely to become infected by this route.

4.5.5.1.2.2.3. Postcyclic transmission of helminths

The secondary aim of the investigation of the diet of Loch Maragan trout was to examine the possibility of postcyclic transmission of *Neoechinorhynchus rutili* involving feeding on vertebrates already harbouring a mature *N.rutili* infection. Walkey (1967) reported finding sticklebacks in the diet of sticklebacks in his study population which was also infected with *N.rutili*, but did not comment on this as a possible route for infection. All the whole

minnows consumed by Loch Maragan trout that were dissected were found to be harbouring plerocercoids of *Ligula intestinalis* in their body cavities but no *N.rutili* infection. However, this was a small sample and it is known that minnows in Loch Maragan harbour *N.rutili* infections (see section 4.6). Therefore, it seems probable that piscivorous trout may be exposed to *N.rutili* and become infected when they feed on infected minnows.

It is also interesting to speculate about the fact that all the dietary minnows examined were parasitized. This observation opens a wider field of investigation involving the role of parasitism in the level of predation of these individuals. Giles (1983, 1987) investigated this relationship in a system involving *Gasterosteus aculeatus* infected with plerocercoids of the pseudophylidean cestode, *Schistocephalus solidus*. He found that infected individuals recovered significantly more quickly from a 'frightening' overhead stimulus than uninfected individuals and that the former group would also feed after the stimulus. These observations were in part explained by the increased metabolic demands placed on the host by the parasite such that the fish had an increased oxygen demand and requirement for food energy. As a result of the increased oxygen demand, infected individuals would swim preferentially in open water and/or close to the surface where the oxygen concentration was higher. A similar observation was also made by Meakins (1974). This factor combined with the more rapid recovery after a frightening stimulus meant that infected individuals were more susceptible to predation by the parasites' avian definitive host than their uninfected counterparts. Similar observations have been made for fish species infected with plerocercoids of *L. intestinalis*. Dence (1958) found that infected shiners (*Notropis cornutus*) were more sluggish than their uninfected counterparts and spent more time in the shallower waters near to the

shore, even when there were bird predators in the area. Holmes & Bethel (1972) reported that infected spottail shiners (*Notropis hudsonius*) and yellow perch (*Perca flavescens*) often swam closer to the lake surface in Alberta. Evidence suggesting the increased susceptibility to predation, as a result of the behavioural changes observed in infected fish, has been given by Dobben (1952). He found that 30% of the roach in the diet of some cormorants (*Phalacrocorax carbo*) in the Netherlands were infected, whereas only about 6.5% of the general population were infected. It seems reasonable that a similar situation occurs in minnows infected with plerocercoids of *Ligula intestinalis* such that they are more susceptible to predation. The plerocercoids cause marked distension of the body and values of up to 39.8% of the body weight have been recorded at Loch Maragan (July 1988). This parasite load is likely to have a detrimental effect upon the swimming ability of the minnow and thus their ability to escape from predators. When minnows infected with *L.intestinalis* are predated upon by trout, transmission to the definitive hosts (*Larus* spp.) does not occur, but for *N.rutili* in fish concurrently infected with *L.intestinalis*, the chances of predation and possible transmission must be increased. To date there are no data to measure the extent of this type of transmission at Loch Maragan but it obviously warrants further investigation. The successful experimental transmission of *N.rutili* from *Gasterosteus aculeatus* to *Salmo gairdneri* (Lassiere & Crompton, 1988) gives support to this view.

4.5.5.2. *Benthic faunal elements absent from the diet*

Taxonomic groups which appeared in the Loch Maragan benthic faunal surveys but not in the brown trout diet included Oligochaeta, Hydracarina and Ostracoda.

4.5.5.2.1. *Oligochaeta*

Oligochaetes appeared to be quite common in the benthic faunal samples from Loch Maragan although none were recovered from the trout stomach samples. Similarly, Ball (1961) reported finding no oligochaetes in the diet of Llyn Tegid trout, and Frost & Smyly (1952) found oligochaetes to be abundant in the fauna of Three Dubs Tarn but none were eaten by trout. They attributed the absence in the diet to their mud-dwelling habit which protects them from foraging trout. However, Crozier (1985) found unidentified specimens in the diet of Lough Neagh brown trout and Pentelow (1932) found 2.9% of the 104 River Tees trout stomachs he examined to contain oligochaetes. The absence of oligochaetes from the Loch Maragan diet may be misleading. It is possible that they are dietary elements but these organisms are likely to be rapidly digested in the trout stomachs and therefore would not be found in the dietary analyses.

4.5.5.2.2. *Hydracarina*

These were found in both of the benthic faunal surveys at Loch Maragan but were never recovered from any of the stomach samples. None were found to be infected with developmental stages of *N.rutili*. Stenson (1979), Frost (1950), Swynnerton & Worthington (1940) and Frost & Smyly (1952) found hydracarinids in the diets of the trout which they investigated. Macan (1949) found 115 m^{-2} in the spring benthic fauna at Three Dubs Tarn in the Lake District but Frost & Smyly (1952) reported only one eaten by trout over a similar spring period. They gave no explanation for this observation but one may be that these quick-moving, small animals are able to avoid potential trout predators. Trout would be unlikely to select such small dietary items which may also be distasteful. At Powder Works Dam Lochan hydracarinids were found in the benthic faunal samples collected in

December 1986 and also in small numbers in the diets of trout in 5 of the 10 monthly dietary samples collected, with up to one specimen per stomach (p.o.). Also single specimens were found in two stomach of trout caught at Gladhouse Reservoir in April 1987 (Lassiere, 1988b).

4.5.5.2.3. *Ostracoda*

A total of 8 ostracods (unidentified) were recovered from the entire benthic faunal material collected in November 1986 and these were found to be uninfected with developmental stages of *N.rutili*. Loch Maragan is an acidic loch with low calcium content and this may explain the observed low density of the population (Hounsome p.c.). In other, more calcium-rich, waters, populations of ostracods are larger and consequently are more likely candidates as intermediate hosts of *N.rutili*. This was probably the case in Monkton Pond where Walkey (1967) found 2 ostracod species acting as the intermediate hosts for *N.rutili*. None of the stomachs nor intestines examined during the dietary survey of Loch Maragan trout were found to contain ostracods. Ostracods are known dietary items of trout and have been seen in trout caught at other sites in Scotland (p.o.) These ostracods were found whole in the lower regions of the gut so inaccuracies in the stomach analysis due to rapid digestion of these animals seems unlikely. In general, however, ostracods do not form an important element of the diet of brown trout at any site. Evidence suggests that ostracods do not form a significant part of the diet of Loch Maragan brown trout and consequently are not suitable for consideration as 'candidates' for transmission hosts of *N.rutili* to Loch Maragan trout.

4.5.5.3. *Stomach fullness*

The results were rather ambiguous as a consequence of the sampling method and therefore do not merit a detailed discussion. However, one conclusion that can be drawn is that gill netting does

not result in the evacuation of trout stomachs, only 12% of the sample having been found empty, and does provide samples from which the dietary components can be studied. Ball (1961) reported that the mean volume of food in the stomachs varied seasonally with a maximum in summer of about 8 times that of the winter level, in Llyn Tegid brown trout. No such pattern was revealed in the Loch Maragan samples.

4.5.5.4. *Proportion of bottom and surface/mid-water fauna in the diet*

When Ball (1961) analysed the relative importance of the surface/mid-water fauna and benthic fauna in the diet of Llyn Tegid brown trout the analysis was based on the percentage representation of each element by number, volume and occurrence. He used these values to compare the relative importance of each faunal element in the diet. Due to the way in which the stomach samples were collected in the present study it was not possible to carry out a similar analysis of the data. Consequently the patterns of dietary change in terms of these 2 subdivisions of the fauna were difficult to identify. However, the general pattern of increasing importance of surface/mid-water fauna was evidenced by the increasing numerical importance of these elements in the Loch Maragan brown trout diet during the spring and summer months, similar to the situation reported by Ball (1961).

In general, a similar pattern to that described by Ball (1961) at Llyn Tegid, Frost & Smyly (1952) at Three Dubs Tarn, Lake District, Stenson (1979) in some Swedish lakes and Nilsson (1955) at Lake Blasjon, Sweden, of the change in the diet of brown trout in the warmer months to a diet including a larger proportion of mid-water and surface faunal elements as compared to the colder months when bottom fauna predominated, was observed in the Loch Maragan trout diet. These seasonal changes in the dietary spectrum must reflect similar changes

in the loch fauna and changes in the feeding strategy of the trout.

4.5.5.5. *Comparison with diets of trout infected with* Neoechinorhynchus rutili *in other Scottish localities*

A summary of the dietary components of trout from other sites of *N.rutili* infection in Scotland is given in Table 4.15. For the most part, the dietary elements indicated are collated from a limited number of gut samples from each site over a restricted range of dates. Only the data for Loch Maragan and Powder Works Dam Lochan represent a significant number of samples over a range of dates and seasons. Despite this limitation, it is clear that there are similarities in the diets of these trout, with trout caught in the small highland lochans showing a much less varied dietary spectrum than those caught in larger lowland lochans, for example, Loch Monzievaird. Most significantly, at 6 of the 9 sites *Sialis lutaria* larvae are found as part of the diet and at 4 of these sites the larvae have also been shown to be infected with *N.rutili*. In addition to these personal observations, Robertson (1953) reported a single specimen of *S.lutaria* to be infected with *N.rutili* at Dunalastair Reservoir, where she also found brown trout infected and this forms part of the diet of the trout there (p.o. 1987). As many as 57 larvae were recovered from a single trout, caught at Lochan Creag nan Caorann in August 1986. However it is not possible from the data to intrepret any seasonal variation in the importance of this element in the trout diets at these sites.

Interestingly, ostracods of the species *Herpetocypris reptans* (Baird, 1835) were also consumed by trout at 2 sites. Dissections of these found no evidence of developmental stages of *Neoechinorhynchus rutili*. Hounsome (p.c.) described this species as a detrital feeder

which spends all of its time on the bottom or climbing on vegetation and as such would be an ideal candidate for a host of *N.rutili*, becoming exposed to acanthors in the benthic sediments during its foraging activities. However, ostracods observed in the trout guts were undamaged, even at the distal end of the gut. This suggests that these ostracods are rather indigestable and, therefore, any *N.rutili* infecting them would have a low establishment rate in the definitive host. Dissection of 231 specimens from Drumore Loch indicated that this species is not an important host of *N.rutili*.

In general, these data support the view that *Sialis lutaria* larvae are an important element in the life cycle of *Neoechinorhynchus rutili* at several Scottish sites in terms of transmission, and that trout become exposed to developmental stages of *N.rutili* as a result of feeding on infected individuals. The role of ostracods at these sites however, remains unclear.

4.5.5.6. *Dietary spectrum*

Ball's (1961) description of the trout as an 'opportune' carnivore seems most apt to express the catholic nature the diet. The diet of Loch Maragan trout is fairly varied including both invertebrate and vertebrate elements. The overall dietary spectrum of trout in its habitat must represent a combination of the availability of food organisms and the selection of various items by the trout. The examination of the benthic fauna at Loch Maragan indicated that a fairly limited range of faunal elements were available to the trout and that on the whole the trout avoided these faunal groups. When compared to the dietary spectra of trout from other localities, the Loch Maragan dietary spectrum appears to be fairly narrow and this in part must be indicative of the available fauna in the loch. The Loch Maragan dietary spectrum bears great similarity to trout caught in

other highland lochans and this supports the latter proposition. In larger, less acidic environments where the calcium content is higher, the occurrence of crustaceans and molluscs is more frequent and this contributes to the wider dietary spectra observed in trout from these habitats. Overall the success of *Neoechinorhynchus rutili* at these various sites will be determined by the availability of suitable intermediate hosts, namely *S.lutaria* and by the selection of these hosts by trout while feeding. One explanation for the high prevalences and intensities of *N.rutili* infections observed in remote sites may be a result of the combination of these 2 factors.

4.6. LOCH MARAGAN MINNOWS

Two hundred and five minnows (*Phoxinus phoxinus*) caught in nets together with 2 individuals that had been consumed by trout (see section 4.3.1.2) were examined for macroparasites (gut and body cavity). For each fish, the total length (mm) and wet weight (g, to the nearest 0.01 g) were measured. The entire gut was removed and examined for parasites and, where possible, the sex was determined by gross examination of the gonads. Minnows ranging from 22 to 98 mm length were examined. Details of the macroparasitic infections of these fish are given in section 4.7.2.

4.7. PARASITES OF LOCH MARAGAN FAUNA

During the course of this study, a number of macroparasite species were found in the haemocoel or viscera, where appropriate, of some of the Loch Maragan fauna. No attempt was made to follow the seasonal dynamics of these parasites (except for *Neoechinorhynchus rutili* in trout).

4.7.1. *Sialis lutaria* larvae

Young adult *Neoechinorhynchus rutili* (Chapter 7) and an unidentified trematode metacercaria were found in *Sialis lutaria* larvae from Loch Maragan. The trematode could possibly be a species of *Crepidostomum* for which the second intermediate host is the nymph of the insect *Ephemera danica* (for *Crepidostomum farionis*). Robertson (1953) found *C.farionis* metacercaria in *S.lutaria* larvae from Dunalastair Reservoir. The first intermediate host is a mollusc, *Pisidium* sp., which was found in the benthic faunal surveys of the loch (section 4.3.1.4).

4.7.2. Minnows

Three macroparasite species were found infecting Loch Maragan minnows. Plerocercoids of the cestode *Ligula intestinalis* in the body cavity, adult *Neoechinorhynchus rutili* (Acanthocephala) and *Crepidostomum* sp. (Trematoda) in the intestine.

4.7.2.1. *Ligula intestinalis*

Both male and female fish of a length range of 25 to 98 mm total length were found to be infected with *Ligula intestinalis* plerocercoids. Twenty nine percent (60/207) were infected by *L.intestinalis* only and 3.38% (7/207) had double infections of *L.intestinalis* and *Neoechinorhynchus rutili*. The overall prevalence of infection was 32.37% (67/207). Between 1 and 10 plerocercoids were found in the body cavity of an individual fish, the mean intensity of infection was 0.63 and the distribution of infection amongst the fish was typically overdispersed where $s^2/x = 2.72$. The value of k for the negative binomial distribution was calculated using the maximum likelihood equation (method in Elliot, 1977b) and the agreement of the observed distribution with the negative binomial was compared using a

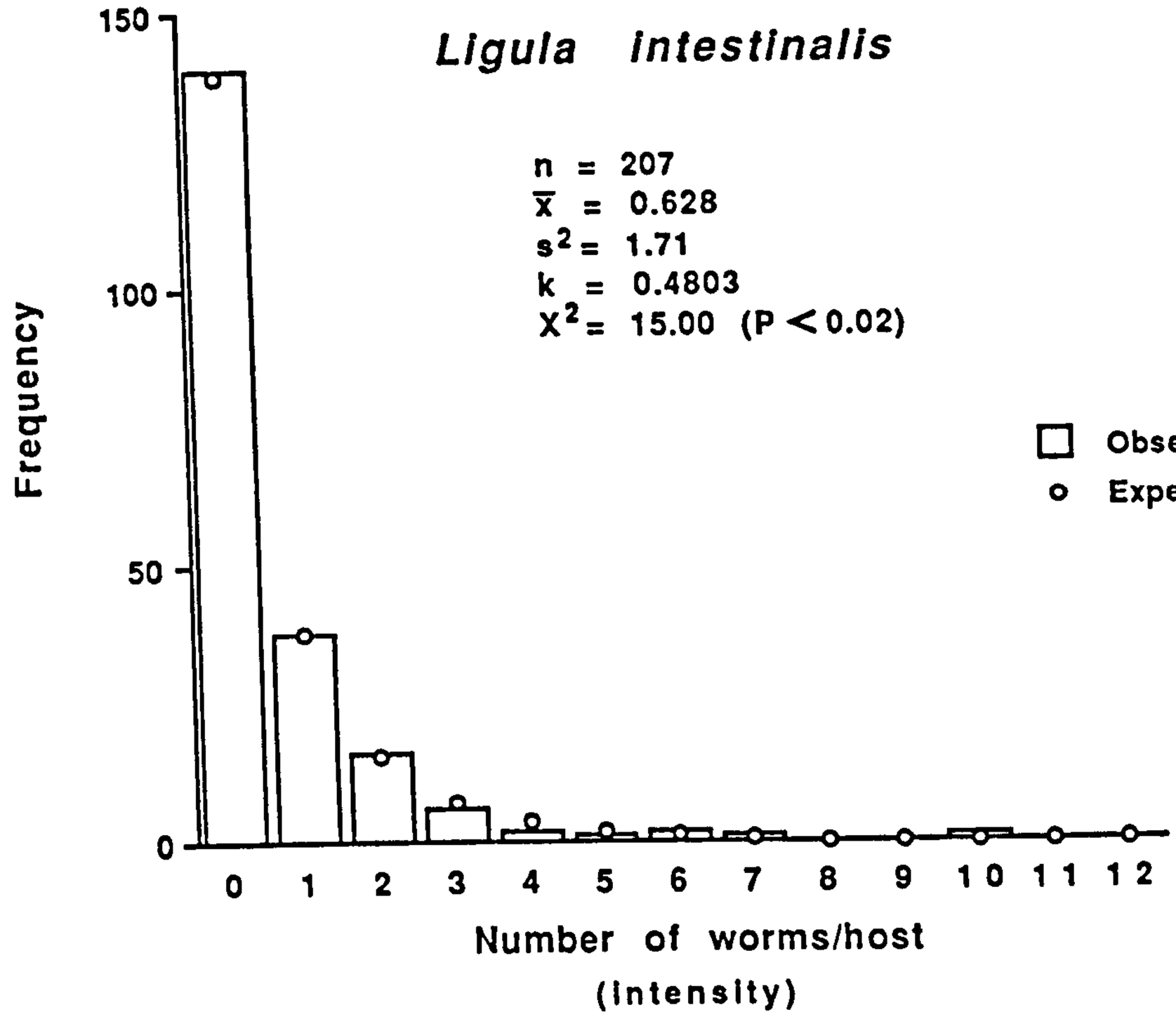
Chi squared test (see Fig 4.10 a). There was good agreement with this mathematical distribution. Kennedy & Burrough (1981) described the distribution of *L.intestinalis* in roach at Slapton Ley as being close to random most of the time and only slightly overdispersed in the summer months. This difference may be explained simply because the majority of minnows at Loch Maragan were sampled in the summer months or may indicate an aggregated distribution of infected copepods either temporally or spatially.

Plerocercoids from individual fish were surface dried with a tissue and weighed to the nearest 0.01 g. The parasite index was calculated using the following formula:

$$\text{Parasite Index} = \text{P.I.} = \frac{\text{total weight of parasites}}{\text{weight of host + parasites}} \times 100$$

(After Arme & Owen, 1968). The maximum P.I. was 41.80 for a 70 mm-long minnow which harboured 5 plerocercoids weighing 1.86 g. The highest value for P.I. for a single infection was 28.07 for a fish 53 mm long and the mean P.I. was 13.38 (n = 29). Infections were found in fish taken in April, May, July, August, September and November. The distribution of infection with respect to fish length is shown along with the distribution of *N.rutili* infections in Fig 4.11. The size of fish infected with *L.intestinalis* was smaller than those infected with *N.rutili*; perhaps this pattern is diet related. *L.intestinalis* utilises copepods as the intermediate host (e.g. *Cyclops strenuus*, *Diaptomus gracilis*) and *N.rutili* relies on *Sialis lutaria* larvae. Orr (1967) described other sites in Scotland where minnows were infected with *L.intestinalis* and found a prevalence of infection of 16.94% (n =177) in minnows collected from Milngavie reservoir. He described the conditions in oligotrophic Scottish lochs as being ideal for the build up of dense copepod populations and hence the transmission of the

a.



b.

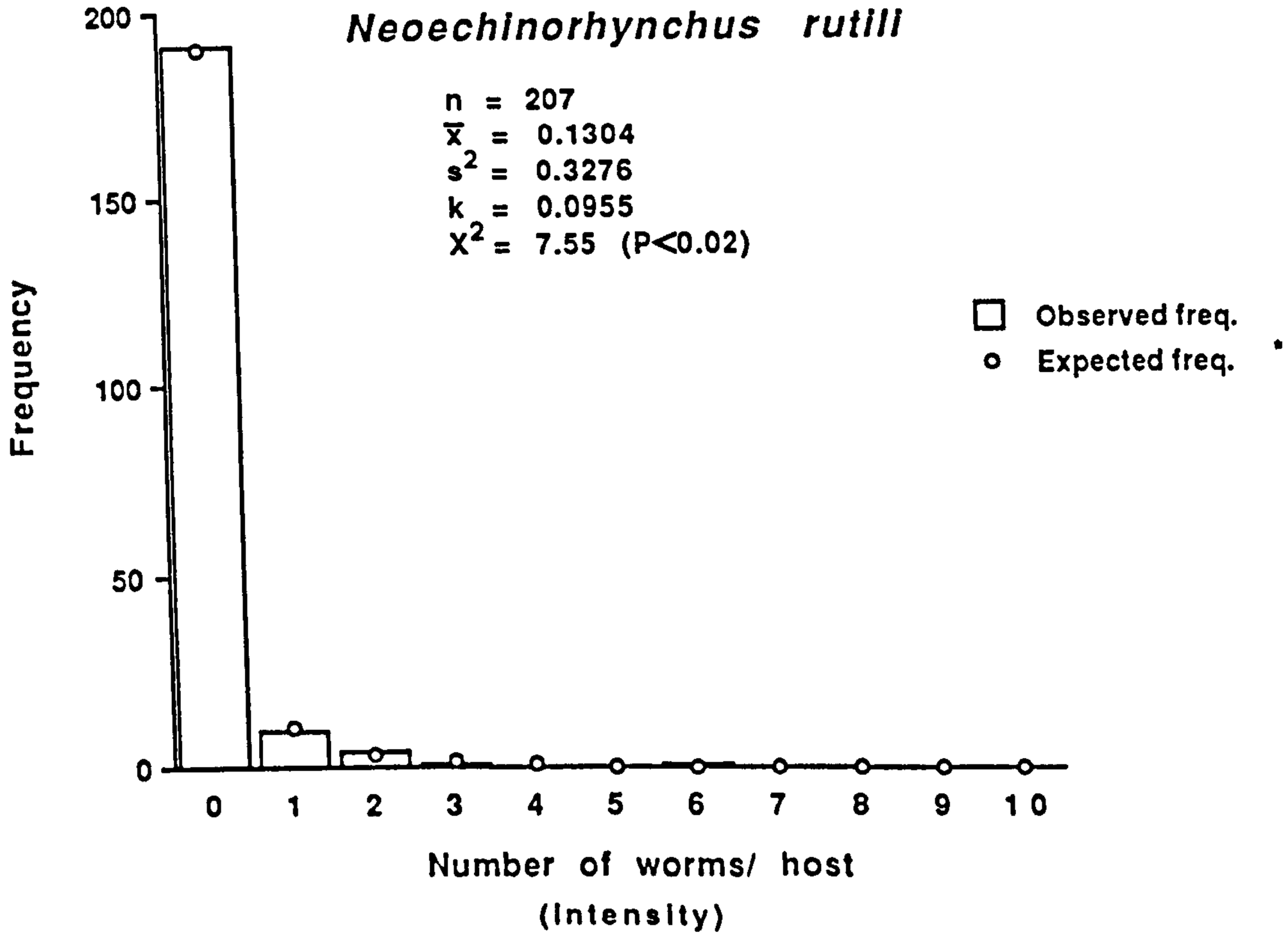


Fig 4.10 Frequency distribution of parasitic infections of minnow from Loch Maragan: a. *Ligula intestinalis*; b. *Neoechinorhynchus rutili*

(* = expected frequency from the negative binomial distribution)

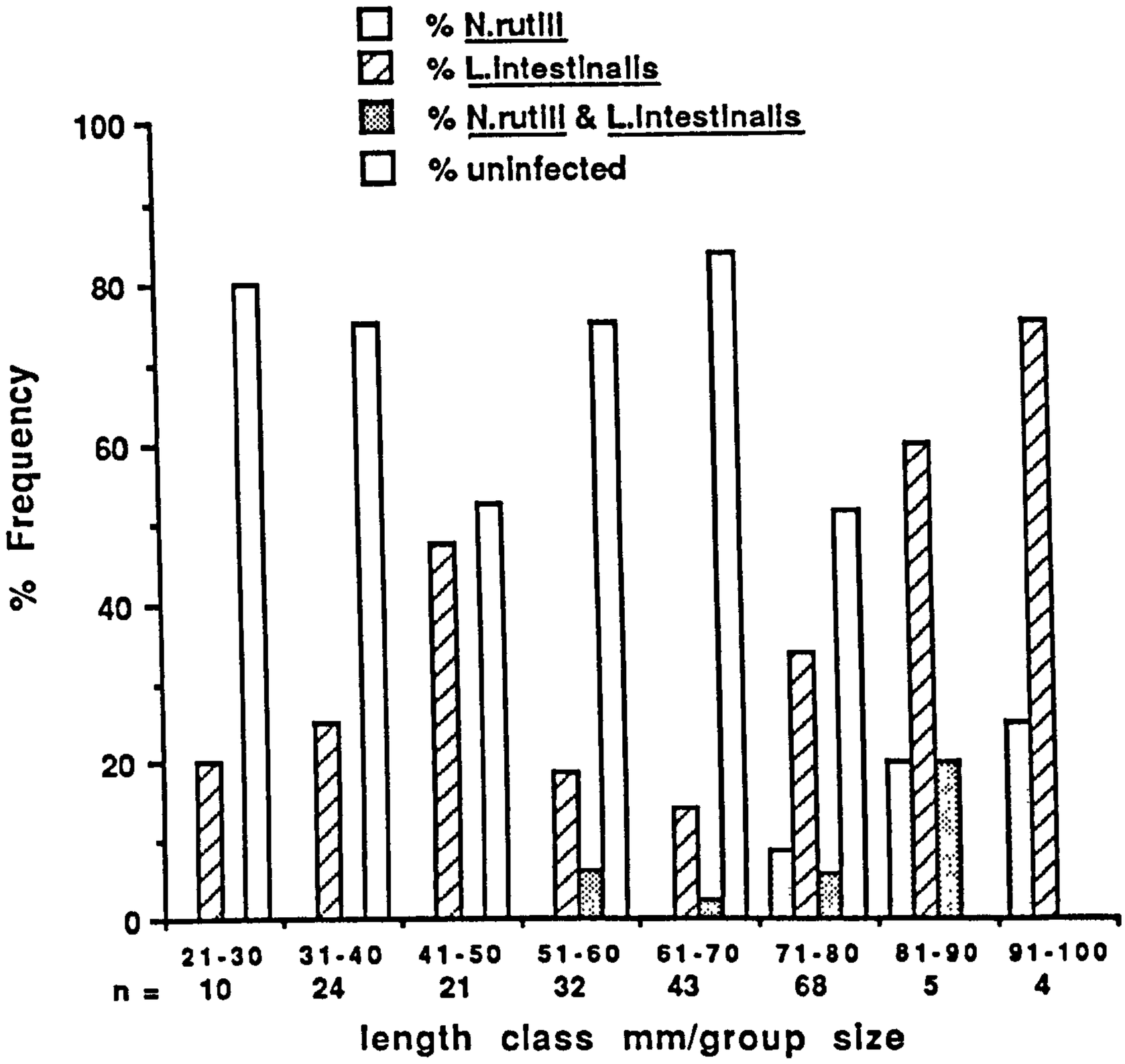


Fig 4.11 Distribution of parasitic infections in Minnows from Loch Maragan

parasite to the fish. Chubb, Pool & Veltkamp (1987) described piscivorous birds and mammals as the definitive hosts. Such birds occur at Loch Maragan.

4.7.2.2. *Neoechinorhynchus rutili*

Both male and female minnows ranging from 57 to 98 mm in length were found to be infected with *Neoechinorhynchus rutili*; 4.35% (9/207) were infected with *N.rutili* only and 3.38% had double infections with *Ligula intestinalis*. The overall prevalence of infection was 7.73% (16/207). Between 1 and 6 worms were found in the intestine of individual fish, the mean intensity of infection was 0.13 and the distribution was typically overdispersed ($s^2/x = 2.51$). The observed distribution showed close agreement with the negative binomial distribution (Fig 4.10b). Infections occurred in the May, July and August samples and the distribution with respect to fish length is shown in Fig 4.11.

The mode of transmission of *N.rutili* to minnows at Loch Maragan is unknown. One minnow 98 mm long was found to have eaten a *Sialis lutaria* larva in May 1987. Consideration of the size range of fish infected (Fig 4.11) suggests that transmission is via 'larger' invertebrate hosts, most probably *S.lutaria* larvae at Loch Maragan, rather than by significantly smaller ostracods.

In May 1987, a single minnow specimen harbouring 6 worms contained gravid *N.rutili*. In August 1987 20 worms were recovered from the fish sample of which 10 were females with free ovaries, 8 were females with free ovaries and shelled acanthors and 2 were males. Only 1 fish had a mixed sex infection, but the occurrence of 4 fish with gravid female worms only in the gut indicates that males had been present. It is probable that the intensity of infection is higher earlier in the year when new infections occur. However, a single

minnow in July 1988 had only one female with free ovaries in its gut. Nevertheless, the *N.rutili* populations within the minnows are obviously reproducing and must contribute to the number of acanthors available to invertebrate hosts. If postcyclic transmission is operating at Loch Maragan, then the observed distribution of *N.rutili* in the minnow hosts will partially influence the subsequent distribution in piscivorous trout hosts.

4.7.2.3. *Crepidostomum* spp.

Two minnows, 77 and 98 mm long respectively, were found to be infected with adult *Crepidostomum* sp. in May and August 1987.

4.7.3. Brown trout

Four genera of macroparasites were found infecting brown trout at Loch Maragan. *Neoechinorhynchus rutili* (Acanthocephala), *Capillaria salvelini* (Nematoda) and *Crepidostomum* sp. in the intestine and *Diphyllobothrium dendriticum* and *D.ditremum* (Cestoda) were associated with the viscera. Of the 190 fish examined 25, 10 and 4 fish harboured single genus infections of *N.rutili*, *Crepidostomum* sp. and *Diphyllobothrium* spp. respectively. Seventy one, 3, 9, 1 and 4 fish had dual infections of *N.rutili* and *Crepidostomum* sp., *N.rutili* and *C.salvelini*, *N.rutili* and *Diphyllobothrium* spp., *Crepidostomum* sp. and *C.salvelini* and *Crepidostomum* sp. and *Diphyllobothrium* spp. respectively. Eighteen and 32 trout had triple infections of *N.rutili*, *Crepidostomum* sp. and *C.salvelini* and *N.rutili*, *Crepidostomum* sp. and *Diphyllobothrium* spp. respectively. Eight fish were infected with all 4 parasite genera and 5 were uninfected.

4.7.3.1. *Neoechinorhynchus rutili* · see Chapter 5.

4.7.3.2. *Capillaria salvelini*

Nematodes, identified as *Capillaria salvelini* by Dr J.C. Chubb, University of Liverpool, were most commonly found in the rectal region of trout, the usual site for this parasite (Moravec, 1980). *Capillaria salvelini* was recovered from trout in January, April, May, June, July, August, October and November. Between 1 and 15 worms were found in an individual fish and the overall prevalence of infection was 15.79% (30/190). Campbell (1974) found the brown trout in Loch Leven to be infected with *C.salvelini*. The nematode was present throughout the year and there was no clear evidence of a seasonal cycle of occurrence.

4.6.3.3. *Crepidostomum* spp.

The species of *Crepidostomum* inhabiting the gut of brown trout from Loch Maragan were not identified further. Elsewhere in Scotland, 2 species, *C.farionis* and *C.metoecus*, have been found infecting brown trout (Campbell, 1974; Bwathondi, 1976; Pike & Edwards, 1983) and it is possible that both species occur in brown trout at Loch Maragan. One hundred and forty four of 185 trout examined were infected with *Crepidostomum* spp. (77.84%). Counts of the numbers of worms in 120 trout were made; the intensity of infection ranged between 1 and 268 per fish. The mean intensity of infection was 12.88 and the s^2/x = 59.26, indicating a strongly overdispersed distribution. Worms were found throughout the entire gut posterior to the stomach, including the pyloric caeca, and were present in all months for which the trout were sampled. Trout from 124 mm total length were infected. Campbell (1974) compared the prevalence of infection of *C.metoecus* in brown trout from Loch Leven that were either more or less than 196 mm long and found that the former group were more heavily infected. At Loch Maragan the equivalent percentage prevalences were 81.14% and 75.65%

respectively which apparently indicates no difference. Rahkonen & Valtonen (1989) list the published possible second intermediate hosts of *C.farionis* and include the ephemeropeteran *Paraleptophlebia* sp. These were found in large numbers in stomach samples and therefore may be acting as transmission hosts at Loch Maragan.

4.7.3.4. *Diphyllobothrium* spp.

Chubb *et al.*, (1987) described the plerocercoids of *Diphyllobothrium dendriticum* and *D.ditremum* as occurring commonly and concurrently in salmonids in montane lakes, encapsulated either in or on viscera. Cysts containing plerocercoids of both species were found to be infecting brown trout at Loch Maragan. The cysts were found on the outer stomach wall, associated with the pyloric caeca and occasionally other parts of the intestine, in the liver and spleen, on the ovary surface and also on the body cavity wall. No attempt was made to dissect out each individual cyst in order to enumerate them, so only a presence or absence score was made for the infection. The overall prevalence of infection was 30% (57/190).

4.8. SUMMARY

4.8.1. The main field site for the examination of the ecology of *Neoechinorhynchus rutili* in brown trout was Loch Maragan, Central Region (Grid Ref. NN 402278). The loch has a surface area of 7.3 ha, a maximum depth of 10.2 m and one outflowing stream, the Inverhaggernie Burn.

4.8.2. The p.H. of the water (in November 1986) was 6.44 (at 17°C), the conductivity was 242 um/cm and the alkalinity was 0.12 meq/1.

4.8.3. Three species of fish were found in Loch Maragan: brown trout (*Salmo trutta*), eels (*Anguilla anguilla*) and minnows (*Phoxinus phoxinus*).

4.8.4. A total of 225 trout were caught in gill nets or with a fly rod between October 1986 and August 1988. Samples were taken in every month between these dates when the loch was unfrozen and during the close season, when permission to fish was obtained from the Department of Agriculture and Fisheries for Scotland.

4.8.5. A total of 205 minnows, ranging from 22 to 98 mm in length, were caught and examined for macroparasites between April 1987 and August 1988.

4.8.6. Two benthic faunal surveys, carried out by a diving team in November 1986 and May 1987 respectively, did not reveal which species of invertebrates were acting as the intermediate hosts for *N.rutili* at Loch Maragan.

4.8.7. The topography of the loch was mapped and the water volume estimated to be 153943 m^3.

4.8.8. Trout between 1 and 5 years old were caught. Maturity was seen in males at 1 year and in females at 2 years of age. The spawning season apparently lasted from late October to May.

4.8.9. The growth rates of trout (males and females together) during the 2nd, 3rd and 4th years of life were 69.14, 40.96 and 25.45 respectively (G_L values = specific growth rate). The mean total lengths for the 1, 2, 3 and 4 year old trout were 75.8, 151.3, 227.9 and 292.0 mm respectively.

4.8.10. The condition factor values for the trout were highest in the summer months and lowest over winter.

4.8.11. An estimate of the size of the brown trout population was made in August 1987 by means of a simple mark and recapture technique. The population (1 to 3 year olds) was estimated to be 2641 (maximum value).

4.8.12. The diet of 168 brown trout, caught mainly by gill netting

between July 1986 and August 1988, was investigated from analyses of 161 stomach samples and 7 entire gut samples.

4.8.13. A total of 19 dietary elements from 16 faunal groups were recovered from the 161 stomach samples: 11 benthic faunal groups and 5 surface-midwater faunal groups.

4.8.14. The diet showed the Loch Maragan trout to be carnivorous, eating a limited range of faunal elements.

4.8.15. The significance of each dietary faunal element was discussed on a seasonal and individual level at Loch Maragan and was compared with reports from other localities and with the available benthic fauna at the loch.

4.8.16. *Sialis lutaria* larvae form an important element of the diet of Loch Maragan brown trout, especially during the spring months.

4.8.17. *Sialis lutaria* larvae of a wide size range were consumed by Loch Maragan trout, specimens of the middle size range being eaten most frequently.

4.8.18. The probability of behavioural changes in *Sialis lutaria* larvae infected with *Neoechinorhynchus rutili* affecting their susceptibility to predation by trout is considered.

4.8.19. Only *Sialis lutaria* larvae were identified as invertebrate hosts of *Neoechinorhynchus rutili* at Loch Maragan and at a number of other sites around Scotland.

4.8.20. Larger trout (220 to 334 mm total length) were piscivorous at Loch Maragan, especially during the summer months.

4.8.21. The postcyclical transmission of parasites via the piscivorous feeding habits of Loch Maragan brown trout is considered.

4.8.22. The proportion of surface/mid-water faunal elements in the diet increased during the summer months. Benthic faunal elements were important in the diet throughout the year.

4.8.23. The dietary spectrum at Loch Maragan is similar to that of trout from other highland lochans and several faunal elements were found in common with the more varied diets of trout from other types of water body.

4.8.24. Various macroparasites were found in the haemocoel of *Sialis lutaria* larvae, and in the viscera of minnows and brown trout collected from Loch Maragan.

4.8.25. *Sialis lutaria* larvae harboured small, apparently adult, *Neoechinorhynchus rutili* and an unidentified metacercaria in their haemocoels.

4.8.26. Minnows were infected with plerocercoids of the cestode *Ligula intestinalis*, in the body cavity and *N.rutili* and *Crepidostomum* sp. (Trematoda) in the intestine. The prevalence of the *L.intestinalis* infection was 32.37%, with each fish harbouring between 1 and 10 worms. The mean intensity of infection was 0.63 and the distribution of infection was typically overdispersed (k = 0.480). The prevalence of *N.rutili* infection was 7.73%, with between 1 and 6 worms per fish. The mean intensity of infection was 0.13 and the distribution of infections was typically overdispersed (k = 0.095). *Crepidostomum* sp. was found in only two minnows.

4.8.27. Brown trout were infected with *Capillaria salvelini* (Nematoda), *Crepidostomum* sp. (Trematoda) and *Neoechinorhynchus rutili* (Acanthocephala) in the intestine and *Diphyllobothrium dendriticum* and *D.ditremum* (Cestoda) on the viscera.

Between 1 and 15 *C.salvelini* specimens were found in the guts of trout collected in January, April, May, June, July, August, October and November. The overall prevalence of infection was 15.79%.

The prevalence of the *Crepidostomum* sp. infection was 77.94%, with intensities of infection as high as 268. Worms were found throughout the entire gut, including the pyloric caeca, in all sampling months.

Cysts containing the plerocercoids of *D.dendriticum* and *D.ditremum* were found on the outer surfaces of various visceral organs. The prevalence of infection was 30% of large trout and as regards the postcyclic transmission of helminths, the larger trout are likely to become infected by this route.

CHAPTER FIVE

POPULATION BIOLOGY OF *NEOECHINORHYNCHUS RUTILI* IN ITS DEFINITIVE HOSTS

5.1. INTRODUCTION

Following the description of seasonal dynamics of acanthocephalan infections in fish by Van Cleave (1916), several other species have been investigated. Chubb (1982) reviewed the seasonal dynamics of 34 acanthocephalan species in definitive hosts and since this review, papers regularly appear indicating the continued interest in this research area (e.g. Amin, 1985a, 1986; Ashley & Nickol, 1989; Brattey, 1988; Diamant, 1989; Hubschman, 1985; Khamees & Mhaisen, 1988; Lasee, 1989; Mhaisen, Al-Salim, 1988; Moravec, 1984 a & b; Scholz, 1986, 1987; Sutherland, 1989; Zdzitowiecki, 1986; Zdzitowiecki & Rokosz, 1986). The earlier studies tended to be descriptive relating observed cycles to environmental conditions; only in the more recent papers is there an attempt to elucidate the underlying features of the host-parasite interactions which may also contribute to the observed dynamics (e.g. spatial distribution in host population, effect on hosts). In the 1970's, a number of mathematical models were developed to predict parasite population dynamics and interpret empirical data. The major question posed was which mechanisms prevented the exponential growth of parasite populations which would have resulted in the subsequent extinction of both parasite and host? Bradley (1974) addressed this problem and recognised 3 basic types of regulation of parasite population numbers:

Type I - Populations determined by transmission: In this case a small change in transmission rate could lead to large changes in population size. However, Kennedy (1977) pointed out that the transmission rate could only regulate population size if it was density-dependent and at the time of writing he concluded that there was no evidence for fish parasites to support this.

Type II - Parasites regulated at the host population level: Large

parasite burdens may induce host mortality and hence reduce the number of parasites available for transmission. Here the pathogenic effect of the parasites brings about their regulation. Evidence for this in natural infections is limited. Conversely the host may have the ability to develop resistance to the parasites and kill or control them. This would only have an effect on the parasite population as a whole if a large proportion of the host population were to mount a successful immune response and hence prevent subsequent reinfection.

Type III - Parasites regulated by host individuals: Hosts develop immunity to superinfection and hence regulate parasite populations. Kennedy (1977) described the extent or degree of these responses as almost always dependent upon the degree of stimulation i.e. intensity leading to a density dependent process. As a result of host immune responses, there may be increased parasite mortality, decreased fecundity, increased generation time and reduced establishment in subsequent infections. Kennedy (1985a) gives examples of these 3 types of regulation by reference to acanthocephalan parasites.

Later Anderson & May (1978) and May & Anderson (1978) devised models to describe the regulatory processes in macroparasite populations and recognised that both stabilizing and destabilizing processes could be operating simultaneously. The stabilizing processes include : overdispersion of parasite numbers per host; non-linear functional relationships between parasite burden per host and host death rate (parasite-induced host mortality); density-dependent constraints on parasite population growth within individual hosts (density-dependent effects on fecundity). The destabilizing processes were thought to include: parasite induced reduction in host reproductive potential; parasite reproduction within the host which directly increases parasite population size and time delays in parasite reproduction and transmission.

Most recently, Dobson & Keymer (1985) extended the basic Anderson & May (1978) model in order to describe the population dynamics of acanthocephalan worms. The model takes the simplest form of the acanthocephalan life cycle with one intermediate and one definitive host species and it is used to test sets of empirical data. In many cases the authors found that there was a lack of certain parameter values e.g. transmission rate to intermediate and definitive hosts and egg production in specific data sets and their general conclusion was that more comprehensive field data on single systems was required. This present study aimed to obtain such data for a *Neoechinorhynchus rutili* suprapopulation, but it soon became clear that to measure all of the life history parameters in the time available was logistically impossible. However, the concentrated study of *N.rutili* in a population of brown trout, *Salmo trutta*, over an extended period and 'snap-shot' data sets from other Scottish metapopulations inhabiting fish hosts and *Sialis lutaria* larvae are presented here in an attempt to extend the present knowledge about this parasite and to compare the observations with other studies in different localities in Britain and abroad.

The population dynamics of *N.rutili* have been considered on a seasonal basis throughout its distribution range (Chubb, 1982). In Britain, the population dynamics in several definitive host species have been investigated to some degree (Table 5.1). The distribution of *N.rutili* in Scotland is fairly widespread and occurs in a number of habitats (Chapter 3). The study by Robertson (1953) at Dunalastair Reservoir was restricted in season to the months between March and September while that of Bwathondi (1976) covered the entire year. However the mean intensity of infection did not rise above 4.00 in any month at either study site so detailed seasonal analysis was not

feasible. Therefore, Loch Maragan was considered as a suitable site for examining the population dynamics over a more extended period where the mean intensity of infection was greater. *Neoechinorhynchus rutili* has been found in fish farm stocks and so it is of interest to determine any effects of *N.rutili* on the condition of its host and see whether any form of control should be enforced upon the stocking of natural waters from such farms.

Table 5.1: Studies of *Neoechinorhynchus rutili* population dynamics in Britain

Country	Definitive host species	Reference
England	*Gasterosteus aculeatus*	Walkey (1967), Chappell (1969b, c), Dartnall (1972)
	Salmo trutta	Thomas (1964a, b)
	Esox lucius, *Leuciscus leuciscus*, *L.cephalus*, *Rutilus rutilus*, *Thymallus thymallus*	Davies (1967)
Wales	*Phoxinus phoxinus*	Bibby (1972)
Scotland	*Salmo trutta*	Robertson (1953), Bwathondi (1976)

Thus the main aim was to examine the population dynamics of *N.rutili* in one of its more common Scottish definitive hosts, the brown trout and to attempt to partially fulfil the plea of Kennedy (1985a) to extend the knowledge of life history parameters for acanthocephalan species.

5.2. TERMINOLOGY

The various terms utilized to describe parasite population parameters in this chapter are defined to facilitate comparisons with other similar studies.

% Prevalence of infection = No. of infected hosts x 100/No. of hosts examined (equivalent to Margolis *et al*, 1982 definition)

Mean intensity of infection = Total no. of individuals of a particular parasite species in a sample of hosts/Total no. of individuals of the host species examined (i.e. includes uninfected hosts (Anderson, 1982) (equivalent to Relative Density or Abundance as defined by Margolis *et al.*, 1982).

Infrapopulation = All individuals of a species of parasite occurring in an individual host (Esch *et al.*, 1975)

Metapopulation = Total no. of individuals of a species of parasite infecting one species of host or the sum of the infrapopulations in a single species of host (Riggs & Esch, 1987; Riggs, Lemly & Esch, 1987).

Suprapopulation = All individuals of a species of parasite in all stages of development within all hosts in an ecosystem (Esch *et al.*,1975)

5.3. MATERIALS AND METHODS

5.3.1. Fish collections

5.3.1.1. *Loch Maragan*

A total of 226 brown trout (*Salmo trutta*) was caught between July 1986 and August 1988 by a number of methods as detailed in section 4.3.1.1 Chapter 4.

5.3.1.2. *Other Scottish sites*

Details of the dates of capture, fish host species harbouring *N.rutili* and numbers of fish that were examined during the course of this work are given in Tables 3.2 and 3.3. Four species of fish from 12 sites of *N.rutili* infection were examined (*Salmo trutta*, *S.gairdneri*, *Gasterosteus aculeatus* and *Phoxinus phoxinus*). These 'snap-shots' from other sites were available between February and August and in October for *Salmo trutta*; January, February and May for *S.gairdneri*; February, March, April, October, November and December for sticklebacks (see Chapter 4 for details of minnows). The majority of the trout were fly caught, apart from those from fish farms. The sticklebacks and minnows were caught in nets (either hand, gill or seine). Many of the trout were caught by sport fishermen and were usually received in a frozen condition.

5.3.2. Laboratory examination of hosts

5.3.2.1. *Loch Maragan trout*

For each trout the gut was removed and divided into 4 sections: stomach, duodenum, ileum and rectum. Each section was examined in 0.9% (w/v) NaCl aqueous solution and the *N.rutili* established in each region were removed and examined separately. In most cases, a number of worms had become detached from the gut mucosa so a more precise measure of worm position was not attempted.

5.3.2.1.1. *Measurement and sexing of worms*

Where possible measurement and sexing was carried out on live worms. Each worm was placed on a slide in a drop of saline and covered with a coverslip such that no excess liquid flowed from beneath it. Thus a standardized pressure, as a result of the weight of the cover slip partially flattened the worms. The trunk length from the base of the proboscis to the posterior end was measured using an ocular

micrometer (magnification x40). Worms were often non-linear in shape so the length was estimated by following the mid-line of the worm and pivoting the ocular micrometer at the points where the worm curved. Every worm recovered from fish samples from Loch Maragan after July 1986 was measured in this way (Total = 4711). In addition, some worms were also measured after having been frozen in the host gut (April, May and June 1988 samples).

5.3.2.1.2. *Assessment of sex and state of maturity of the worms*

The sex and state of maturity of *N.rutili* was recorded as follows:

m = males

f1 = females with unfragmented ovarian tissue

f2 = females with free ovaries (ovarian balls)

f3 = females with ovaries and shelled acanthors (either immature or mature)

These abbreviations are used throughout in subsequent text, tables and figures. When present, copulatory caps on worms were recorded.

5.3.2.1.3. *Estimation of the dry weight of the worms*

To ascertain whether trunk length was a reliable indicator of worm biomass, 48 worms (25 m, 4 f2 and 19 f3), collected fresh from a number of trout hosts in July and August 1988, were measured and then freeze-dried for 24 h. Individual dry weights were measured in mg (accuracy to 4 decimal places).

5.3.3. Data analysis

In all statistical tests, the significance level was set at $P<0.05$ and Student's t-test is abbreviated to t-test. Where necessary an F-test was carried out to compare the variances of the 2 samples.

All t and z tests were unpaired 2-way tests. In 2 x 2 X^2 tests the Yates' correction factor was applied.

5.3.3.1. *Infection parameters*

5.3.3.1.1. *Worm size*

For a comparison of the trunk lengths of worms of different sex and developmental stage from Loch Maragan brown trout, all worms occurring in age class 2 trout from January, May, August and November 1987 (27 fish: 383 worms), which had been measured in a fresh condition, were utilized in the analysis. The mean lengths of 14 f1, 49 f2, 167 f3 and 153 m worms were compared by means of t-tests on transformed length data ($y = \log_{10} x$). The standard error of the mean for each worm class was also calculated. The relationship between trunk length and dry weight of worms was calculated as the least squares regression line for each group of worms separately. The correlation coefficient was also calculated.

5.3.3.1.2. *Dispersion pattern of worms amongst hosts*

Using the intensity of infection data for all 226 brown trout caught in Loch Maragan, the mean intensity of infection, the total number of worms and the variance to mean ratio were calculated. The value of k was calculated by the maximum likelihood method as described by Elliot (1977b) and the goodness of fit with the negative binomial distribution was examined by means of a X^2 test on the first 155 frequency values (i.e. d.f = 154).

5.3.3.2. *Temporal variation in prevalence and intensity of the* Neoechinorhynchus rutili *infection in brown trout at Loch Maragan*

For each month, the total numbers of worms of each sex and stage and the sum of worm trunk lengths in each group was calculated and the

prevalence, mean intensity (+/- s.e.) and the female to male ratio for the metapopulation of worms per month were determined. Variance to mean ratios were calculated to provide a rough measure of the degree of overdispersion of the worms in their hosts in each monthly sample.

For seasonal comparisons, the year was divided into 4 periods: winter (Jan, Feb, Mar = W); spring (Apr, May, Jun = Sp); summer (Jul, Aug, Sep = Su); autumn (Oct, Nov, Dec = A). The designation of season was based on the winter period when the loch was ice covered. The % prevalence and mean intensity of infection (+/- s.e.) was calculated for each season. Percentage prevalence differences between seasons in different years were compared by means of either X^2 or Fisher Exact Probability tests. No comparison was made between the Su seasons of 1987 and 1988 because the values were 100% in both years. The mean intensity of infection was compared by means of either t-tests performed on transformed data, where comparisons between only 2 years of data were required or by a Model II 1-way ANOVA in the case where 3 years of data were available for comparison. When no significant difference was found, data for seasons from different years were combined and used for comparisons between seasons. If the difference was significant a t-test was performed on the total length of host data to determine if the difference could be attributed to the nature of the fish sample.

Pairwise t-tests (on transformed data: $y = \log_{10} [x + 1]$) between seasons were carried out to determine when the mean intensities were significantly different. The female to male ratio between years and between the same seasons in different years was tested by means of a 2x2 X^2 contingency test. Since no significant differences were found in these analyses the significance of any difference in ratios between adjacent seasons was tested using the same test.

For the analysis of changes or apparent growth in worm trunk length, data from 1987 was used (because some of the 1988 material had been frozen and therefore may not have been directly comparable). Thus 1660 worms from monthly samples (Jan 3, Apr 468, May 481, Jun 232, Jul 133, Aug 263, Sep 5, Oct 55 and Nov 20) were used in the analysis. The mean +/- s.e. of trunk length for each worm class for each month was calculated. In order to examine any apparent 'growth' trends these data were combined into seasons and the mean lengths were compared by means of a t or z test, depending on sample sizes, on transformed data ($y = \log_{10} x$).

5.3.3.3. *Intestinal distribution of* Neoechinorhynchus rutili *in its host*

The intestinal distribution of 3893 worms from trout caught between November 1986 and August 1988 was investigated. This excluded the sample of 755 worms from the 36 fish caught over an extended period between January and April 1987 because the net was trapped in ice. Their overall distribution, from all seasons was expressed as the percentage occurring in each of the 4 anatomically distinct regions of the gut. The single worm recovered in the stomach was not included in any further analysis because it was probably not established and had just been released from a prey item. *Neoechinorhynchus rutili* was rarely found in the pyloric caeca so no separate analysis of this distribution was made.

Differences between the distribution in the duodenum, ileum and rectum between seasons, within years were compared by means of X^2 tests. In order to express numerically the distribution pattern in each season the 'mean gut index' was calculated by multiplying the worm number in each region (for the entire sample of hosts) by a factor of 1 for duodenal worms, 2 for ileal and 3 for rectal. These

numbers were arbitrarily chosen and did not intend to reflect the relative lengths of these gut regions in the fish. The % representation of the *N.rutili* metapopulation in each gut region, during each season was also calculated.

Seasonal differences in the distribution of male and female worms were compared by means of X^2 tests (only when no more than 20% of the cells of the contingency table had values <5). The percentage values in each gut region for males and female worms in each season were also calculated. Separate X^2 tests were carried out to compare female (f1,2 and f3) and male distributions between seasons and for f1,2 and f3 within seasons. Here f1 and f2 worms were treated together since both stages were found in *Sialis lutaria* larvae which presumably was the origin of these worms in the trout.

The effect of intensity of infection on worm distribution was considered utilizing the spring data of 1987 from all hosts in the age 2 year class. Thus 588 worms from 16 hosts were included in the analysis. The boundary between light and heavy intensities of infection was set at 50 worms per host and the distribution was compared by means of a X^2 test. A similar test was carried out on summer 1987 data which consisted of 271 worms from 23 hosts, but the intensity boundary was set at 20.

To examine the effects of worm density on worm length the same spring 1987 data was used and the mean worm trunk lengths were compared within worm classes by means of t and z tests on transformed data ($y = \log_{10} x$). The possibility of effects on worm fecundity is examined in Chapter 6.

5.3.3.4. *Effect of host sex on prevalence and intensity of* Neoechinorhynchus rutili *infection*

Statistical comparisons were made between each age class separately. Percentage prevalences between sexes were compared by

means of a X^2 test (2 x 2 contingency table) or by the Fisher Exact Probability test when the conditions of the X^2 analysis were not met (see Pickering & Christie, 1980). Since sufficient data was available for year classes 2 and 3, seasonal comparisons between the sexes were made. In this case, the seasons adopted were those described by Thomas (1964a) to allow for comparison with his results. The seasons were January to March, April to September and October to December. Mean intensities of infection between the sexes within age classes were compared by means of a t-test on transformed data (y = log (x + 1)).

5.3.3.5. *Effect of host age on the prevalence and intensity of* Neoechinorhynchus rutili *infection*

As it was established that there was no significant difference between % prevalence values of *N.rutili* in either male or female fish, all data were combined (see section 5.4.1.5). Fisher exact probability or 2 x 2 X^2 contingency tests were used to compare percentage prevalences between age classes.

Mean intensities of infection of different age classes were compared by means of either t-tests on transformed data (y = log x + 1) when an F test indicated that there was no significant difference between the variances of the samples. When either sample had more than 25 data points a z test was performed (Fowler & Cohen, 1987). The standard errors of the means for each age class and a correlation coefficient between mean intensity and age class, up to age 4, were calculated.

5.3.3.6. *Effect on host fish of* Neoechinorhynchus rutili *infection*

During dissections of fish guts any changes of the gut appearance, likely to be associated with the presence of *N.rutili*, was noted. The regression line (by least squares) and the correlation

coefficient was calculated between the condition factor and intensity of infection for all 226 trout. In addition, the correlation coefficients for the same relationship for only immature trout were calculated to remove any confounding effects of reproductive products or the stress associated with reproduction (method after Valtonen, 1980a).

5.3.3.7. *Estimation of the size of the* Neoechinorhynchus rutili *metapopulation in Loch Maragan trout*

The attempt to estimate the population structure of the brown trout in Loch Maragan provided the opportunity to estimate the *N.rutili* metapopulation size. The maximum worm numbers were estimated by considering the spring data and the numbers of reproducing females using the summer 1987 data. Estimates were made by multiplying the mean intensity for each age class by the corresponding host subpopulation count (max. values). Worms inhabiting age class 4 and older trout were excluded from the estimation because they probably represented only a small proportion of the host population.

5.3.3.8. Neoechinorhynchus rutili *in other definitive host species in Scotland*

For each sample of fish, the range and mean intensity of infection was noted and the female to male ratio calculated for comparison with the Loch Maragan data. The presence of gravid f3 *N.rutili* and parasite species, other than *N.rutili* was also recorded.

5.4. OBSERVATIONS

5.4.1. *Neoechinorhynchus rutili* in Loch Maragan brown trout

5.4.1.1. *Worm size*

5.4.1.1.1. *Trunk length*

The range of worm trunk lengths of the 1987 sample of 383 worms is shown on Table 5.2. All pairwise combinations for the comparison of mean trunk length between worm classes were significant ($P<0.05$) apart from f1 x m for which $P = 0.123$, 165 d.f.

Table 5.2: Trunk lengths of *Neoechinorhynchus rutili* from Loch Maragan brown trout

Sex	Developmental stage	Trunk length um Range	Mean +/- s.e.
f	1	1024-3520	2070.9 183.4
f	2	1088-7040	3341.1 174.3
f	3	1920-9344	5104.9 134.5
f	all	1024-9344	4544.4 122.3
m	all	800-6240	2507.9 76.3

5.4.1.1.2. *Biomass*

There were strong correlations between the values for trunk length and dry weight for different stages and sexes of *N.rutili* indicating that trunk length was a good measure of worm size (Table 5.3).

Table 5.3: Regression equations for trunk length v dry weight of *Neoechinorhynchus rutili*

n	Sex/ Dev. stage	Regression equation	Correlation coefficient
25	m all	$Y = -4.30 \times 10^{-2} + 3.24 \times 10^{-5} X$	r = 0.815
19	f3	$Y = -6.09 \times 10^{-2} + 5.59 \times 10^{-5} X$	r = 0.730
4	f2	$Y = -9.88 \times 10^{-2} + 4.49 \times 10^{-5} X$	r = 0.975

Y = dry weight mg, X = trunk length um

5.4.1.2. *Dispersion pattern of worms amongst hosts*

The distribution of numbers of *N.rutili* in Loch Maragan brown trout was observed to be overdispersed where the variance to mean ratio was 55.83 and k = 0.7893 (see Fig. 5.1). The expected values of the negative binomial distribution are superimposed upon the observed frequencies. A X^2 value of 166.93, P<0.05 d.f. 154 indicates a good agreement of the observed values with this theoretical distribution. The relatively low value of k indicates that the degree of overdispersion is relatively great as indicated by the range of infection intensities from 0 to 324 in these fish.

5.4.1.3. *Temporal variation in prevalence and intensity of the* Neoechinorhynchus rutili *infection in brown trout at Loch Maragan*

Established male and female *N.rutili* were found in Loch Maragan brown trout in all of the sampling months. A total of 4858 worms were collected from 226 trout, with individual fish harbouring between 1 and 324 worms in the gut. Overall 87.6% (198/226) of the trout were infected. Of these 3977 were measured and the 126 worms from July 1986 and the 755 worms from Jan-Mar 1987 were not measured. The prevalence

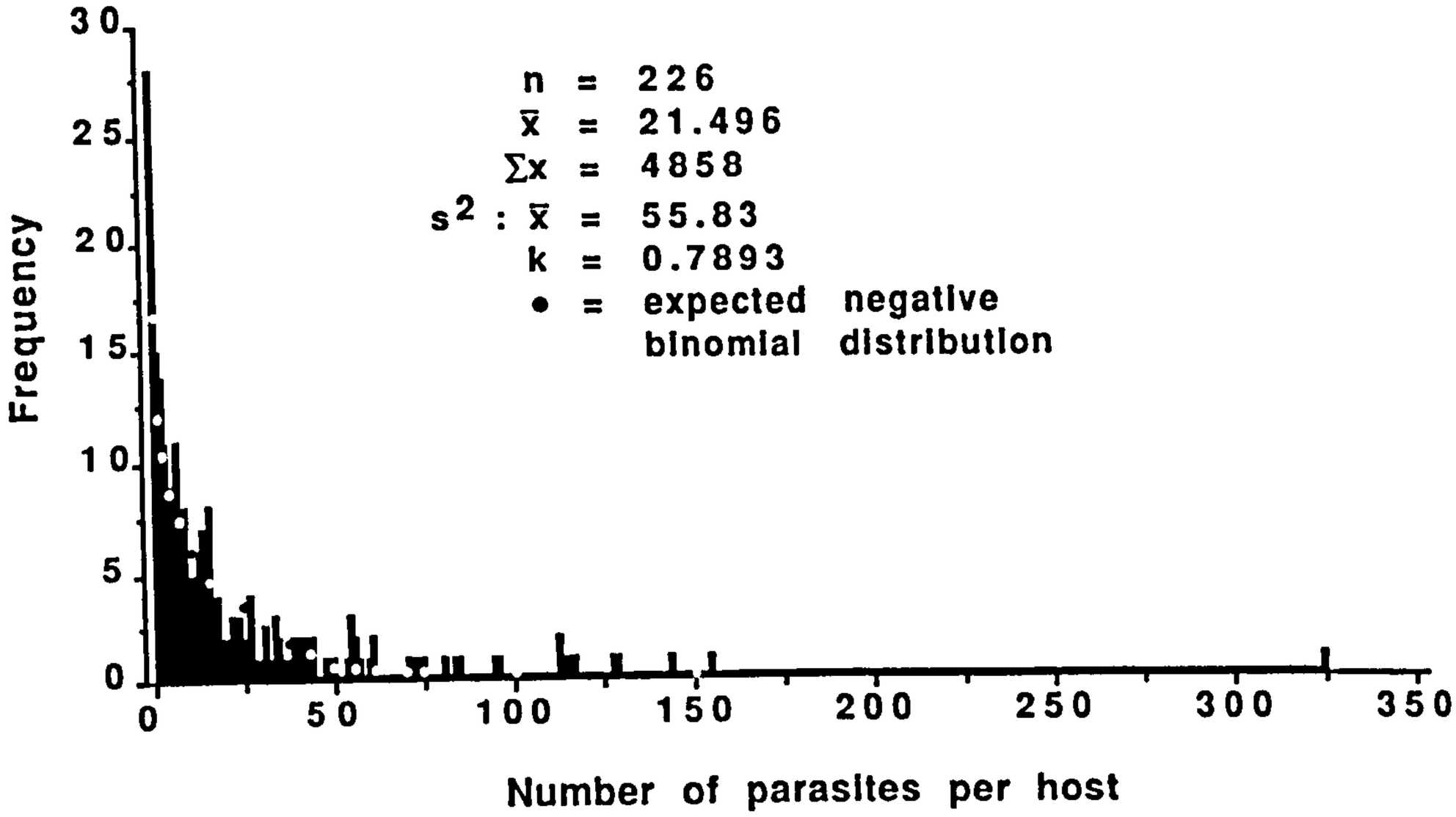


Fig 5.1 Frequency distribution of *Neoechinorhynchus rutili* in Loch Maragan brown trout

and mean intensity of infection in each monthly sample is shown in Table 5.4 and Fig. 5.2 (The values for the fish caught in the net over a 3 month period are ommitted: n=190). The prevalence of infection never fell below 50% (Nov 1986) and was 100% in 10 of the sampling months. Despite a lack of data for the winter months it appears that there was a seasonal cycle in prevalence, with prevalence values at 100% as early as January and staying high throughout the spring months and early summer until July and then dropping down to lower values from August to November. This pattern was observed for 2 consecutive years, 1986 and 1987 and the beginning of this pattern again in 1988. In order to incorporate the 3 month collection (Jan-Apr 1987) the entire data set for 226 fish was considered in 3 month blocks as described in section 5.3.3.2. These 'seasons' were chosen to correspond with major temperature changes in the environment. Taking sample size and structural differences into account there was a remarkable similarity between seasons from different years (Fig. 5.3). The X^2 tests indicated that these observed differences between seasons were not significant.

There also appears to be a seasonal cycle in mean intensity of infection which approximately follows the percentage prevalence pattern (Figs 5.2 and 5.3). In January the intensity was observed to be fairly low and increased during the first months of the year until highest intensities were observed in May 1987 and June 1988 with a steady drop off to lower intensities in August and September. Winter levels dropped off towards zero but worms were still present. Although the seasonal data showed remarkable similarities in intensities of infection between years significant differences were found between years for the Sp ($t = 12.39$, 45 d.f.) and W samples ($t = 4.22$, 40 d.f.). Accordingly, t-tests were performed on uncombined seasonal

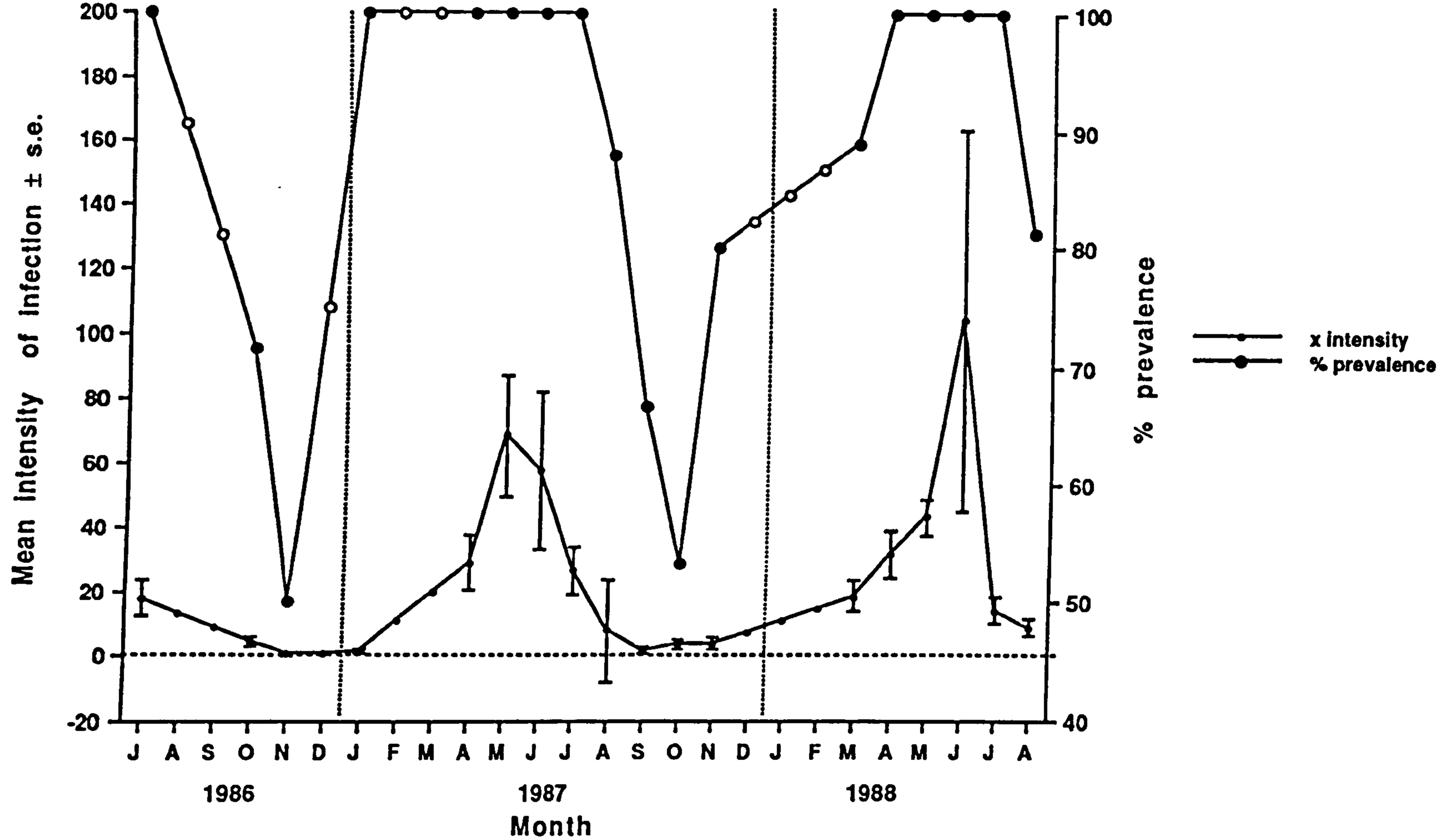


Fig 5.2 Monthly changes in prevalence and intensity of *Neoechinorhynchus rutili* infections of brown trout from Loch Maragan

Note: Where data was unavailable for some months direct extrapolation between points was made. These months are indicated by the open circles on the prevalence plot.

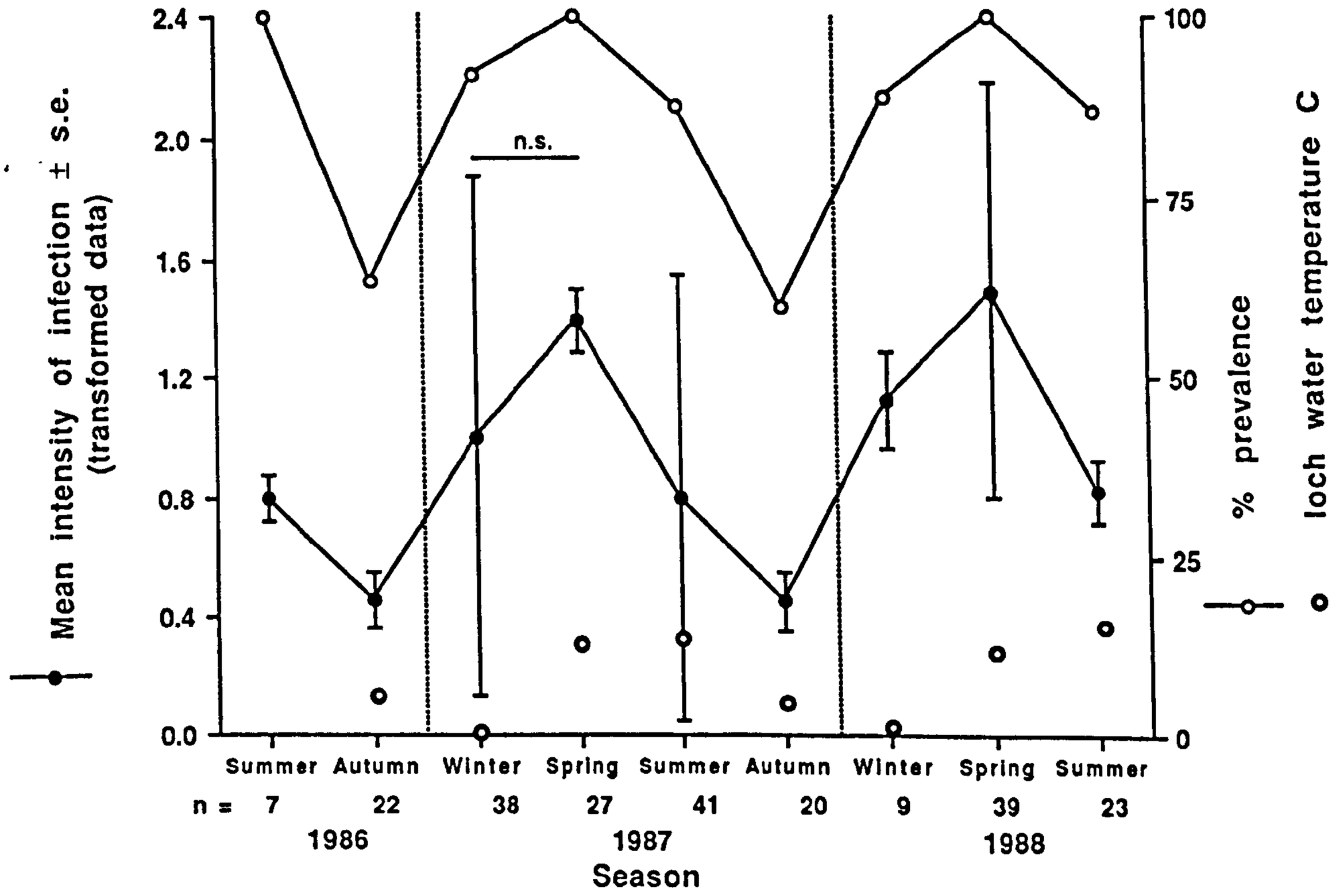


Fig 5.3 Seasonal changes in a. prevalence and intensity of *Neoechinorhynchus rutili* infections of brown trout b. water temperatures in Loch Maragan

Note: n.s. = no significant differences between means (t = 0.284, 63 d.f.). Mean temperature values for each season shown.

transformed data. The differences between means of adjacent months were all significant apart from between W and Sp 1987. This may be explained in part by the fact that there was a higher proportion of age 3 trout in the W sample which would increase the mean intensity of infection (see section 5.4.1.6).

Table 5.4: Monthly collections of *Neoechinorhynchus rutili* from brown trout

Date	Number of Worms						Fish		
	f1	f2	f3	fa	m	total	% prevalence of infection	Mean intensity	n
1986									
Jul	-	-	-	88	38	126	100.0	18.00	7
Oct	1	19	29	49	35	84	66.7	4.67	18
Nov	0	2	0	2	0	2	50.0	0.50	4
1987									
Jan	1	1	0	2	1	3	100.0	1.50	2
Jan-Apr	73	296	4	373	382	755	91.7	20.97	36
Apr	24	193	0	217	251	468	100.0	29.25	16
May	12	55	210	277	204	481	100.0	68.70	7
Jun	26	62	47	135	97	232	100.0	58.00	4
Jul	4	9	79	92	41	133	100.0	26.60	5
Aug	4	36	150	190	73	263	87.9	7.96	33
Sep	0	0	3	3	2	5	66.7	1.67	3
Oct	7	7	23	37	18	55	53.3	3.67	15
Nov	3	6	1	10	10	20	80.0	4.00	5
1988									
Mar	32	53	2	87	82	169	88.9	18.78	9
Apr	42	218	2	262	248	510	100.0	31.88	16
May	60	274	93	427	357	784	100.0	43.55	18
Jun	15	112	143	270	252	522	100.0	104.40	5
Jul	1	6	62	69	30	99	100.0	14.14	7
Aug	0	3	102	105	42	147	81.3	9.19	16
Total	305	1352	950	2695	2163	4858	87.6	21.50	226

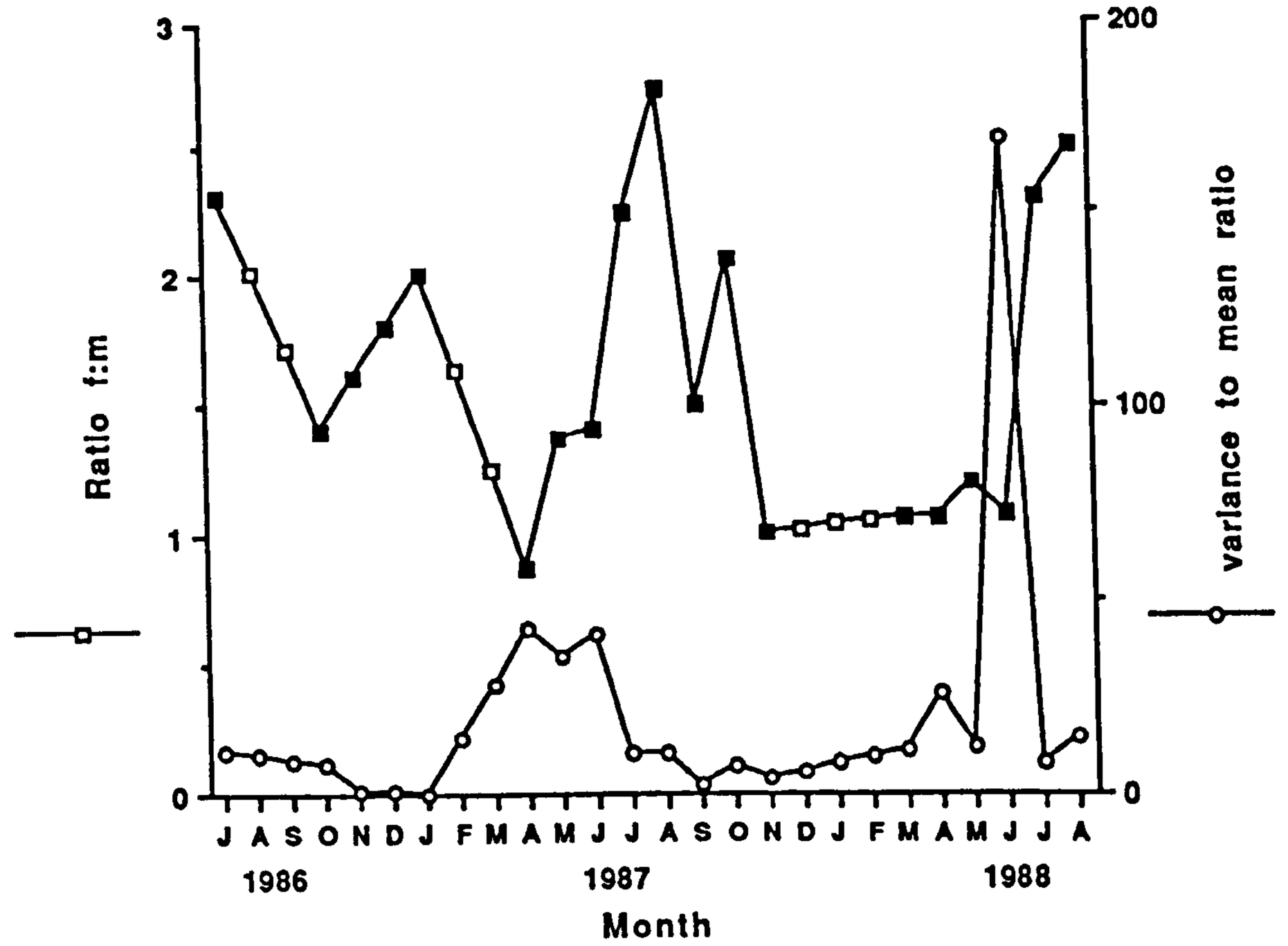
(n = no. of trout in sample)

Table 5.5: Seasonal distribution of *Neoechinorhynchus rutili* in Loch Maragan brown trout

Season	Mean intensity of infection +/- s.e.	% prevalence	No. of *N.rutili* f	m	all	f:m	s^2:x	n
1986								
Summer	18.00 5.35	100.00	88	38	126	2.316	11.11	7
Autumn	3.91 1.24	63.64	51	35	86	1.457	8.67	22
1987								
Winter	19.95 5.11	92.10	375	383	758	0.979	49.79	38
Spring	43.74 8.36	100.00	629	522	1151	1.139	43.16	27
Summer	9.78 1.82	87.80	285	116	401	2.457	14.00	41
Autumn	3.75 1.10	60.00	47	28	75	1.679	6.42	20
1988								
Winter	18.78 4.84	88.89	87	82	169	1.061	11.23	9
Spring	46.56 8.71	100.00	959	857	1816	1.119	63.48	39
Summer	10.70 2.36	86.96	174	72	346	2.417	11.93	23

The changes observed in the variance to mean intensity ratio and the female to male ratio are shown in Table 5.6 and Fig 5.4a for monthly samples and the seasonal data is shown in Table 5.5 and Fig 5.4b. No statistically significant differences in the proportion of female and male worms within seasons between years (X^2 test) was found so data from different sampling years were combined and used to compare seasonal differences. Highly significant differences were found between Sp and Su ($X^2 = 78.35$, $P< 0.001$), Su and Au ($X^2 = 5.65$, $P< 0.05$), A and W ($X^2 = 6.25$, $P< 0.05$) but not between W and Sp ($X^2 = 2.69$, n.s.). The variance to mean ratio remained above unity in all months except November 1986 and January 1987 when the sample sizes were very small. The highest values were found during Sp when the concurrently high values for mean intensity indicated that this was a recruitment period. The high variance to mean ratios infer that the

a.



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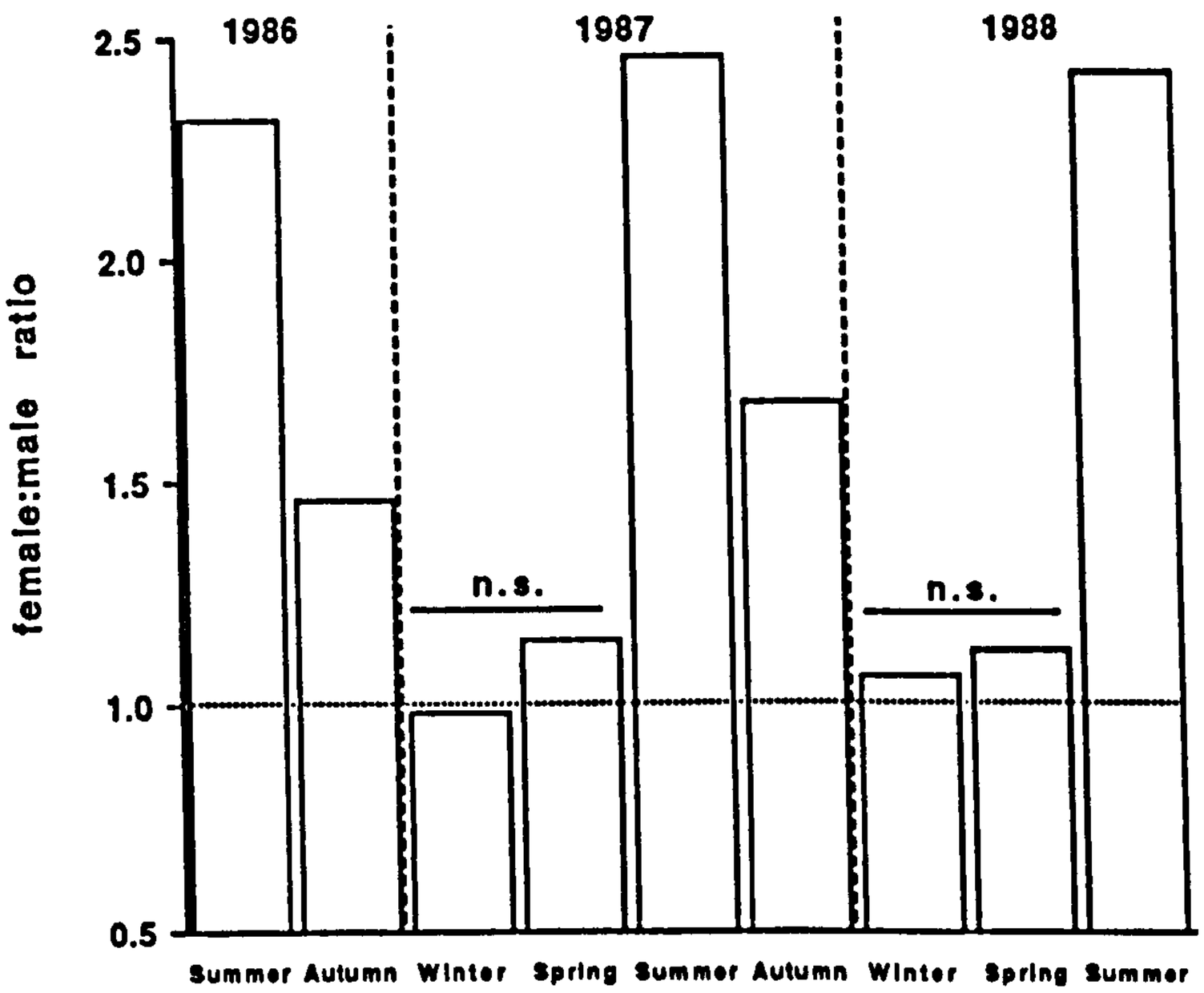


Fig 5.4 Female to male ratios of *Neoechinorhynchus rutili* metapopulations in Loch Maragan brown trout a.monthly b.seasonal

Note: Fig 5.4 a Where data was unavailable for some months direct extrapolation between points was made (indicated by open squares on the f:m ratio plot). Fig 5.4b n.s. = no significant difference between ratios.

recruitment is not random or equal among all members of the trout host population. Later in the year the values are lower suggesting a decreased recruitment rate and a reduction in the size of the range of intensity values.

Table 5.6: Monthly population parameters for *Neoechinorhynchus rutili* in Loch Maragan Brown

Date	Variance to mean intensity ratio	Ratio f:m
Jul 1986	11.111	2.316
Oct 1986	8.247	1.400
Nov 1986	0.667	(all f)
Jan 1987	0.333	2.000
Apr 1987	42.573	0.865
May 1987	35.692	1.358
Jun 1987	41.115	1.392
Jul 1987	10.462	2.244
Aug 1987	10.322	2.725
Sep 1987	2.600	1.500
Oct 1987	7.623	2.056
Nov 1987	4.125	1.000
Mar 1988	11.234	1.061
Apr 1988	26.014	1.056
May 1988	12.334	1.196
Jun 1988	168.623	1.071
Jul 1988	8.354	2.300
Aug 1988	14.371	2.500

The apparent 'growth' of developmental stages of *N.rutili* in 1987 is shown in Fig 5.5. Statistically significant differences were found between mean lengths of f1 worms between Sp and Su ($t = -3.75$, $P = 0.004$, 68 d.f.) and between Sp and A ($t = -2.136$, $P = 0.0362$, 70 d.f.) but not between Su and A ($t = 1.728$, $P = 0.1033$, 216 d.f.). No comparisons with W lengths were made because only 2 worms were found.

A similar pattern was found for f2 worms with no comparisons with W lengths possible because of too few worms. Significant differences were as follows: Sp x Su $t = -9.459$, $P = 0.0001$, d.f. 353; Sp x A t =

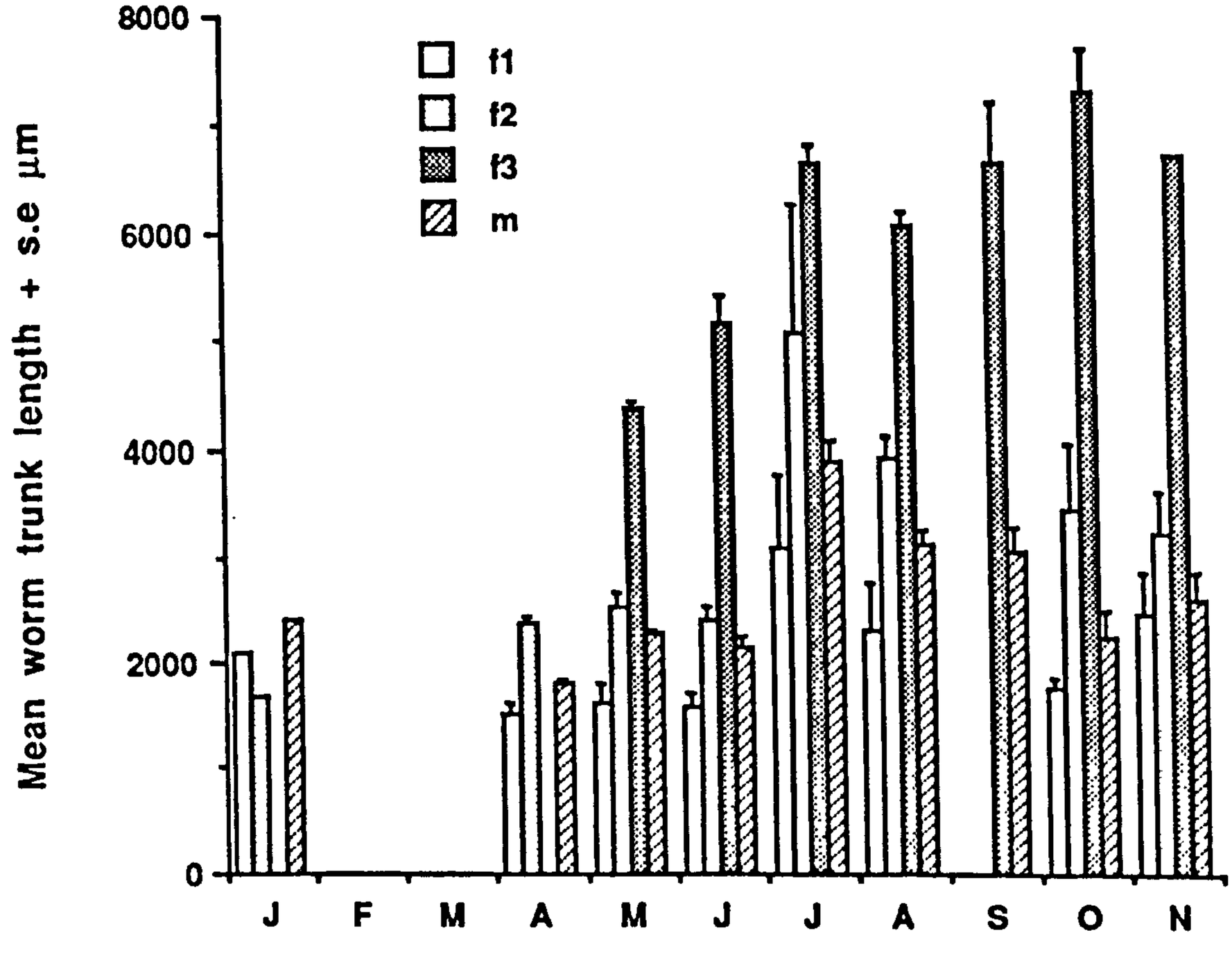


Fig 5.5 Mean trunk lengths of developmental stages of *Neoechinorhynchus rutili* from Loch Maragan brown trout in 1987

-3.684, P = 0.0001, d.f. 320; not significant Su x A t = 1.319, P = 0.1925, 55 d.f.

No winter sample was available for the f3 worms. Results of seasonal comparisons were as follows: Sp x Su t = -12.033, P < 0.01, d.f. 487; Sp x A t = -4.55, P < 0.01, d.f. 280; not significant Su x A t = -0.940, n.s., 256 d.f. No winter sample was available for the male worms. Results of seasonal comparisons were as follows: Sp x Su t = -13.11, P < 0.01, d.f. 666; Sp x A t = 3.948, P < 0.01, d.f. 142; not significant Su x A t = -1.19, n.s., 578 d.f.

5.4.1.4. *Intestinal distribution of* Neoechinorhynchus rutili *in its host*

The overall distribution of *N.rutili* observed in Loch Maragan brown trout suggests that the ileum and rectum are the preferred gut regions for its establishment (Table 5.7). However this is not a static phenomenon. An examination of the monthly and seasonal changes in distribution shows it to be seasonally dynamic (Tables 5.8 and 5.9, Fig 5.6). Chi squared tests of the proportion of worms in each gut region within seasons between years indicated that there was a significant difference in the distributions. Therefore comparisons were made between adjacent seasons, for each season. A significant difference between each pairwise comparison showed that worms dominated in the duodenum and ileum in W and Sp, in the rectum in Su and were distributed almost 50:50 between the duodenum + ileum and the rectum in A. The mean gut indices for W, Sp, Su and A 1987 were 1.5, 2.03, 2.65 and 2.41 respectively. For W, Sp and Su 1988 they were 2.09, 2.04, 2.82 respectively. Although these values give no indication of the overall spread of the distribution throughout the gut it numerically identifies them.

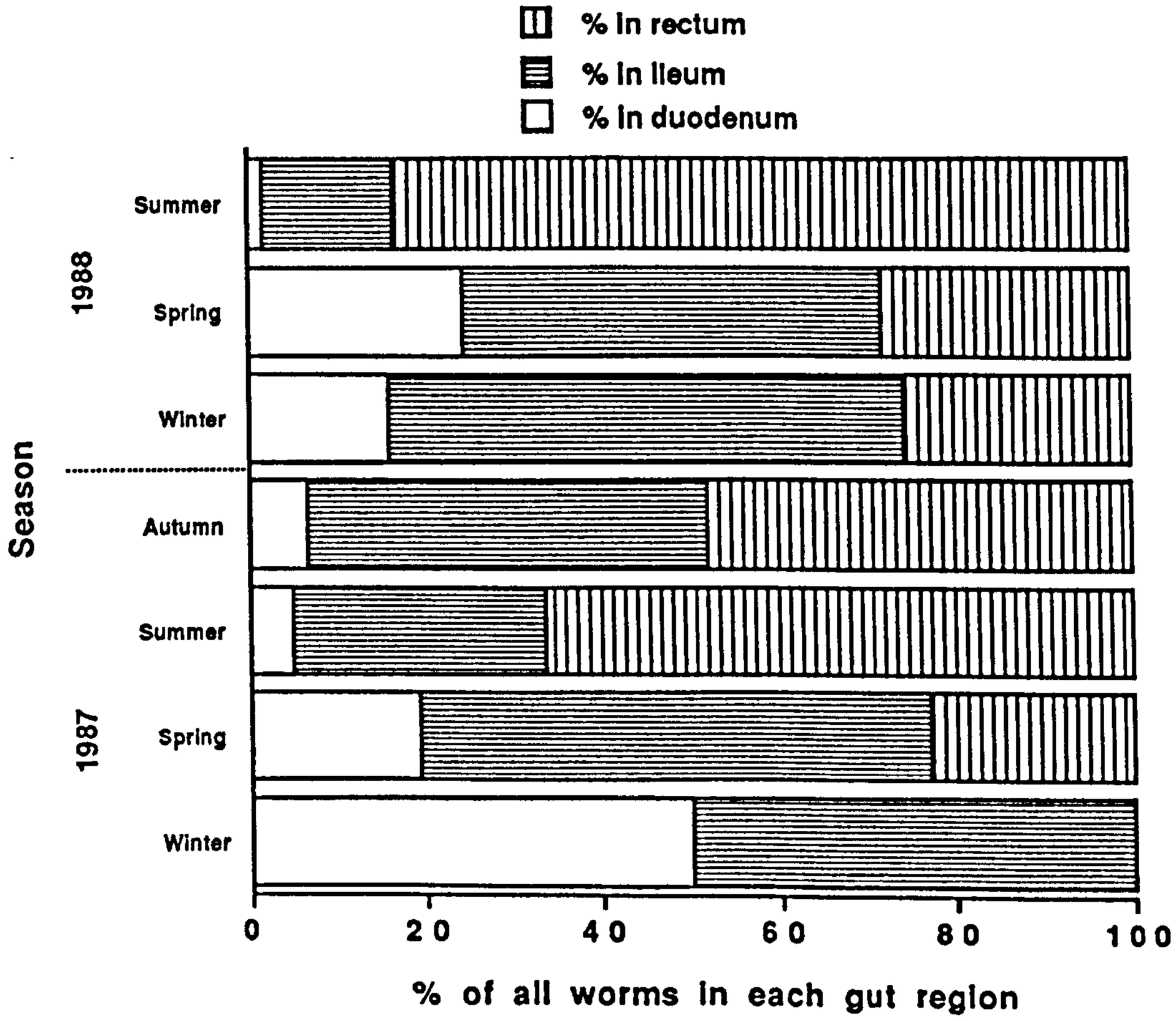


Fig 5.6 Seasonal distribution of *Neoechinorhynchus rutili* in the guts of Loch Maragan brown trout

Table 5.7: Overall distribution of *Neoechinorhynchus rutili* in brown trout guts

Gut region	Number collected	% representation
Stomach	1	0.03
Duodenum	729	18.73
Ileum	1833	47.08
Rectum	1330	34.16
Total	3893	

Table 5.8: Monthly distribution of *Neoechinorhynchus rutili* in the guts of Loch Maragan brown trout

Date	Gut Region											
	Duodenum			Ileum			Rectum			All		
	f	m	a	f	m	a	f	m	a	f	m	a
1986												
Nov	0	0	0	1	0	1	1	0	1	2	0	2
1987												
Jan	0	1	1	1	0	1	0	0	0	1	1	2
Apr	57	69	126	117	146	263	43	36	79	217	251	468
May	19	17	36	190	143	333	68	44	112	277	204	481
Jun	46	20	66	49	38	87	40	39	79	135	97	232
Jul	10	2	12	31	16	47	51	23	74	92	41	133
Aug	7	1	8	46	20	66	137	52	189	190	73	263
Sep	0	0	0	1	0	1	2	2	4	3	2	5
Oct	2	2	4	9	10	19	26	6	32	37	18	55
Nov	1	0	1	6	9	15	3	1	4	10	10	20
1988												
Mar	16	11	27	51	48	99	20	23	43	87	82	169
Apr	86	73	159	93	103	196	83	72	155	262	248	510
May	88	63	151	209	157	366	130	137	267	427	357	784
Jun	65	69	134	158	144	302	47	39	86	270	252	522
Jul	1	0	1	14	4	18	54	26	80	69	30	99
Aug	3	0	3	12	7	19	90	35	125	105	42	147

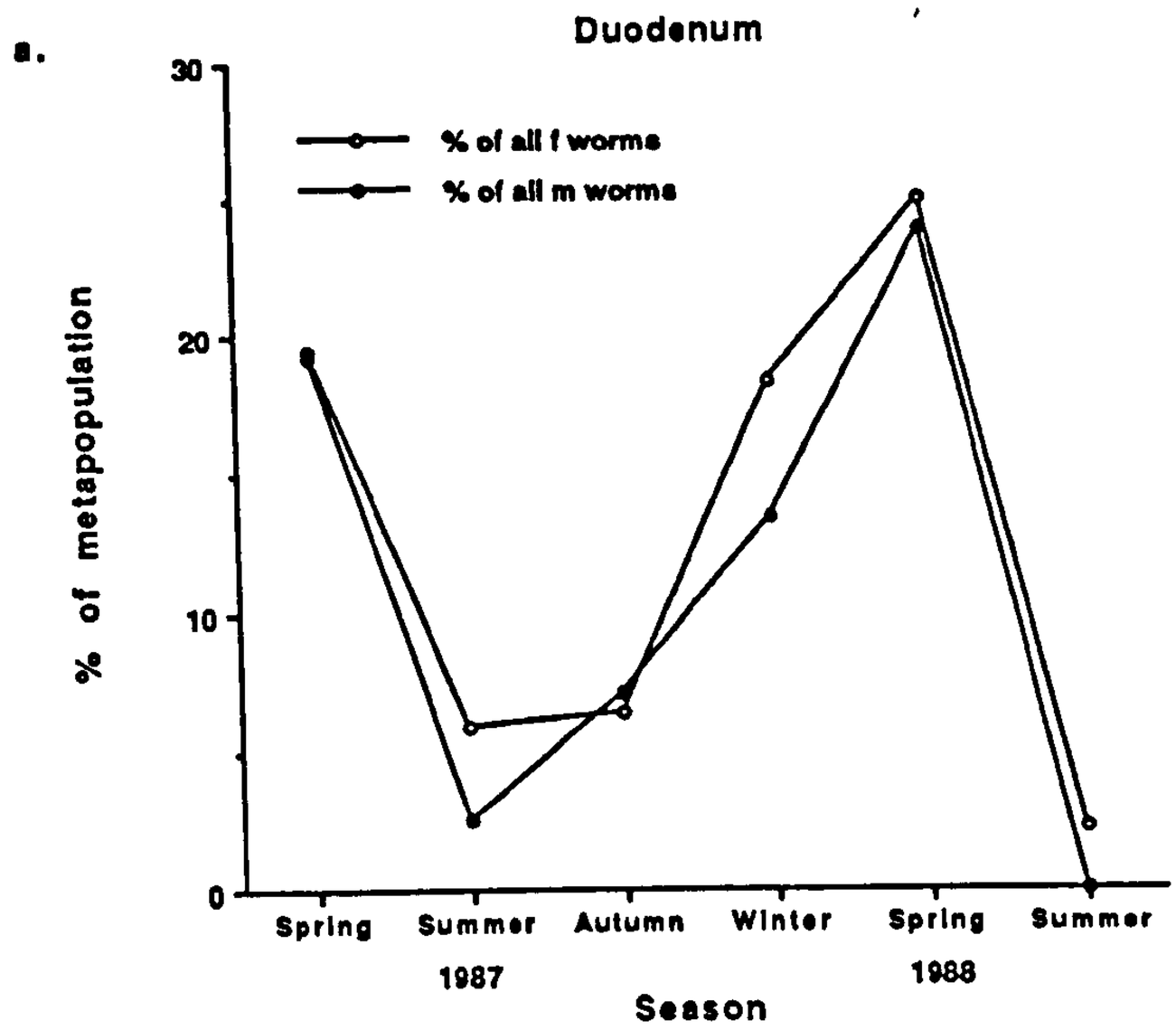
Chi squared tests examining the seasonal distribution of female and male worms indicated that within seasons there was no significant

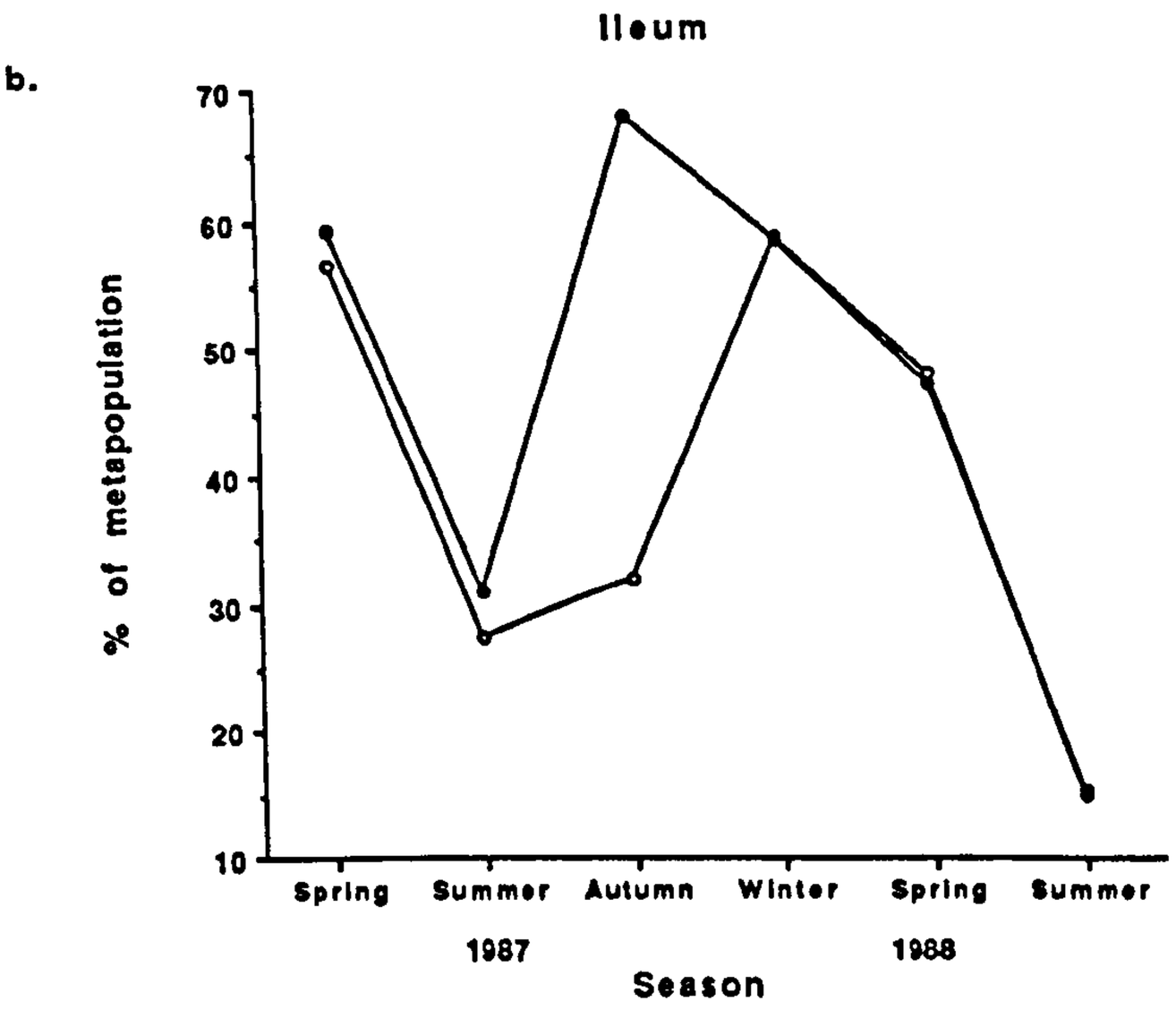
difference in their distribution in Sp 1987 and 1988, Su 1987 and W 1988. Significant differences were found between the distributions in the ileal and rectal regions in Su 1988 (X^2 = 6.08 and 33.60 respectively) and in the rectal region in A 1987 (X^2 = 13.44) and this tends to suggest that there were differential movements or distribution preferences of the sexes at these times (Fig 5.7 a, b, c).

Table 5.9: Seasonal gut distribution of *Neoechinorhynchus rutili*

Season	Duodenum			Ileum			Rectum			All		
	f	m	a	f	m	a	f	m	a	f	m	a
1987												
Winter	0	1	1	1	0	1	0	0	0	1	1	2
Spring	122	106	228	356	327	683	151	119	270	629	552	1181
Summer	17	3	20	78	36	114	190	77	267	285	116	401
Autumn	3	2	5	15	19	34	29	7	36	47	28	75
1988												
Winter	16	11	27	51	48	99	20	23	43	87	82	169
Spring	239	205	444	460	404	864	260	248	508	959	857	1816
Summer	4	0	4	26	11	37	144	61	205	174	72	246

This apparent spatial change in distribution or 'movement' was considered for each sex separately between seasons. For male worms significant differences were observed in the distributions (Fig 5.7d) between Su and A 1987 (X^2 = 13.34, $P < 0.01$); Sp and Su 1987 (X^2 = 95.57, $P < 0.01$); and again between Sp and Su 1988 (X^2 = 55.88, $P < 0.01$). No significant differences were found between the distributions in A 1987 and W 1988, W and Sp 1988 and A 1987 and Sp 1988 indicating that little change in male distribution occurred between A and Sp and that between Sp and Su significant changes were observed. This observation is further substantiated by the mean gut indices for these seasons (Table 5.10).





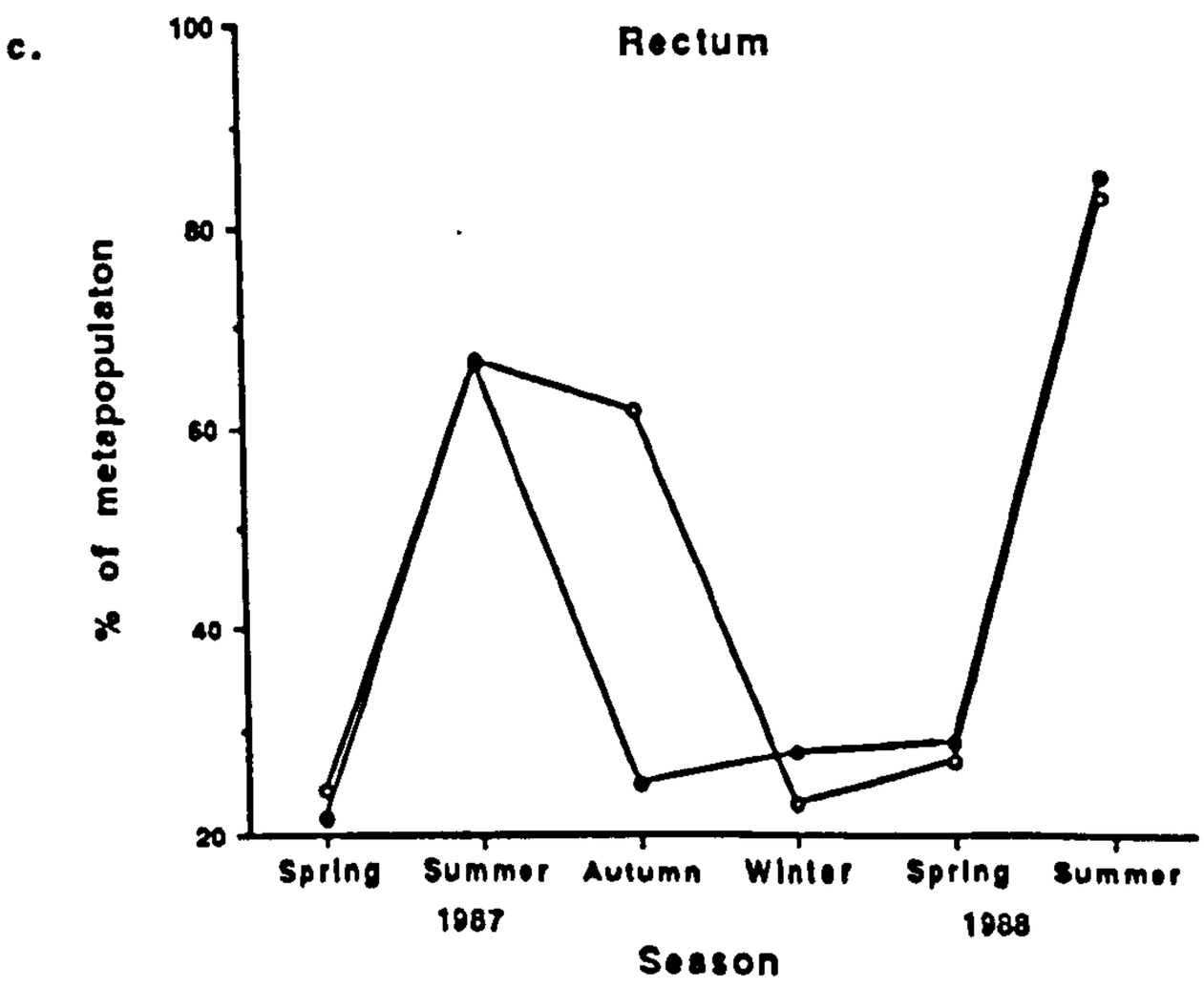
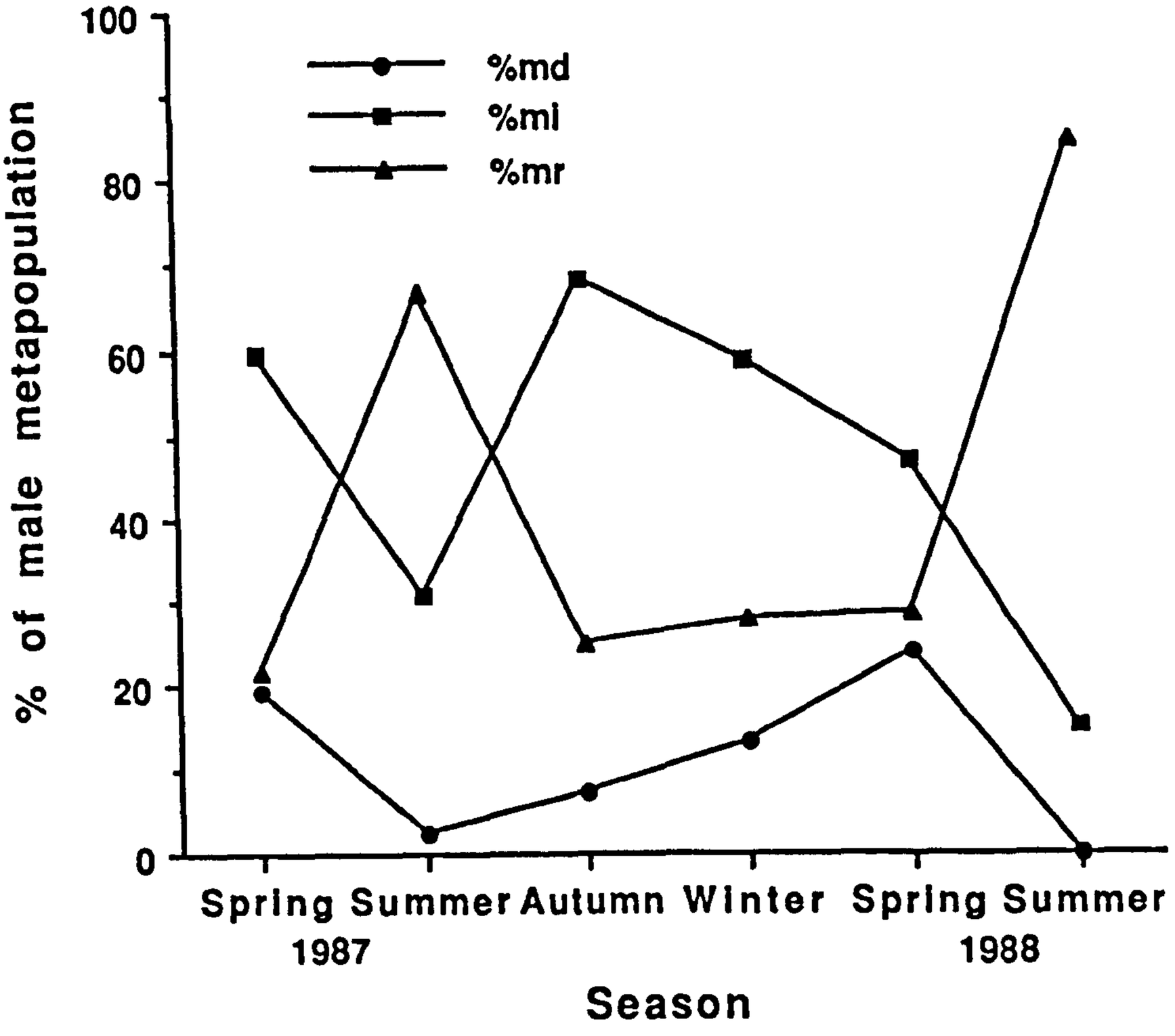


Fig 5.7 Seasonal intestinal distributions of metapopulations of *Neoechinorhynchus rutili* in Loch Maragan brown trout: a. duodenal worms; b. ileal worms, c. rectal worms; d. all male worms; e. all female worms

d.


All male worms
%md
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100
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% of male metapopulation
Spring Summer Autumn Winter Spring Summer
1987
1988
Season

e.

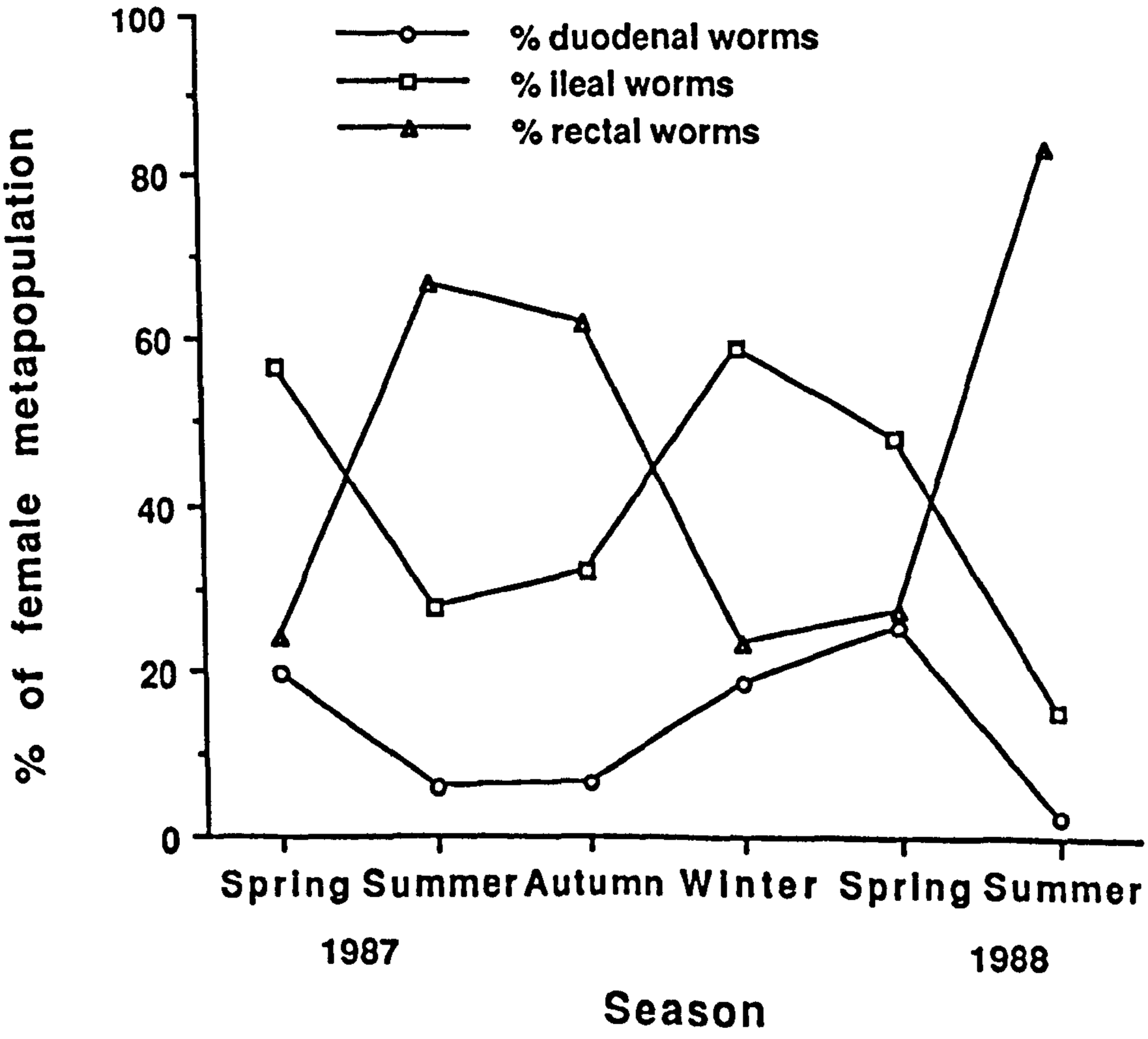

All female worms
% duodenal worms
% ileal worms
% rectal worms
100
80
60
40
20
0
% of female metapopulation
Spring Summer Autumn Winter Spring Summer
1987
1988
Season

Table 5.10: Seasonal gut indices for *Neoechinorhynchus rutili* distribution

Season	Gut Index				
	All worms	f1,2	f3	fall	mall
1987					
Winter	1.50	-	-	0.50	0.50
Spring	2.04	1.86	2.32	2.05	2.02
Summer	2.62	2.42	2.65	2.61	2.63
Autumn	2.41	2.09	3.00	2.55	2.18
1988					
Winter	2.09	2.04	2.50	2.05	2.14
Spring	2.04	1.93	2.31	2.02	2.05
Summer	2.82	2.50	2.82	2.81	2.85

The observed overall seasonal distribution of female worms is shown in Fig 5.7 e and was similar to that of the male worms apart from in A 1987. The distribution of different stages of female was considered within seasons and was found to be statistically different in all seasons (Sp 1987, $X^2 = 84.06$, $P = 0.01$; Su $X^2 = 6.71$, $P = 0.05$). In A 1987 f3 only occurred in the rectum whereas the f1,2 occurred throughout the gut. No tests could be carried out on the W and Sp 1988 data because too few f3 worms were present. However the gut indices for Sp 1988 indicate that f3 adopted a more posterior position. The general conclusion from all comparisons was that the f3 were distributed more posteriorally than the f1,2 within seasons.

Between adjacent seasons, the distribution of f1,2 worms was significantly different apart from between A 1987 and W 1988 ($X^2 = 0.472$). In Sp the worms dominated in the duodenum and ileum (82.8% of worms) and in Su 88.7% of the worms were in the ileum and rectum indicating a posterior movement with season. This movement is also

evident from the mean gut index values (Table 5.10).

For f3 worms no tests could be carried out for Su x A 1987 or W 1988 because too few worms were found. However significant differences were found in the distributions between Sp and Su 1987 (X^2 = 72.41) and the same seasons in 1988 (X^2 = 97.06). This difference is manifested by a posterior movement of the worms in Su. Comparison between the Sp of 1987 and 1988 showed the distribution to be similar but for Su between years a significant difference was found (X^2 = 10.95). This difference may be explained by the fact that the 1988 Su sample did not include individuals from September. Thus the pattern appears to be a stable repeating one from year to year.

The effect of intensity of infection was examined in trout aged 2 from 1987. In Sp 1987 the comparison between the gut distributions of lightly and heavily infected hosts (‹50›) indicated that there was a significant difference between them (X^2 = 21.69). The mean gut indices were 2.22 and 1.95 for lightly and heavily infected hosts repectively indicating a more anterior mean position of worms in heavily infected hosts. This is bourne out by the percentage of worms occurring in the duodenum: 12% in light infections and 21.7% in heavy. This higher proportion of worms in the duodenum would be expected to bring about greater mating success in heavily infected hosts and the percentage representation of f3 worms of 17.69 and 11.14% respectively (X^2 = 17.64, P ‹0.01) in heavily and lightly infected hosts indicated that this was probably the case. Interestingly, the proportion of male worms in the equivalent host groups were 45.48 and 48.69% respectively and this similarity suggests that the males are more successful when in dense populations at finding and inseminating females. These results suggest that in higher intensity infections worms tend to settle in the duodenum despite the fact that in general this does not

seem to be the preferred gut region. This congregation of worms appeared to have the advantage of increasing mating success and indicates an obvious advantage for a worm to be in a heavily infected host.

In Su 1987 the distribution of worms was found to be significantly different between lightly and heavily infected hosts (X^2 = 6.22) and worms had an overall more anterior position in the latter, as confirmed by the mean gut indices (L = 2.68, H = 2.47). This difference may simply be a 'throwback' of the original Sp distribution but may also reflect a lower concentration of nutrients more posterior in the guts of heavily infected hosts which prevents a movement of worms down the gut. The way in which this is manifested is unknown, but it could depend upon chemical stimuli from other worms in the gut or a loss of worms which attempt to establish in more posterior regions already inhabited by individuals i.e. intraspecific competition.

The effects of worm density on worm length were examined in the 1987 Sp data (Table 5.11). Significant differences were found between worm lengths (within sex/stage classes) between heavily and lightly infected hosts for all worms apart from f2. In these cases the worms in the latter type hosts were significantly larger indicating possible density dependent effects upon growth. The lack of difference between f2 mean lengths in the two classes of host can be explained. If we imagine that an f1 arriving in the gut must use nutrients to develop to the f2 stage without growth. In the lightly infected hosts these nutrients will be utilized for development first in preference to growth to accelerate its attainment of reproductive maturity, hence the small size of the worms in these hosts. However in heavily infected hosts, where nutrients could be limiting new f1 worms may not have enough suitable nutrients to develop into f2's (therefore there

is a higher proportion of these individuals in the population) and the f2s already in the population may represent the 'first come first served individuals' which arrived in the gut first and followed the same growth pattern as the f2 worms in the lightly infected hosts.

Table 5.11: Mean lengths of *Neoechinorhynchus rutili* in lightly and heavily infected hosts

Stage/sex	Mean worm trunk lengths +/- s.e. um n low intensity		n high intensity		Test result	P
f1	5	56.00 11.34	31	48.97 2.86	t=4.24	<0.01
f2	51	69.39 3.19	113	70.73 2.53	z=0.08	n.s.
f3	45	153.78 6.94	66	128.00 4.91	z=3.09	<0.01
m	83	64.46 2.57	194	57.36 1.44	z=2.38	<0.05

5.4.1.5. *Effect of host sex on the prevalence and intensity of* Neoechinorhynchus rutili *infections*

No effect of host sex was observed on the prevalence or intensity of infection of *N.rutili* in trout aged from 1 to 3 years (Table 5.12). In trout of age class 4 there were no differences in prevalence values but the mean intensities were significantly different, males being more heavily infected. The samples of age class 4 fish were similar in terms of season of capture (f: Mar x 1; Apr x 3; May x 1; Jul x 1) (m: Mar x 1; Apr x 1; May x 2; Aug x 1) so there appears to be a real difference in the susceptibility of the sexes.

Table 5.12: Percentage prevalence and intensity of *Neoechinorhynchus rutili* infections of male and female brown trout from Loch Maragan

Age	Sex	n	% prevalence	P	x intensity	d.f.	t.	P
1	f	8	75.00	0.40	10.75	14	0.561	>0.05
	m	8	87.50		17.63			
2	f	75	86.67	$x^2 = 0.34$	19.13	31	0.330	>0.05[a]
	m	36	83.33	>0.05	23.89	z = 0.845		>0.05[b]
3	f	32	87.50	0.75	24.59	5	-1.418	>0.05[c]
	m	12	83.33		22.83	30	1.720	>0.05[b]
4	f	6	100.00		17.83	9	-5.429	<0.001
	m	5	100.00		54.60			
5	f	0	-		-			
	m	1	100.00		-			

a = Oct-Dec; b = Apr-Sep; c = Jan-Mar

5.4.1.6. *Effect of host age on prevalence and intensity of* Neoechinorhynchus rutili *infections in brown trout*

No significant difference (Fisher test) was found between the percentage prevalence of year class 1 (81.25%) and year class 4 (100%) trout (P = 0.19) indicating the similarity in all age classes(see Table 5.13 and Fig 5.8). Fish caught between January and April 1987 were not included in this analysis because the prolonged period in the net may have affected the worm numbers. There was a good correlation, r = 0.979 between mean intensity of infection and increasing age, up to age 4 (P<0.01). No significant difference was found between the

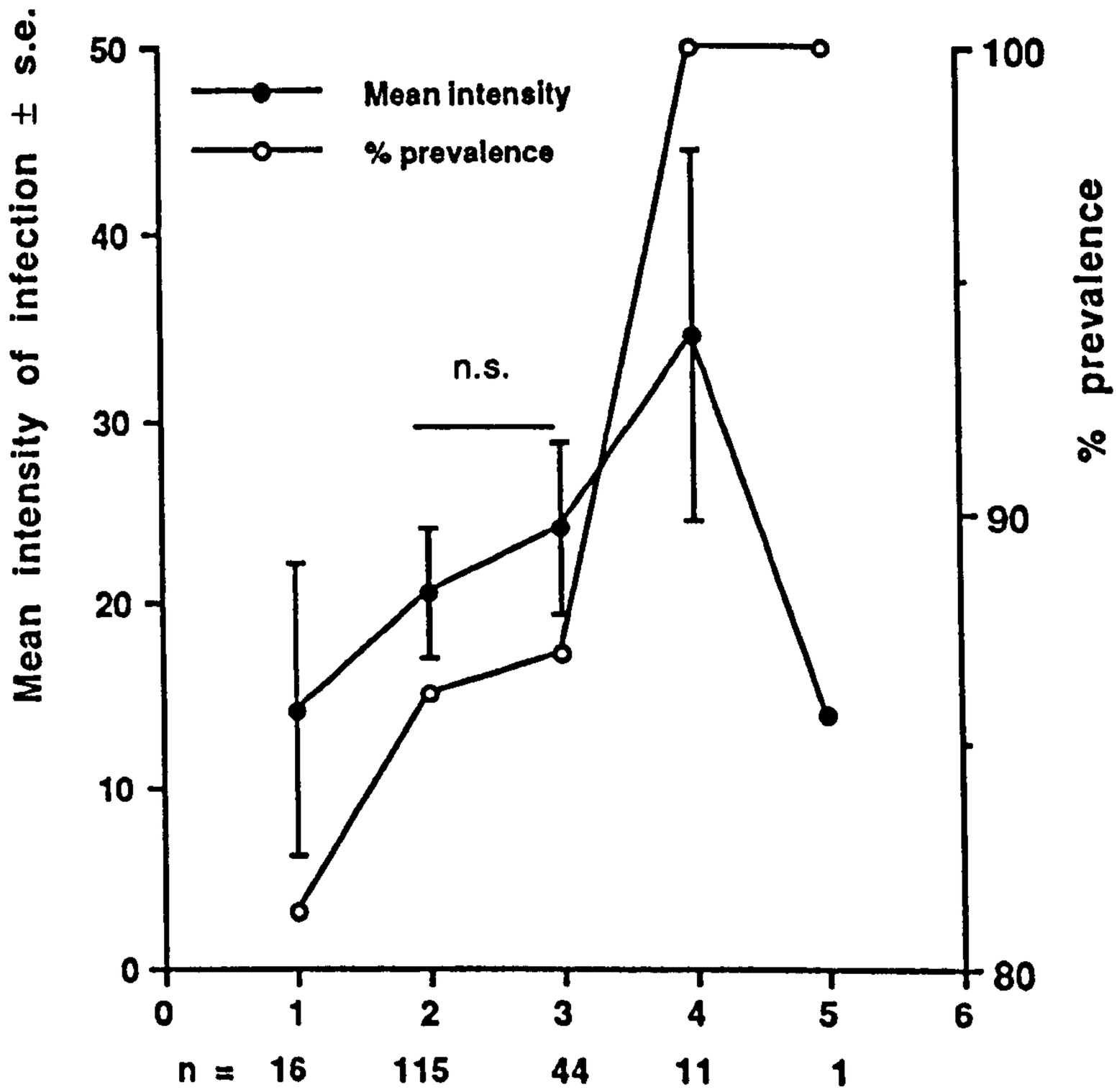


Fig 5.8 Prevalence and mean intensity of *Neoechinorhynchus rutili* infection in different age classes of brown trout from Loch Maragan (sexes combined)

Note: There was no significant difference in % prevalence between age classes (X^2 test).
n.s. = no significant difference between mean intensities of infection (Student's t-test on transformed data).
Mean intensity of infection for Age class 4 ± s.e. = 17.83 ±4.95 (females); 54.60 ±22.30 (males).

intensities of infection of age classes 2 and 3 where there was considerable overlap in the range covered by the standard errors of the means.

Table 5.13: Prevalence and intensity of *Neoechinorhynchus rutili* infection in hosts of different age

Age	n	% prevalence	Intensity x	Intensity s.e.	x	s^2:x	d.f.	t	P
1	16	81.25	14.19	7.96	227	71.52			
							129	2.71	<0.01*
2	115	86.09	20.68	3.48	2378	66.82			
							z = 0.587		>0.05
3	44	86.36	24.11	4.70	1061	40.37			
							53	2.97	<0.01*
4	11	100.00	34.58	10.05	380	41.61			
5	1	100.00	14.00	-	14	-			

(Year 2 x Year 4, 124 d.f., t = 3.95, P<0.0001, * = significant difference).

5.4.1.7. *Effect of* Neoechinorhynchus rutili *infection on host fish*

Occasionally inflammation was observed at the point of attachment of some worms to the host's intestinal mucosa. When large numbers of worms were present in a small gut area the whole tissue appeared reddened. This phenomenon was not examined histologically because of the unsuitability of the material.

No correlation was found between intensity of infection and condition factor = C.F. (r = 0.063). The range of C.F. values in the uninfected fish appeared much greater than for infected fish (see Fig 5.9) and the most heavily infected fish (n = 324) had a relatively high C.F. No statistically significant correlations were found between intensity of infection and condition factor for immature male and female fish although the regression lines had negative slopes. The regression equations were as follows: females (n=122) Y = -2.954X +

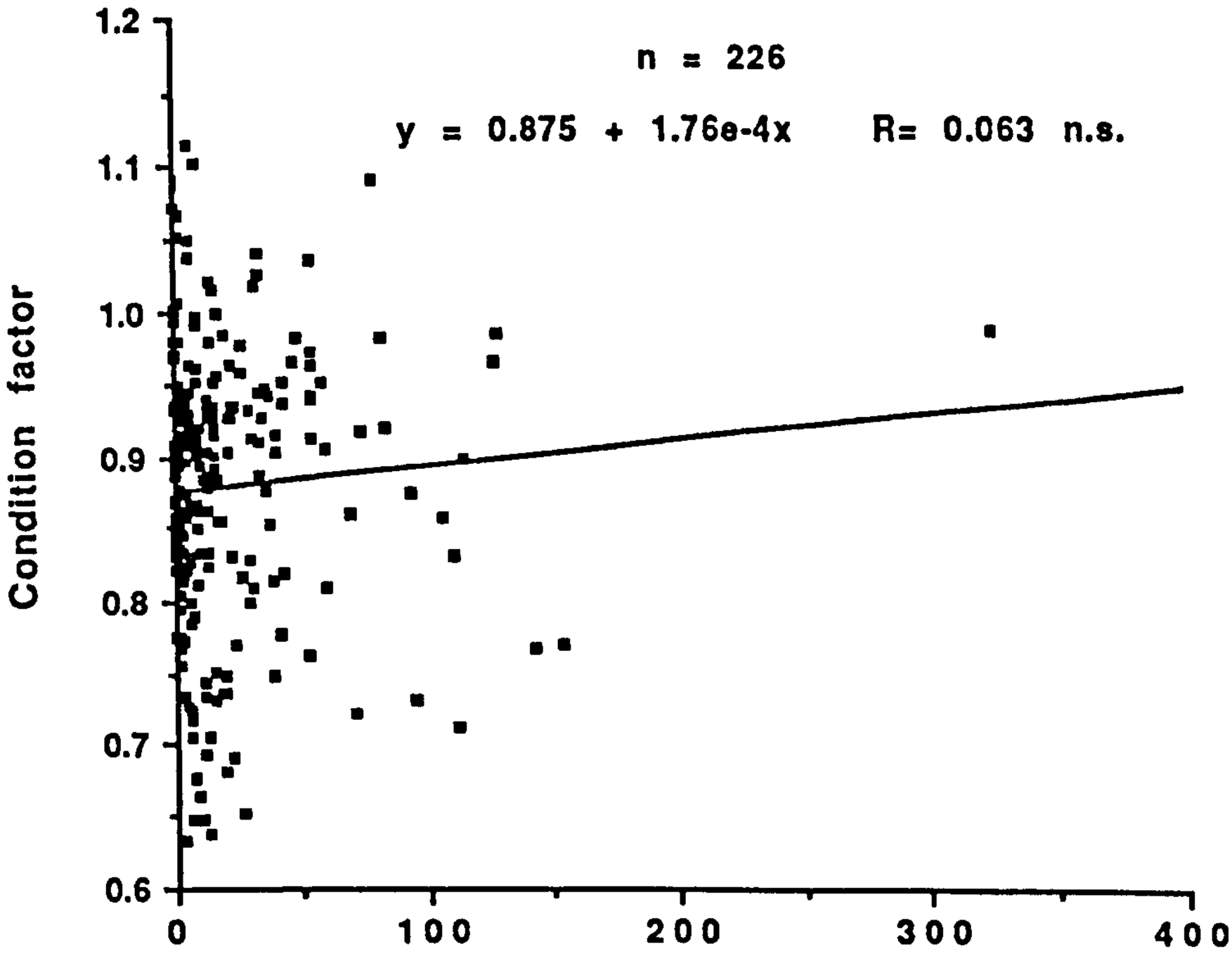


Fig 5.9 Relationship between intensity of *Neoechinorhynchus rutili* infection and condition factor in Loch Maragan brown trout

28.307 r=0.0613; males (n=48) Y = -0.721X + 22.465 r=0.0397. Despite this apparent lack of effect on the trout hosts 2 dead fish were observed lying on the Loch Maragan substrate (3m depth) in June 1987. The light nature of the substrate made it impossible to collect these individuals so any adverse effects of parasites could not be assessed.

5.4.1.8. *Estimate of the Loch Maragan* Neoechinorhynchus rutili *metapopulation size in brown trout*

The maximum estimates +/- s.e. for the entire Sp 1987 metapopulation (trout of year classes 1 to 3) and the f3 metapopulation in Su 1987 are presented in Table 5.14.

Table 5.14: Estimates of *Neoechinorhynchus rutili* metapopulations in brown trout in 1987

Trout age class	Max. population estimate for trout	Metapopulation size +/- s.e. spring (all worms)	summer (f3 only)
1	2,018	5,045 +/- 1,009	2,017 +/- 0
2	501	19,578 +/- 4,650	2,801 +/- 732
3	122	11,531 +/- 1,666	748 +/- 267
Total	2,641	36,154	5,566

5.4.2. Observations on *Neoechinorhynchus rutili* infections at other Scottish sites

5.4.2.1. *Black Clauchrie Burn (Site 5)*

Eleven brown trout caught in August 1986 were found harbouring between 1 and 3 *N.rutili* per host (mean = 1.45), including gravid females. The sex ratio was 7.00. These trout also harboured *Crepidostomum* spp..

5.4.2.2. *Bridge of Weir (Site 6)*

Forty percent of 25 rainbow trout examined in May 1987 were found to be infected with between 1 and 15 *N.rutili* (mean = 1.32). The female to male ratio was 1.54 and gravid f3 worms were recovered in the trout with the highest intensity of infection. *Echinorhynchus truttae* was also seen in these trout. In January 1988 the % prevalence was 81.8% (n = 11) and the range of intensity of infection was 1-891 (mean = 154.45). In the individual infected with 891 worms the lumen of the gut was completely obstructed and worms were also found attached within the pyloric caeca. There was a noticeable degree of redness in the most heavily infected gut regions. This trout was a mature male, 248mm long and 176.96g. *Echinorhynchus truttae* and *Cyathocephalus truncatus* were also found infecting these fish. In February 1988 a single rainbow trout was found harbouring 6 worms and was also feeding upon *Sialis lutaria* larvae.

Two brown trout caught in February 1988 harboured 25 and 36 worms respectively. *Echinorhynchus truttae*, *Crepidostomum* spp. *Cystidicola farionis* were also present in the trout which had been feeding upon *Sialis lutaria* larvae.

Three-spined sticklebacks caught at this site were also infected with *N.rutili*. In February 1988 34.5% (n=29) were infected with 1 to 4 worms per individual (mean = 0.79). f1, f2 and worms were recovered and the female to male ratio was 1.636. Fish in the 51-60mm length class had the highest prevalence of infection. The tapeworm *Proteocephalus filicolis* was also present in the guts of some fish.

In March 1988 the % prevalence of infection was 42% (n=7) with between 1 and 4 worms per fish (mean = 0.86). The female to male ratio was 1.5.

5.4.2.3. *Carbeth Loch (Site 8)*

Two brown trout caught in May 1988 had 25 and 44 *N.rutili* in their guts (mean = 34.5) respectively and the sex ratio was 5.27. Gravid female worms were present and represented 86% of the female population.

5.4.2.4. *Drumore Loch (Site 11)*

Gravid female worms were recovered in October, November and December 1987 and further details of the *N.rutili* infection in sticklebacks are given in Chapter 8.

5.4.2.5. *Lochan Creag nan Caorann (Site 18)*

Brown trout were found to be infected with both *N.rutili* and *Crepidostomum* spp. In August 1986, the range and mean intensity of infection were 1-119 and 29.0 (n=6) respectively and the female to male ratio was 2.00. In July 1987 and 1988 the range and mean intensity of infection were 1-6 and 1-34, and 1.57 (n=7) and 15.5 (n=4) respectively. The sex ratio in July 1988 was 1.70 with f3 worms representing 69% of the female population.

5.4.2.6. *Lochan Duin (Site 19)*

A single rainbow trout caught in July 1987 was found to harbour 56 *N.rutili*.

5.4.2.7. *Loch Essan (Site 23)*

The prevalence, range, mean intensity and sex ratio of *N.rutili* infections of brown trout in October 1986 and June 1987 were 50% and 100%, 0-1 (n=4) and 1-26 (n=6), 0.5 and 13.33 and 1:1 and 0.95 respectively.

5.4.2.8. *Loch Monzievaird (Site 30)*

Only 1 brown trout caught in March 1987 was infected with *N.rutili*

(3 worms, 5 fish examined). Other parasites found in the brown trout included *Echinorhynchus truttae*, *Crepidostomum* spp., and *Eubothrium crassum*. A three-spined stickleback caught at the same time had 1 *N.rutili* specimen in the gut.

5.4.2.9. *Loch Oss (Site 31)*

Two brown trout caught in July 1987 had 111 and 168 *N.rutili* in their guts (mean = 139.5) and also harboured *Crepidostomum* spp.. These trout had relatively low condition factors of 0.67 and 0.60 respectively.

5.4.2.10. *Powder Works Dam Lochan (Site 35)*

Gravid female worms were present in June 1986 and the female to male ratio was 1.58. Further details of *N.rutili* infections in brown trout are given in Chapter 4.

5.4.2.11. *River Doon Trout company, Dalrymple (Site 40)*

Only 1 rainbow trout out of 18 examined in May 1987 was found harbouring a single f2 *N.rutili* specimen.

5.5. DISCUSSION

5.5.1. The size of *Neoechinorhynchus rutili* from Loch Maragan brown trout

Neoechinorhynchus rutili from Loch Maragan brown trout exhibited the typical acanthocephalan feature as described by Parshad & Crompton (1981) of sexual dimorphism in terms of size, female worms being longer than males. Although the mean lengths of the 2 sexes were statistically different there was a marked overlap in the range of worm trunk lengths. Despite the fact that these measurements were made on fresh worms, the size ranges were similar to those described by Meyer (1932), Van Cleave & Lynch (1950), Bykhovskaya *et al.* (1962) and

Yamaguti (1963). The similarity in the mean lengths of f1 and m worms and the overall larger size attained by f2 and f3 worms suggests that the growth of worms in the trout host is not equal among the sexes. A similar observation was made by Crompton (1974) for *Moniliformis dubius* (=*M.moniliformis*) infecting rats, where the females grew more than the males, regardless of whether insemination had occurred or not. For *N.rutili* and other acanthocephalans this may be an indication that the optimal size for reproduction in the sexes is different and that females have a requirement to be larger in order to produce the maximum number of acanthors in her lifetime. More simply, Valtonen (1980b) suggested that males be smaller because of their shorter lifespan.

The fact that fresh trunk length appears to be a reliable measure of biomass (as dry weight) is a useful tool for investigations of *N.rutili* population dynamics since the measurement of the former is quick and easy and the worm is subsequently in a state suitable for other histological purposes. The regression equations indicated that for a given increase in trunk length, males showed the lower and f3 females the higher apparent rate of increase in dry weight. This difference could be dependent upon the differential densities of body components or a genuine difference in growth rate between the different classes of worm.

The analysis of trunk lengths between seasons showed a trend for increase in length for all worm classes as the year progessed between spring and summer and none in autumn. The former growth period must be linked to increasing ambient temperatures and hence increased metabolic rate of worms which will find themselves in a more nutrient rich environment due to increased host feeding at this time. The apparent autumnal cessation of growth can be explained by the fact that as the temperatures decrease further recruitment results in small

worms entering the population and this coupled with the loss from the gut of mature worms results in no apparent net increase in worm length within each class. Muzzall (1984) found that gravid *N.limi* also increased in length between spring and summer (March & June). The fact that growth of f1 and f2 worms in spring is accompanied by a decrease in their proportion in the metapopulation (see section 5.5.3 for details) suggests that both growth and maturation occur simultaneously. A similar pattern was observed by Amin (1986) in *N.prolixoides*. By contrast, Valtonen (1980b) found no evidence of growth at all in male *Metechinorhynchus salmonis* from white fish although females increased in length as they matured.

The observation of apparent density dependent effects on worm length manifested as a reduction in length in heavily infected hosts is interesting and has been seen in other acanthocephalan species. Kennedy (1977) found that in heavy infections of *Pomphorhynchus laevis* there was a reduction in worm length. Devine (1988) found an apparent density dependent reduction of worm length in *N.rutili* from brown and rainbow trout with differing infection intensities. If this effect is to have any influence upon the parasite population dynamics then this length reduction must also reduce the fecundity of the worms. This possible stabilizing effect is considered in Chapter 6.

5.5.2. Dispersion pattern of *Neoechinorhynchus rutili* amongst Loch Maragan brown trout

The overall distribution of *N.rutili* in Loch Maragan brown trout was overdispersed and there was good agreement with the negative binomial model. This appears to be a general pattern in fish acanthocephalans (Kennedy, 1985a) and has also been reported for other metapopulations of *N.rutili* in other definitive host species (e.g. in three-spined sticklebacks by Walkey, 1967 and Chappell, 1969b & c and

in carp by Moravec 1984a). The value for the variance to mean ratio greater than unity (55.83) and the low value for k (0.7893) indicates that the overdispersed nature of the distribution (Anderson & Gordon, 1982). Pennycuick (1971a) also discussed some of the factors which could bring about overdispersion. This type of distribution is not a static phenomenon it being the result of the dynamic relationship between factors tending to cause underdispersion e.g. parasite mortality, density dependent processes and parasite induced host mortality and those tending to cause overdispersion eg. heterogeneity in host susceptibility to infection, direct reproduction within the host and heterogeneity in the ability of hosts to kill parasites (Anderson & Gordon, 1982). This dynamic property of distribution is evidenced by the change in the variance to mean ratios between months and seasons at Loch Maragan. A similar dynamic pattern of changing dispersion patterns, as indicated by seasonal k values was seen for *Pomphorhynchus bulbocolli*, infecting the fish *Hypentelium nigricans*, in Kentucky (Gleason, 1984) and in *Acanthocephalus lucii* infecting *Perca fluviatilis* (Brattey, 1988). Differences between the distributions of parasites in different age classes of hosts was also seen. Therefore some or all of the above mentioned factors must be playing a role in bringing about the observed distributions.

Taking factors which could cause underdispersion first. There was no evidence of parasite mortality in any of the material and the lack of effect upon trout condition factor indicated that the effect of the parasite upon the host was also minimal. Anderson & Gordon (1982) state that if the parasite has an adverse effect upon the host the age intensity curve should be bell shaped which was not the case for the Loch Maragan brown trout data (see Fig. 5.8). The only apparent factor operating to bring about underdispersion was the density dependent

effect upon worm length (section 5.2.1). Secondly, considering factors which could cause overdispersion. No direct reproduction of *N.rutili* is possible in the definitive host gut so this factor can be discounted and from the sample evidence it appears that the host is unable to kill the parasites. Harris (1972) found that although chub (*Leuciscus cephalus*) was able to produce antibodies to *Pomphorhynchus laevis* they apparently had no effect on the parasites. It seems reasonable to assume that the situation is similar for *N.rutili* in brown trout. Therefore, the main factor bringing about overdispersion in this system will be the heterogeneity in host susceptibility to infection. This can be manifested in numerous ways as a result of host age, sex, state of maturity, differential ability to react to parasites, which may possibly have a genetic basis, feeding behaviour and also any environmental factors (e.g. temperature and day length) which have an effect upon the host.

All of these factors are termed as environmental stochasticity by Anderson & Gordon (1982). Examining each element in turn. Host age plays a role in increasing overdispersion since different size classes have different mean intensities of infection and the possible mechanisms bringing this about are discussed in section 5.5.6. Sex and state of maturity had no apparent effect upon the distribution within age classes at Loch Maragan. Any heterogeneity in the hosts ability to react to the *N.rutili* infections is difficult to assess from the field data but was probably insignificant. Behavioural factors will be associated with parameters that include age, sex, temperature and day length. Firstly, age may determine hierarchies amongst the trout and hence the ability of an individual to hold a territory and its spatial location within the loch which will have consequences for the availability of prey species harbouring *N.rutili*. The distribution of *N.rutili* in the 2 prey sources identified at Loch Maragan, minnows and

Sialis lutaria larvae, were themselves overdispersed and this will again serve to increase overdispersion in the trout metapopulation. Both day length and temperature will have an influence upon the state of maturity of the trout hosts which will in turn bring about heterogeneity in feeding patterns amongst immature and mature trout. These environmental factors will also effect the availability of the host species in the loch on a seasonal basis, particularly *Sialis lutaria* larvae, which exhibit a spring migration towards the loch shore to pupate (Elliot, 1977a). Also, if there is heterogeneity in the occurrence of trout forming search images for potential prey species this again will increase the degree of overdispersion. Finally, there is evidence that temperature can affect the establishment success of acanthocephalan worms (Kennedy, 1972) and this form of regulation may also be operating in *N.rutili* at Loch Maragan.

Anderson & Gordon (1982) describe how environmental stochasticity is itself superimposed upon demographic stochasticity. In essence demographic stochasticity describes the equilibrium between the processes of parasite birth, death, immigration and emmigration and apparently is usually Poisson in form. It may therefore be the additional effects of environmental stochasticity which bring about the overdispersed distribution observed in nature. Thus the picture at Loch Maragan is one where the factors likely to bring about overdispersion in the *N.rutili* metapopulation inhabiting brown trout appear to be more prominent than those that bring about underdispersion. Hence the observed high variance to mean ratio and low k value. On Dobson & Keymer's (1985) nomogram showing the predicted contours for various proportions of the female population likely to be mated for various combinations of k and N (average worm

burden per host) the point for the Loch Maragan trout data comes far over to the right, compared with the point for Walkey's (1967) data for *N.rutili* in sticklebacks and predicts that 99% of the worm population should be mated. This example perfectly illustrates the advantage of the overdispersed distribution to the parasite.

5.5.3. Temporal variation in the *Neoechinorhynchus rutili* infection of Loch Maragan brown trout

The seasonal population dynamics of *N.rutili* around the world in its definitive hosts has been adequately reviewed by Chubb (1982) and should be referred to where necessary. He found a number of different patterns of occurrence within climatic regions and attributed these to the specific conditions in each locality and the evolutionary adaptability inherent in the genetic makeup of the species. In the context of the present study it seems appropriate to make most comparisons with relevant studies on *N.rutili* which occurred in the same climatic region, that is, Marine West Coast, as adopted by Chubb (1982). The seasonal reproductive strategies are discussed in detail in Chapter 6 and so will only be referred to in passing here.

Most studies of acanthocephalan population biology consider percentage prevalence and intensity values separately e.g. Walkey (1967), Robertson (1953), Lasee (1989), Chappell (1969c), Moravec (1984a) and Valtonen (1979). Diamant (1989) and Hooper (1983) apparently tried to tidy up ambiguities caused by using these 2 population parameters by using the single z index. This index was developed by Janion (1968) to combine percentage prevalence and intensity values for individual hosts, to compare these statistics in groups of hosts, e.g. of one age class or between seasons. However, it appears that the z statistic is designed for utilization with data from individual hosts which are successively recaptured and their

parasite burdens estimated on each capture occasion. This was clearly not possible for the analysis of acanthocephalan population dynamics carried out by Diamant (1989) and the index was therefore wrongly used. It was unfortunate that this index was used because it makes comparisons with his work complicated. Therefore, the z index was not used in the present analysis and I have looked at the 2 parameters separately.

Firstly, considering the general pattern of prevalence and intensity of infection at Loch Maragan in the brown trout, *N.rutili* occurred throughout the year and there was an apparent seasonal pattern of changes in percentage prevalence (although no statistically significant differences were detected) and intensity (statistically significant), the highest values being observed in late W, throughout the Sp and in early Su. Chubb (1982) summarized the factors which could influence acanthocephalan population dynamics and these fell into 2 categories, biotic and abiotic factors. In order for a seasonal pattern to arise the sum of these factors must also vary seasonally although individual factors may not.

Chubb (1964) postulated that temperature might play a major part in determining the presence or absence of a well defined seasonal periodicity in the development of some acanthocephalan populations. He found examples of cases where distinct seasonal patterns arose at aquatic sites which froze during the winter for significant periods and none where freezing did not occur. Therefore, at Loch Maragan, which freezes over in winter (up to 3 months) such a seasonal periodicity would be expected. Seasonal changes in temperature would have a direct influence on the rate of physiological processes of all hosts and the parasites themselves since they are all poikilothermic. An examination of the temperature and intensity seasonal patterns at Loch Maragan showed that although the form was similar, the

temperature graph was shifted forwards such that peaks appeared in summer, whereas *N.rutili* intensity peaks occurred in spring. At increased temperatures there is an increase in trout feeding activity (Ball, 1961) and trout also show a preference for *Sialis lutaria* larvae probably because they migrate towards the shore at this time (Ball, 1961; Frost & Smyly, 1952). By the summer the increased availability of aerial food results in trout tending not to feed on the *Sialis* larvae, which are likely to be less common at this time. The analysis of the available seasonal prevalence data for *N.rutili* in *S.lutaria* larvae in Scotland suggested that values would peak in spring (see Chapter 7). Thus an increased feeding rate in trout, specifically on *S.lutaria* larvae and migration of these larvae, mediated by temperature (and possibly day length), coupled with the expected peak in *N.rutili* prevalence in the insect larvae should bring about the observed spring peak in intensity. In summer, the drop off could be caused by reduction in available *S.lutaria* larvae because of pupation of the oldest instars, a change in the trout dietary preferences toward aerial food and also a decreased establishment rate of worms at the higher temperatures. Kennedy (1972) reported this phenomenon for *Pomphorhynchus laevis* infection dynamics. The autumn and winter drop off in intensity values was probably again mediated by temperature. At this time the mature worms would be leaving the gut having successfully reproduced and the lower temperatures will result in reduced feeding of trout and hence chance of acquisition of new infections. The fact that young small worms occurred throughout the year suggested that continuous recruitment was occurring. This implies that worms in an invasive condition are present all through the year in the intermediate hosts. Alternatively occurrence of invasive *N.rutili* in *Sialis lutaria* larvae is seasonal and the apparent winter

recruited worms are in fact quite long established individuals whose metabolism and hence growth rate has been slowed down due to the low winter temperatures. Lack of host feeding should have no effect upon established worms at this time since other *Neoechinorhynchus* species are known to survive long periods of host fasting (Van Cleave & Ross, 1944).

A similar pattern of no real seasonal prevalence cycle was found by Walkey (1967) in three-spined sticklebacks in County Durham, England, and he also found continuous invasion of the fish throughout the year. Continuous invasion of hosts was also seen by Thomas (1964b), Bibby (1972), Aderounmu (p.c. to Chubb, 1982), Chappell (1969c), Halvorsen (1972) and Mamer (1978) (see Chubb, 1982 for species and site details). Moravec (1984a) also found the same pattern in carp in Czechoslovakia. Maximal mid-year occurrence was also found by Bibby (1972) in minnows in Wales (May to October) and Robertson (1953) in brown trout (May to June).

An examination of the female to male ratios, the variance to mean ratios and the mean lengths of the various worm classes gives an insight into the form of the pattern of the recruitment and maturation of *N.rutili* in Loch Maragan brown trout. In Sp the male to female ratio was about 1:1, the worms are relatively small and no f3 worms were present indicating that recruitment was occurring, most probably via *Sialis lutaria* larvae, which would have been migrating to the shore at this time. During this season the variance to mean ratio was at its highest value for the year indicating that the recruitment was not equal amongst the trout population hence leading to increased overdispersion of worms in the host population (see section 5.5.2). By Su the worm metapopulation was dominated by females presumably because some males had already been lost from the gut and f3 females were present indicating that successful mating had occurred. No exponential

increase in mean intensity of infection was observed which may be a function of temperature on establishment success. During this period there was apparent growth of all worm classes probably as the result of increasing ambient temperatures and consequent increase in food availability. By A the female to male ratio was still biased towards females although less so than in Su suggesting that recruitment of new worms of both sexes was occurring via continued feeding upon *N.rutili* infected hosts by the trout. Further evidence for recruitment at this time was the lack of any significant difference in the worm length (m, f1 and f2) between Su and A, the effect of large worms on the calculation being diluted by large numbers of new small worms. For the f3 worms the non significant difference could have been the result of newly inseminated, smaller f3 worms entering the subpopulation or of the f3s reaching an optimum length and simply increasing their acanthor output. In W the ratio fell back to 1:1 when most f3 worms were lost from the gut and only newly recruited worms were left in the gut. Unfortunately no comparisons with the lengths of the worms during the W could be made because of a lack of material.

The remarkable similarity in the patterns of prevalence and intensity and in the changes of female to male ratio in the Loch Maragan *N.rutili* metapopulation inhabiting brown trout between years must indicate the stability of the interactive forces in this host parasite system as suggested by Kennedy (1974) for *Pomphorhynchus laevis*. Indeed, Aho Camp & Esch (1982) suggested that long-term consistency of seasonal patterns in aquatic systems may often be the result of ecosystem stability rather than other factors such as density dependent regulation. They felt this this was especially true of parasites which complete their life cycle within an aquatic system. There were some similarities with other studies in the same climatic

region but the patterns were not identical. One conclusion that can be drawn from this is that at any one site the complex interaction of both biotic and abiotic factors leads to a unique dynamic seasonal pattern of occurrence of *N.rutili* in its hosts.

5.5.4. Intestinal distribution of *Neoechinorhynchus rutili* infections of Loch Maragan brown trout

The adults of most species of Aanthocephala exhibit a preference for a particular region of their hosts' alimentary canal (Crompton, 1973) and *N.rutili* appears to be no exception. In the Loch Maragan brown trout data, which considered the distribution of 3893 worms, the preferred sites were the ileum and rectum. Kennedy & Lord (1982) critised the lack of precision of many acanthocephalan data sets on intestinal distribution, but it was felt that in this case the simple division between the anatomically distinct regions of the trout gut would more adequately take regional differences in intestinal topography and biochemistry into account than an arbitrary percentile consideration (Amin, 1985b). The effects upon worm gut distribution as a result of the host being caught in gill nets were discounted as important by Hubschman (1985) for *Tanaorhamphus longirostris* in gizzard shad and has therefore been assumed to be the case here. Kennedy (1985b) claimed that post-mortem migrations of Acanthocephala in fish should be of 'little significance' especially if the host is examined soon after death. In the present study the brown trout were kept on ice and dissected within a few hours. My observation was that although specimens remained alive in the gut after the death of the host, very little movement of the worms themselves occurred and therefore I was satisfied that the observations were representative of the distribution *in vivo*.

The available information on the distribution of *N.rutili* in

brown trout is limited and consequently Kennedy (1985b) did not consider its distribution in this or any other species of host. Descriptions of the distribution in brown trout are given by Thomas (1964a), Bwathondi (1976) and Devine (1988). None of these studies found *N.rutili* established in the stomach, which Thomas described as physiologically unfavorable for this species, but all 3 described *N.rutili* as a parasite of the pyloric caeca and intestine although no more than 10% of the total worm population was found in the former region. By contrast, very few worms were found in the pyloric caeca of the Loch Maragan brown trout. Thomas (1964a) claimed that there was no requirement for the worms to be located in the relatively stable pyloric caecal environment because their proboscides provided adequate anchorage for life in the main intestinal lumen. He also found that the proportion of *N.rutili* inhabiting the pyloric caeca was reduced in older trout and thought that this could either be related to interspecific competition with other gut parasites or due to changes in host physiology. Bates (1989) found that in experimental infections of rainbow trout with *Acanthocephalus anguillae* and *Pomphorhynchus laevis*, the worms avoided the pyloric caeca because it reduced their probability for mating. This may also explain the apparent shunning of this region by *N.rutili* in brown trout.

Unfortunately, Thomas (1964a) only considered the distribution in 2 regions of the gut, the pyloric caeca and the post-pyloric caecal region so no comparisons could be made with the present study. Both Bwathondi (1976) and Devine (1988) divided the gut into 5 sections of equal length (posterior to the pyloric sphincter of the stomach) but Devine (1988) did not consider worms in the pyloric caeca. Bwathondi (1976) found that the overall distribution, based on 84 specimens collected over a 1 year period, were mainly distributed in the first 4 sections of the gut and the pyloric caeca, about 20% of the worm

population occurring in each region and none in the most posterior region 5. Devine (1988) only considered the distribution in individual hosts and made no attempt to calculate the general pattern in the brown and rainbow trout which he examined in October 1987. My own recalculations from his original data gave results as follows: for regions 1, 2, 3, 4 and 5 repectively the percentage representation of the worm population was 22.7, 23.0, 22.2, 18.3 and 13.8 (pyloric caeca excluded) for 704 worms recovered from 2 brown trout. The equivalent values for 4425 worms from 8 rainbow trout were as follows: 12.6, 20.9, 32.9, 25.5 and 8.2. Devine (1988) stressed the extreme individual variation in distribution between hosts, which Kennedy (1985b) noted as a general pattern in British fish acanthocephalans, and this was probably why he did not attempt to compute a general host species distribution pattern. A direct comparison with the Autumn 1987 distribution data from Loch Maragan brown trout indicated that the Drumore Loch brown trout distributions were somewhat different, the former showing a more posterior distribution pattern (see Table 5.9). Devine also examined the spatial relationship of female and male worms in the gut of brown trout and concluded that the distribution was entirely random.

In general, the available data for comparison with infected brown trout from other locations is poor and based on either few worms over a long period or on many at one point in time. The Loch Maragan data gives a more comprehensive view both in terms of worm numbers and seasonal coverage so it is likely that the picture of distribution is fairly accurate. Nevertheless, in all cases it appears that *N.rutili* exhibits no more precision in site selection than any other British fish acanthocephalans (Kennedy, 1985b) although there are some seasonal changes in distribution which are discussed below. Although

it is likely that the worms will be released from some prey items, e.g. *Sialis lutaria* larvae in the stomach, their preference for regions posterior to the p.c. may indicate their greater suitability, both morphologically and physiologically. In the transmission experiments with *Sialis lutaria* larvae and rainbow trout, *N.rutili* established more anteriorly, but their positions may change as the infection ages. Worms derived from a piscine source (i.e. postcyclic transmission) may be released more posteriorally in the gut due to the longer digestion period required for these food items, although for sticklebacks this appears not to be the case, and thus bring about the observed distribution.

In other hosts the intestinal distribution of *N.rutili* is quite variable. In carp, Moravec (1984a) found worms occupying the first quarter of the intestine whereas Tesarcik (1972) found it throughout the intestine but concentrated in the middle region. In minnows, Bibby (1972) found 75% of worms in the intestinal bulb, 20% in the anterior and 1% in the posterior intestine. In *Coregonus nasus*, Valtonen (1979) found *N.rutili* distributed as follows: 79.1% in the p.c. area, 16.4% in the second third of the gut and 4.5% in the posterior third. In three-spined sticklebacks, Devine (1988) found worms distributed in the posterior half of the intestine in October and Walkey (1963) found worms throughout the gut with 20.3% in the upper intestine, 53.25 in the lower intestine and 26.3% in the rectum. He even found that large specimens that were attached in the lower intestine or rectum protruded from the anus of the fish host which was also seen in infections of Drumore Loch sticklebacks (p.o.). Steinstrasser (1936) described the distribution in rainbow trout as including the entire intestine with the pyloric caeca also invaded in heavy infections. He also noted that young worms tended to be located more anterior to the more mature worms. In all of these cases the distribution of *N.rutili*

has been considered in isolation and it must be remembered that interspecific interactions with other gut parasites may affect the observed distributions, as was found by Chappell (1969a). Experimental infections of various hosts would give a true insight into the spatial preferences of this parasite. It is worth noting here that at some of the other sites of infection fish were concurrently infected with *Echinorhynchus truttae* (see Table 3.3) and material from these sites would provide information about possible interspecific effects between acanthocephalans on spatial distribution.

Although Kennedy (1985b) described seasonal changes in distribution as not very important considerations in his generalized description of acanthocephalan distributions in fish, Kennedy & Lord (1982) thought that most acanthocephalans would exhibit posterior migrations during the course of infection, so the data from the present study obviously merits some comment. The spatial distribution of *N.rutili* was seasonally dynamic, worms dominating in the duodenum and ileum in W and Sp and showing an apparent posterior migration in Su and A, resulting in a high proportion of the population being found in the rectum. Amin (1986) found a significant posterior movement of *Neoechinorhynchus cylindratus* in *Micropterus salmoides* and *N.prolixoides* in *Erimyzon sucetta* between spring and summer in populations inhabiting a lake in Wisconsin. This is quite different to Muzzall & Bullock's (1978) finding that gravid *Neoechinorhynchus saginatus* dominated in the region 20 to 40% of the distance along the gut. In the Loch Maragan trout population of *N.rutili* the distribution of male and female worms was similar in all seasons apart from in A when the females occupied a more posterior position than the males (see Table 5.10). By contrast, Bibby (1972) found no differences in the gut distribution of male and female *N.rutili* in his year long

study on minnows. Notably, this apparent more posterior position of females was due to the distribution of both f1,2 and f3 worms. This type of migration has been observed for other fish acanthocephalan species, including *Neoechinorhynchus* spp., details of which are given by Kennedy & Lord (1982). These authors attributed the migration to the maturation of worms, gravid females tending to occupy the posterior part of the preferred gut region. The fact that this observation is linked to the seasons at Loch Maragan and the proportion of f3 was greatest during the summer months suggests that these processes might be mediated by temperature changes. Similar observations of a posterior migration of *N.rutili*, specifically of mature specimens, were noted by Walkey (1967) in three-spined sticklebacks, Bwathondi (1976) in brown trout and by Tesarcik (1970, 1972) in carp.

An examination of the seasonal changes in sex ratios helps to explain the observed differences in female and male distributions in A. At this time the population was dominated by females, mostly gravid f3's which occupied a posterior position. The males which these females would have mated with, presumably had a shorter life span and had left the gut resulting in the female bias in the population. Consequently the small number of males were therefore probably from new infections which were established more anteriorly in the preferred ileal region. This dynamic migration therefore leads to the situation where f3 worms have the opportunity to monopolize the posterior gut region, absorb nutrients and produce large numbers of acanthors.

The effects on intestinal distribution of parasite abundance have been examined and Kennedy & Lord (1982) indicated that in heavy infections the mean worm position will be more anterior. This was the case in the *N.rutili* infections of Loch Maragan trout in both Sp and Su of 1987 when a greater proportion of worms was found in the

duodenum of these hosts than in lightly infected ones. The way in which this is mediated is unknown but could be associated with nutrient concentration gradients or chemical by-products released by previously established worms. The occurrence in the p.c. could possibly be linked with the high intensities of infection since *N.rutili* was found in this gut region only in heavily infected trout from Bridge of Weir. However, Bwathondi's (1976) observation of preference for p.c. distribution in lightly infected hosts apparently challenges this hypothesis.

5.5.5. Effect of host sex on *Neoechinorhynchus rutili* infections of brown trout

At Loch Maragan, trout sex did not appear influence the prevalence of infection in any age class and a significant difference in mean intensity of infection was only found in 4 year old individuals caught in Winter, Sp and Su, males being more heavily infected than females. Thomas (1964a) found the same pattern of infection in brown trout from the River Teify, West Wales, the significant differences in means occurring between the 4-year-old fish caught between January and March. Thomas found that the female fish were more heavily infected than the males, the converse of the situation in Loch Maragan. Thomas (1964a) observed the reverse pattern for other parasite genera, with male trout being the more heavily infected. Bibby (1972) reported that the sex of minnows had no effect on the prevalence of *N.rutili* infection at his site in Wales. Valtonen & Crompton (1989) found various patterns of infection in Bothnian Bay fish. For example, females had a higher prevalence of infection in pike and salmon and in males in ruffe (*Gymnocephalus cernua*). The intensity of infection was higher in male salmon and lower in male pike. Unfortunately only male brown trout were examined in this study

so no comment can be made on the distribution in this species with respect to sex in this location.

Examination of the distribution in other neoechinorhynchid infections of fish has not yet revealed any consistent pattern. No differences in either prevalence or intensity of infection in hosts of different sex were found by Lasee (1989) for *N.pungitius* in brook sticklebacks (*Culaea inconstans*), by Muzzall & Bullock (1978) for *N.saginatus* in fallfish (*Semotilus corporalis*), by Muzzall (1984) for *N.limi* in mudminnows (*Umbra limi*), by Amin (1985b) for *N.robertbaueri* infecting *Erimyzon sucetta*, or by Khamees & Mhaisen (1988) for *N.agilis* in the cyprinid *Carasobarbus luteus*. No differences were found in the prevalence of infection of *N.pungitius* in brook sticklebacks by Font (1983) or by Jilek (1978) for *Gracilentis gracilentis* or *Tanaorhamphus longirostris* in gizzard shad (*Dorosoma cepedianum*). Additionally no differences were found in intensity values for *N.cylindratus* in *Micropterus salmoides* by Amin (1986) or by Muzzall (1980) for *N.cristatus* infecting white suckers, *Catostomus commersoni*. Observations of significant differences between the sexes are not so common. Mhaisen *et al.* (1988) found that the prevalence and intensity of infection with *N.agilis* was higher in female *Liza abu* than male hosts in Iraq. Amin (1986) found that the intensity of infection of *N.prolixoides* in *Erimyzon sucetta* was higher in male hosts and the reverse pattern for prevalence values. Eure (1976) found that only during spring were female largemouth bass (*Micropterus salmoides*) more heavily infected with *N.cylindratus* than males (intensity).

This phenomenon needs to be investigated in more controlled conditions because field samples are often small and the origin and state of maturity of hosts is not fully reported. The small size of

the Loch Maragan 4-year-old trout sample may have contributed to the observed difference. Loch Maragan trout are known to mature before this age (Chapter 4) so the link with the effect of reproductive hormones seems unlikely. Thomas (1964a) explained that female fish should exhibit lower intensities of infection because of their greater physiological resistance mediated by the hormone oestrogen. The immunosuppressive effect of testosterone in males could also produce the same effect. To resolve this question of host sex effecting worm burden in fish, both immature and mature specimens caught in all seasons without other parasites would need to be examined. Nevertheless, the general view from the available data is that for the majority of fish infected with *N.rutili* and other closely related species, no differences in the intensity of infection with host sex should be expected.

5.5.6. Effect of host age on *Neoechinorhynchus rutili* infection patterns

Diamant (1989) describes the increase of abundance of acanthocephalans with increasing size of the host as a familiar phenomenon and lists the following factors which are likely to bring this about: host habitat, feeding habits, extended exposure to infections, age-related segregation, larger available microhabitats for parasites and changes in physiological conditions with maturity. Data describing this relationship according to the age of the host is much more scarce and Dobson & Keymer (1985) claimed that none was available despite the fact that the studies by Thomas (1964a), Awachie (1965), Pennycuick (1971b) and Jilek (1978) had relevant data.

This pattern of increase in the *N.rutili* infection with age of Loch Maragan brown trout was observed for both prevalence and intensity values (The age 5 data was not considered here because only

1 fish was examined). This pattern is similar to that of *N.rutili* in minnows (*Phoxinus phoxinus*) as reported by Bibby (1972) and in three-spined sticklebacks (*Gasterosteus aculeatus*) (Walkey, 1967; p.o. at Drumore Loch). This increase in prevalence and intensity can be explained by the increase in the number of *N.rutili* (of all stages) in the diet of potential hosts. The analysis of the diet of Loch Maragan brown trout indicated that there was no statistical difference between the intake of *Sialis lutaria* larvae by fish greater or less than 200mm in length. This raises the question of why the larger trout should be more heavily infected and can most satisfactorily be explained by the fact that piscivorous feeding on minnows was only observed in larger trout. In this case, postcyclic transmission of *N.rutili* inhabiting the minnows would act as an extra source for infection of larger trout. Additionally, larger trout will obviously hold larger territories and may therefore increase their possible exposure to prey species harbouring *N.rutili*. Interestingly, there was a noticeable jump in the prevalence values between age classes 3 and 4 and the adoption of piscivorous feeding and the ability to maintain larger territories seems to adequately explain this.

The observations in Loch Maragan trout can be attributed to aspects of their feeding rate and habit but they are not consistent with the pattern of *N.rutili* infections seen at other sites. Valtonen (1979) found that the middle sized whitefish (*Coregonus nasus*) caught in the Bothnian Bay, 175-214mm in length, were most heavily infected because these fish exhibited the most intense feeding upon the ostracod intermediate hosts. This pattern of infection was also reported in sticklebacks by Devine (1988) at Drumore Loch in October 1987 and by Chappell (1969b) in Yorkshire, in brown trout by Thomas (1964a) and in carp (*Cyprinus carpio*) by Moravec (1984a) in Czechoslovakia, although the prevalence values followed the same

pattern as in Loch Maragan brown trout at the latter site. The most common explanation in these reports for the observations was one of a change in feeding habit with size or age.

For both patterns of infection distribution with age, the feeding habits of the fish provide an adequate explanation. The pattern observed in Loch Maragan trout fits well with the hypothesis proposed for *Echinorhynchus salmonis* infections by Amin & Burroughs (1977). They suggested that it was the increased volume of food consumed by larger fish, coupled with postcyclic transmission as a result of adopting piscivorous feeding habits, that resulted in their heavier infections. Interestingly, Moravec (1984a) proposed that smaller fish species (e.g. sticklebacks, minnows and young carp) should show a steady increase in mean intensity of infection with size as a result of increased food intake and larger fish (e.g. larger carp and brown trout) should show a decrease as they get older as the proportion of ostracods in the diet decreases. Clearly, 2 patterns can exist for *N.rutili* infecting different aged hosts and these will be site and species specific due to the variability in the transmission hosts present and the age related dietary preferences for them.

5.5.7. Effect of *Neoechinorhynchus rutili* infections on host fish

Little information is available about pathogenic effects of *N.rutili* on its fish hosts. Nevertheless, the disease effects of *Neoechinorhynchus* spp. are named as neoechinorhynchosis in the standard nomenclature of animal parasitic diseases (SNOAPAD) by Kassai *et al.*(1988). Steinstrasser (1936) reported that *N.rutili* can cause lesions of the intestinal mucosa in mass infections. He reported the case where *N.rutili* infections were the cause of death for rainbow trout yearlings at a trout farm in Germany. He described the pathological effects of this parasite as "dead tissue in the immediate

area around the proboscis spines where the flesh is torn" and that these parasites could only be dangerous to the trout at the beginning of the recruitment season when many worms bored their proboscides through the epithelium into the submucosa of the gut wall simultaneously. He found that an infection of 50 to 70 *N.rutili* had no effect on fish growth and noticed that it was impossible to distinguish infected fish which often appeared "fat". But because of the effect of mass infections he felt that fish farmers should not neglect these worms as possible agents for epizootics. Notably at Bridge of Weir fish farm in Scotland, where trout harboured up to 891 worms, the farmer did not mention any such parasite related deaths. Moravec (1984a) described *N.rutili* as a highly pathogenic "serious parasite" and noted that cases of mortalities of pond carp and trout in Central Europe are due to heavy infestations by this worm. Thomas (1964a) claimed that *N.rutili* elicited a strong host reaction in the brown trout which he examined.

At Loch Maragan, the only evidence of pathology in infected brown trout was reddening of the intestinal mucosa near the point of attachment and this was also seen in a heavily infected trout from Bridge of Weir. This is quite different to the nodule formation seen at the point of attachment of *Neoechinorhynchus capiodi* in quillback (*Carpiodes cyprinus*) intestines as reported by Szalai & Dick (1987). No strong effect upon the host condition factor was evident, as for brown trout from Drumore Loch, Scotland (Devine, 1988), although there was a slightly negative relationship between intensity of infection and condition factor in immature fish from Loch Maragan. Interestingly, Devine (1988) found a strong positive correlation between intensity and condition factor for wild rainbow trout caught in 1987 at Drumore Loch ($r=0.764$), but this result was probably

anomalous because rainbow trout caught in other years at the same site showed no such relationship. The results of my study may have been somewhat confounded by the fact that other parasites were present in some of the trout, and the specific effects on condition should be examined experimentally to clarify the situation. If condition is a good measure of how "fit" a fish is then there appears to be no function relating the probability of a host dying and its parasite burden, that is there was no Bradley Type II effect and the effects are therefore density independent. Similarly, Hine & Kennedy (1974 a & b) found that there was no effect on host growth or mortality in any of the host species infected with *Pomphorhynchus laevis*. They concluded that the infrapopulation sizes were dependent primarily upon transmission processes. The effects on host fecundity of Loch Maragan trout could not be examined because too few mature individuals were caught. Robertson (1953) found only 2 trout infected with *Echinrorhynchus truttae* to be sterile. If this is also the case for *N.rutili* infected trout then it is likely that any effect will be manifested in a small proportion of the fish population.

In general, *N.rutili* appears to have little effect on its host, except when numerous lesions are caused by the simultaneous recruitment of many new worms during particular times of the year. Deaths in these cases may only be indirectly linked to the acanthocephalan infection and may in fact be caused by secondary bacterial infections of these lesions. It was unfortunate that the dead trout observed on the Loch Maragan substrate in June 1987 could not be retrieved and examined as this may have provided some evidence to settle the question of the pathological effects of *N.rutili* at this site. The distribution pattern of *N.rutili* in Loch Maragan trout was overdispersed and this will tend to ensure that any pathological effects will be restricted to a small proportion of the whole trout

population. To use Hayunga's (1989) expression in this case, *N.rutili* "can be visualized as a successful parasite, its hallmark being the lack of severe pathogenicity".

5.5.8. The *Neoechinorhynchus rutili* metapopulation in trout

The maximum *N.rutili* metapopulation size was calculated in order to have an indication of the numbers of worms, given their distribution in the fish population, required to ensure that a new generation of worms occurred the fish host population in the following year. This population estimate is utilized in Chapter 6 in order to discuss aspects of the basic reproductive rate of the parasite (see Dobson & Keymer, 1985). The estimated population of 5,566 f3 worms in 1987 was apparently fecund enough to ensure that a reproducing population of worms occurred in 1988, although the rate of loss of acanthors in the environment was not known.

5.6. SUMMARY

5.6.1. *N.rutili* from Loch Maragan brown trout exhibited typical acanthocephalan dimorphism, females being longer than males.

5.6.2. Trunk length was found to be a reliable indicator of worm biomass (dry weight).

5.6.3. All worms exhibited apparent growth between spring and summer as indicated by changes in trunk lengths in worm classes.

5.6.4. In heavily infected trout worms of all classes were significantly shorter.

5.6.5. *N.rutili* was found to be overdispersed in its brown trout hosts (k = 0.7893, variance to mean ratio = 55.83). Heterogeneity in host susceptibility to infection was thought to be the main factor bringing about this form of parasite distribution at Loch Maragan.

5.6.6. *N.rutili* exhibited a definite seasonal cycle in terms of intensity, with maximum values occurring during the summer months when water temperatures were highest and feeding upon hosts of *N.rutili* was also at a peak. It is suggested that temperature indirectly influenced this pattern at Loch Maragan. Apparent recruitment occurred throughout the year and gravid females were only found during the warmer months of the year.

5.6.7. The similarity of the seasonal pattern of occurrence between years at Loch Maragan was attributed to the stability of the interactions of abiotic and biotic fators.

5.6.8. Analysis of the distribution of worms in brown trout gut suggested that *N.rutili* is typically a parasite of the ileum and rectum and rarely occurs in the pyloric caeca.

5.6.9. Intestinal distribution was seasonally dynamic, a posterior shift in position being seen in the warmer months of the year. In summer, female worms were positioned more posteriorly than male worms.

5.6.10. In high density infections there was an anterior shift in mean position of all worms in the gut.

5.6.11. The sex of the brown trout host had apparently no effect upon the prevalence and intensity of *N.rutili* infection.

5.6.12. Both the prevalence and intensity of *N.rutili* infection in Loch Maragan brown trout increased with host age and this was attributed to factors affecting feeding rate upon infected hosts and dietary preferences for them.

5.6.13. No real evidence for the adverse effect of *N.rutili* on its brown trout host in Loch Maragan was found.

5.6.14. In summer 1987, the *N.rutili* metapopulation in Loch Maragan brown trout (aged 1 to 3 years) was estimated to be 36,154 of which 5,566 (15.4%) were gravid females (maximum numbers).

CHAPTER SIX

REPRODUCTIVE BIOLOGY OF *NEOECHINORHYNCHUS RUTILI* IN ITS DEFINITIVE HOSTS

6.1. INTRODUCTION

Parasite persistence depends on the rate of reproduction of the parasite per generation balancing or exceeding the cumulative losses occurring at each stage of the life history (Dobson & Keymer, 1985). Anderson (1980) used the index R_0 to allow for comparison between the basic reproductive rates of difference parasite species, a value being greater than unity will ensure parasite persistence. This index has been defined specifically for the Acanthocephala as the average number of female offspring, produced throughout the lifetime of a mature female worm, which would achieve reproductive maturity in the next generation in the absence of density-dependent constraints on establishment, survival or reproduction (Dobson & Keymer, 1985). Although R_0 is a dimensionless parameter its calculation requires knowledge of the rates of transmission, reproduction and lifespan of the parasite at all stages of the life cycle. Thus, one important aim in an ecological study is to estimate these values and attempt to predict the value of R_0 for specific host-parasite systems in specific localities.

The general definition of reproduction of Cohen (1977) was utilized by Crompton (1985) in the acanthocephalan context, as the replacement of one pair or population of parents with the next and that the reproductive process in practical terms culminated in the release of shelled acanthors or 'eggs' from the mature worms. Therefore, the reproductive currency of a parasite generation will be some function of the size of the present population of female worms and the numbers of eggs they produce. However, the entire reproductive process is a dynamic one and will be affected by various physiochemical and biotic factors which include: the parasite population structure, host nutrition, immune responses, interactions

with other parasites and seasonal and climatic variability (Crompton, 1985). It is obvious that some measure of acanthocephalan fecundity is an essential to understanding of their population dynamics. Although rates of 'egg' production have been estimated for some acanthocephalans e.g. *Moniliformis moniliformis* (Crompton, Arnold & Barnard, 1972) and *Macracanthorhynchus hirudinaceus* (Kates, 1944) there is apparently no data available for any eoacanthocephalan species, including *N.rutili*. One problem is associated with deciding how to measure fecundity, since there are often logistical problems in measuring output of eggs in the faeces of infected hosts, particularly aquatic hosts like fish. The necessity for measuring this 'rate' hinges on the assumption that mature egg release via the uterine bell is the main source of shelled acanthor output into the external environment. As pointed out by Parshad and Crompton (1981) there is some controversy about the function of this organ as a sorting device although there is apparently more evidence to support this proposed function than not. I have observed apparently mature acanthors in the uterine bell of *N.rutili* in living specimens outside the host but the contribution of this form of egg release to the total is unknown. An alternative or complementary form of egg release may operate in some acanthocephalan species (Whitfield, 1989 Acanthocephalan Workshop). He hypothesized that the entire gravid female acanthocephalan could be envisaged as an 'egg package' or quantum of infection, to be released whole into the external environment at some point in time. Thus, a concentrated focus of acanthors would be available as food for potential intermediate hosts in the external environment. This form of egg release has been suggested for *Acanthocephalus lucii* by Brattey (1982).

The overdispersed distribution of *N.rutili* in its hosts (ostracods and *Sialis lutaria* larvae) may be an indirect product of

such a reproductive strategy. If this form of egg release actually occurs, then analysis of whole worm contents from field collections should be the appropriate way of gaining an impression of the seasonal dynamics of acanthor output in the worm metapopulation taking confounding factors e.g. other parasites, density-dependent effects into account. Combining a measure of parasite fecundity and an estimate of the worm population size, a reproductive success rate can be calculated. Subsequently estimates of the factors which bring about losses between generations can be calculated and fitted into this numerical framework. This type of approach is seen as a developmental stage of all parasite population biology research (Pronin, Timoshenko & Sanzhieva, 1989).

Since reproduction can be influenced by numerous physiochemical and biotic factors it is essential to examine the effects of these upon the population dynamics of the parasite. Thus, the epidemiological question is concerned with the type of strategies adopted to ensure that a new generation of reproducing parasites is produced. It appears that studies of the ecology of naturally occurring acanthocephalan-fish interations have not revealed density dependent constraints upon the dynamics of the adult parasite in the final host (Keymer, 1982). Since some apparent density dependent effects were found in the *N.rutili* metapopulation inhabiting brown trout at Loch Maragan (see Chapter 5) an examination of the apparent fecundity as indicated by 'freeze-frame/ snap shot' analysis of the female worm population was undertaken in order to shed some light upon Kennedy's (1977) statement that 'on the evidence available at present it must be concluded that the majority of fish parasite populations are unregulated and hence unstable'. This appears to be a valid approach since density-dependence in a single rate parameter, if

operative over the naturally observed numerical range, is sufficient to regulate parasite population flow throughout the life-cycle, whether direct or indirect (Anderson, 1976). There is some data pertaining to the reproduction of *N.rutili*. Most studies were concerned with the timing of egg release and the appearance of gravid female worms (e.g. Steinstrasser, 1936, Walkey, 1967 and Bwathondi, 1976 in the Marine West Coast climatic region and Tesarcik, 1970, 1972 and Moravec, 1984a & b in the Humid Warm Summer climatic region. See Chubb, 1982 for climatic region definitions). However, until now there has been no attempt to estimate worm fecundity, the success rate of transmission and therefore no attempt to place this species on the r-k selection spectrum (see Esch *et al.*, 1977). Fecundity in the Acanthocephala has been defined as the number of shelled acanthors produced per female worm (Crompton, Arnold, Walters, Keymer & Marrs, 1988). The importance of having a measure of this parameter was cited along with survival as being one of the 2 which brought about the generation of epidemiological patterns and the determination of evolutionary fitness (Keymer, 1982). Clearly, the scope of the present study only allowed for some aspects of reproduction to be considered, but when integrated with data from future studies should lead to a greater understanding of parasite regulation at both the infrapopulation and suprapopulation levels (Esch *et al.*, 1977).

6.2. MATERIALS AND METHODS

6.2.1. Examination of the reproductive activity and maturation patterns of *Neoechinorhynchus rutili* in its Scottish fish hosts

6.2.1.1. *Loch Maragan brown trout metapopulation*

The material was collected as described in section 5.3.2.1 and in addition when present, copulatory caps on worms were also recorded in

order to assess possible mating frequencies in different individual trout. The treatment of the data for each sex and developmental class of worms in each month and season was described in section 5.3.3.2. In order to assess if there was any seasonal maturation pattern cycle in the worm population, only females were considered. The values for the f1 and f2 worms were combined since both of these stages were found in *Sialis lutaria* larvae (see Chapter 7). Data for the same seasons in different years were also combined and compared pairwise, by means of X^2 tests to establish whether any observed differences in proportions of developmental stages were statistically significant.

The effects of intensity of infection on worm length, gut distribution and mating probability were considered in Chapter 5. Since it was established that there were apparent density-dependent effects upon both worm length and mating probability, as indicated by the proportion of f3 worms in trout from hosts with different intensities of infection, the effects on potential fecundity were examined by estimating the numbers of acanthors in worms from different hosts (see section 6.2.2 for details).

6.2.1.2. *Other Scottish sites*

When available, data on the presence of gravid worms (data in section 5.4.2) in hosts from other sites were compared with the Loch Maragan data to assess whether the timing of maturation of *N.rutili* in different localities was similar. In some cases, comparisons with the proportion of shelled acanthors in worms from Loch Maragan and worms from other site were also made (X^2 test).

6.2.2. Measurement of potential fecundity of *Neoechinorhynchus rutili* from brown trout in Loch Maragan

6.2.2.1. *Selection of worms for analysis*

From May 1987 to August 1988 gravid female worms (f3), when

present in the monthly metapopulation sample, were randomly selected from specific gut regions of individual hosts for use in estimating their acanthor content. Thus, gravid worms from both the ileal and rectal gut regions were examined from both heavily and lightly infected hosts in Sp, Su and A samples. In total 141 worms were examined and the sample origins are summarised in Table 6.1.

Table 6.1: Origin of gravid *Neoechinorhynchus rutili* utilized for acanthor counts

Season	Month	Number of worms examined		
		Ileal	Rectal	Total
Spring 1987	May	12	4	16
	Jun	5	4	9
Summer 1987	Jul	7	14	21
	Aug	2	10	12
	Sep	1	1	2
Autumn 1987	Oct	0	12	12
	Nov	0	1	1
Spring 1988	May	7	7	14
	Jun	7	7	14
Summer 1988	Jul	5	15	20
	Aug	5	15	20
Total		51	90	141

6.2.2.2. *Estimation of free ovary and acanthor content of female worms*

Female gravid specimens of *N.rutili* were removed from the gut and placed in 0.9% NaCl solution in groups from each region of the gut as follows: duodenum, ileum and rectum. Worms for shelled acanthor counts were randomly selected from each of these pots for analysis. Individual worms were placed on a glass slide under a cover slip and the trunk length was measured with an ocular micrometer at x40

magnification as described in 5.3.2.1.1. The individual worms were then placed in exactly 0.5ml of 0.9% NaCl solution in a solid watch glass. The body wall was disrupted using fine seekers in order to release the acanthors and ovarian tissue. The worm was thoroughly broken up to ensure complete removal of body contents into the solution. The sample of body contents and worm pieces was thoroughly agitated with a Pasteur pipette to create a 'suspension', prior to a small sample being removed. Drops of this sample were placed onto a Neubauer Brightline improved double haemocytometer and 10 repeat counts were made for each worm. The number of acanthors per worm was calculated as follows:

$$\text{Total no. of acanthors in whole female worm } (T_a) = \text{Mean no. of acanthors per count } (X) \times \frac{\text{Volume of sample}}{\text{Volume of counting chamber}}$$

$$T_a = X \times 500mm^3 / 0.1mm^3$$

$$= X \times 5000$$

The numbers of shelled and unshelled acanthors were estimated, as well as the number of free ovaries, in some cases. Shelled acanthors were the mature specimens with 3 shell layers. For each individual worm the mean and standard error (s.e.) of the acanthor estimate and the ovarian ball estimate (to the nearest 100) were calculated. Since free ovaries only appeared occasionally in the counting chamber, the estimates may not be accurate. To determine if there was any relationship between trunk length and estimated acanthor content, the least squares regression line and correlation coefficient for the relationship were calculated for the entire sample (n = 141).

For each season (Sp, Su and A), the effect of gut position on total acanthor number and worm length was compared by means of a t-

test on transformed data ($y = \log_{10} x$). The proportion of shelled acanthors in worms from different gut regions were compared by means of a X^2 test.

To assess any possible density dependent effects on potential fecundity as reflected in free ovary or acanthor number, worms collected from heavily and lightly infected hosts (<20<) in the Su season were considered. Any gut regional differences were examined by means of a t-test on log transformed data. Since no significant difference was found in worms between gut regions, the data values were combined and any difference in the mean number of free ovaries or acanthors in worms from the 2 groups of hosts was again compared (t-test). In addition the proportion of shelled acanthors in worms from the two host classes was compared (X^2 test) (gut regions separate).

Between season differences in total acanthor counts, worm length and free ovary counts were compared by means of t-tests on log transformed data and the proportion of shelled acanthors by means of X^2 tests between pairs of seasons.

6.3. OBSERVATIONS

6.3.1. Seasonal maturation patterns of *Neoechinorhynchus rutili* in its Scottish fish hosts

6.3.1.1. *Loch Maragan brown trout*

The monthly and seasonal changes in the proportion of various female developmental stages in the *N.rutili* metapopulation in brown trout are shown in Figs 6.1 and 6.2 respectively (original data in Table 5.4). The mean water temperatures (see Chapter 4) have been superimposed on the graphs. Uninseminated females (f1 and f2) were present in every monthly sample, except September 1987, indicating that recruitment was probably occurring throughout the year. By contrast, gravid f3 worms were only recovered during the warm months,

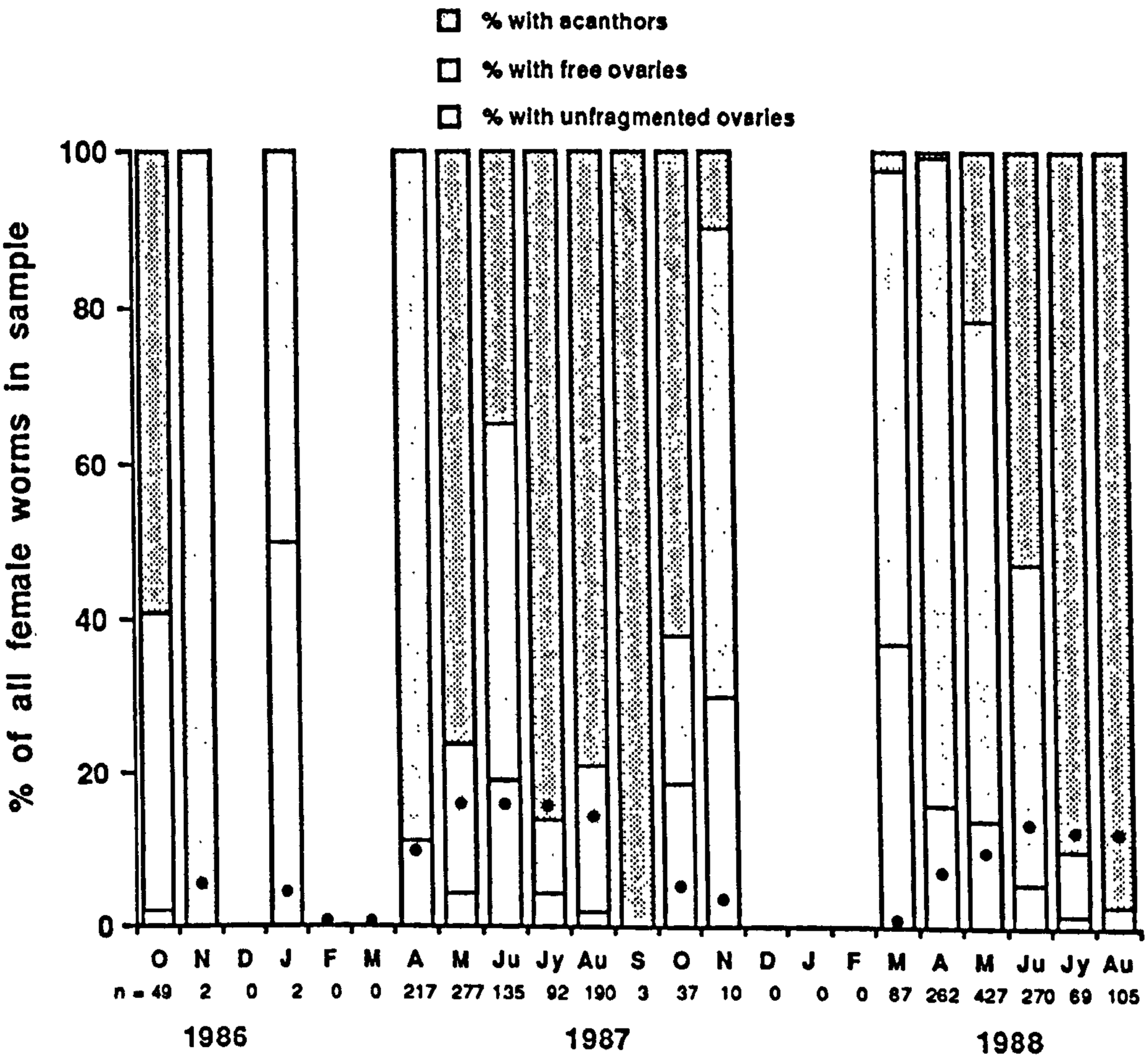


Fig 6.1 Monthly changes in the proportion of female developmental stages *Neoechinorhynchus rutili* in the Loch Maragan brown trout metapopulation

n = number of worms in sample. Dots represent mean monthly water temperatures (°C)

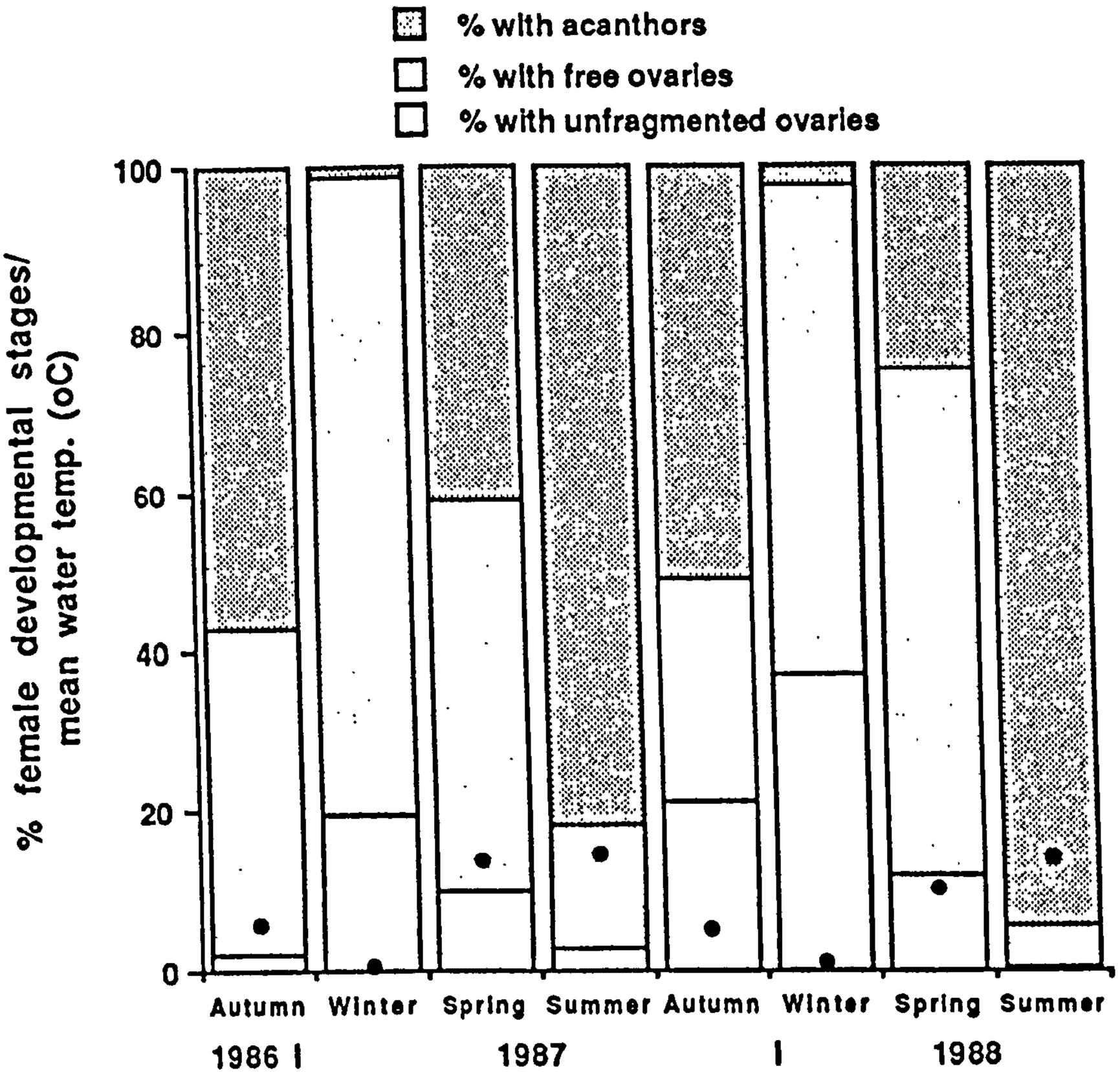


Fig 6.2 Seasonal changes in the proportions of female developmental stages of *Neoechinorhynchus rutili* in the Loch Maragan brown trout metapopulation

Note: Dots represent mean water temperature (°C)

none being found in the November 1986 and the January and April 1987 samples. However, in 1988 the March and April samples contained f3 worms and this may be explained by the fact that the loch thawed earlier (March instead of April) in that year. There was a noticeable similarity in the patterns of increasing water temperature (mean) and the proportion of f3s in the female subpopulation. In both years this proportion was over 80% in the Su season (94% in Su 1988). In October of both years there was a decrease in the proportion of f3 worms with a concurrent increase in that of f1 and f2 worms. This could have been due to a massive loss of f3s from the gut with a simultaneous recruitment of new worms as a result of feeding upon infected *Sialis lutaria* larvae. By late A (November) and throughout the W months, the water temperatures were much lower and the f3 worms formed an insignificant proportion of the metapopulations which were consequently dominated by the f1 and f2 stages. Presumably, the lower water temperatures and host food intake resulted in a reduction in the general metabolic activity of the worms and therefore also sexual activity, resulting in a lower f3 count. In January 1987 and May 1988 the proportions of f1 and f2 worms were almost equal. Later in the year the proportion of the f1s dropped with a concurrent increase in that of the f2s suggesting that the environmental changes were stimulating the f1s to mature into f2s at this time (i.e. ovarian fragmentation). Another explanation could be the possible change in the relative frequency of f1 and f2 worms in the *N.rutili* from dietary elements consumed by trout.

In summary, the general seasonal pattern of maturation (Fig 6.2) showed a clear parallel with the changes in ambient temperature. The Sp water temperatures rose very quickly after the thaw of the W ice and this seemed to be the cue for increased sexual activity and the consequent appearance of more f3 worms in the female population.

Despite the fact that the Sp thaw occurred earlier in 1988, the proportion of f3 worms was significantly lower than in 1987 when the loch did not thaw until late April (X^2 = 45.3, P <0.01: Sp 1987 = 40.85%; Sp 1988 24.8%). A significant difference in the proportions of female developmental stages was also found between Su 1987 and 1988 (X^2 = 14.7, P<0.01: 1987 = 81%; 1988 = 94%). However this result must be treated with caution since the 1988 sample did not include any worms collected in September. No significant differences were found in the female population stucture between years in either A or W (X^2 = 0.9 and 0.3 respectively) but X^2 tests between seasons showed significant differences between adjacent seasons in all cases (P <0.01). These results can be interpreted as representing a seasonal cycle of maturation of female worms at Loch Maragan. A similar annual pattern, repeating over 2 years, with apparent recruitment throughout the year was found with acanthor production restricted to the warmer months of the year.

6.3.1.2. *Other Scottish sites and hosts*

Although data from other Scottish sites and hosts was patchy it indicated the timing of reproductive activity of *N.rutili* in the region (see 5.4.2). In general, f3 worms were seen during the warmer months of the year at most sites e.g. Black Clauchrie in August 1986 (brown trout), Bridge of Weir in May 1987 (rainbow trout), Carbeth Loch in May 1988 (brown trout), Lochan Creag nan Caorann in July 1988 (brown trout) and Powder Works Dam Lochan in June 1986 (brown trout). Minnows from Loch Maragan harboured gravid *N.rutili* in May and August 1987 but not in July 1988. By contrast, f3 worms were recovered from three-spined sticklebacks in Drumore Loch in October, November and in December 1987 when the Loch was frozen over, but this may have been the result of post-capture maintenance conditions. By contrast, gravid

worms were not recovered from rainbow trout in January 1988, brown trout in February 1988 or three-spined sticklebacks in March 1988 from Bridge of Weir.

Comparison of the proportion of f3s in the sampled metapopulations from these sites with the Loch Maragan brown trout data showed some similarities and differences. In May 1988, the relative proportion of gravid female worms in the female subpopulation at Loch Maragan and Carbeth Loch of 21.8 and 86.0% respectively were significantly different (X^2 = 102, P<0.01). In July 1988, the values for Loch Maragan and Creag nan Caorann brown trout were 89.8 and 69.0% respectively (X^2 = 6.38, P<0.05). A similar comparison between the metapopulations inhabiting brown trout (78.9%) and minnows (44.0%) at Loch Maragan in August 1987 indicated that there was also a significant difference in the proportion of f3s present (X^2 = 13.8, P<0.01).

6.3.2. Occurrence of copulatory caps on *Neoechinorhynchus rutili* inhabiting Loch Maragan brown trout

In all cases only terminal copulatory caps (c.c.) were observed on *N.rutili* from Loch Maragan brown trout. No worms were seen *in copula*. Only limited data was available for the 1986 season: one female and 1 x f3 were recovered in July 1986, from hosts harbouring 29 and 30 worms respectively. In the 1987 season, c.c.s were first seen in May on 11 females recovered from the duodenal, ileal and rectal regions of the host gut and on a single male found in the rectum. The females came from hosts with intensities of infection of 116, 94, 143 and 54 respectively and the male from a host infected with 38 worms. Interestingly, none of the female worms from the latter host had c.c. This first seasonal record in 1987 of the presence of c.c.s coincided with the appearance of the first f3 females.

Throughout the rest of the 1987 season, caps were only seen on single females from the ileum in June and in October on a rectal worm. The intensities of infection in these hosts were 56 and 8 respectively. In 1988, f3s were first seen on 18th March but no c.c.s were observed at this time. Copulatory caps were observed as follows (d = duodenum, i = ileum, r = rectum; values in brackets are for intensity of infection): in April 1988; 2 x f2 i (22), 1 x f2 r (114), 3 x f2, 1 x m (84); in May: 1 x f2 r, 1 x m (54) 1 x f2 i (49); in June: 1 f2 d, 5 x f3 i (324) and in August: 1 x m r (34) and 1 x f3 i (23).

From the 1987 and 1988 data, 4 males and 30 females (12 x f2, 18 x f3) from a total worm sample of 3891 worms (2183 female and 1708 males) had c.c.s. This represents 0.23 % and 1.37 % occurrence on male and female worms respectively and suggests that either a very low rate of c.c. production and/or retention. Females with c.c.s were found in all gut regions but capped males were only found in the rectum. The fact that capped f3 worms were found may indicate that in some cases the cap may stay on the worm for some time after copulation and successful insemination.

6.3.3. Acanthor estimates

The relationship between worm trunk length and estimated acanthor content is shown in Fig 6.3. Despite the wide scatter of values a fairly strong positive correlation between these variables was found ($r = 0.513$). The equation for the least squares regression line was $y = 0.016x - 42.769$. This line predicted that worms below 2.67 mm in length should not contain acanthors. The shortest f3 worm found in the entire worm sample, collected in May 1987, was 2.02 mm long.

6.3.3.1. *Effect of gut position on estimated acanthor content*

Analysis of the Sp data for differences in total acanthor number

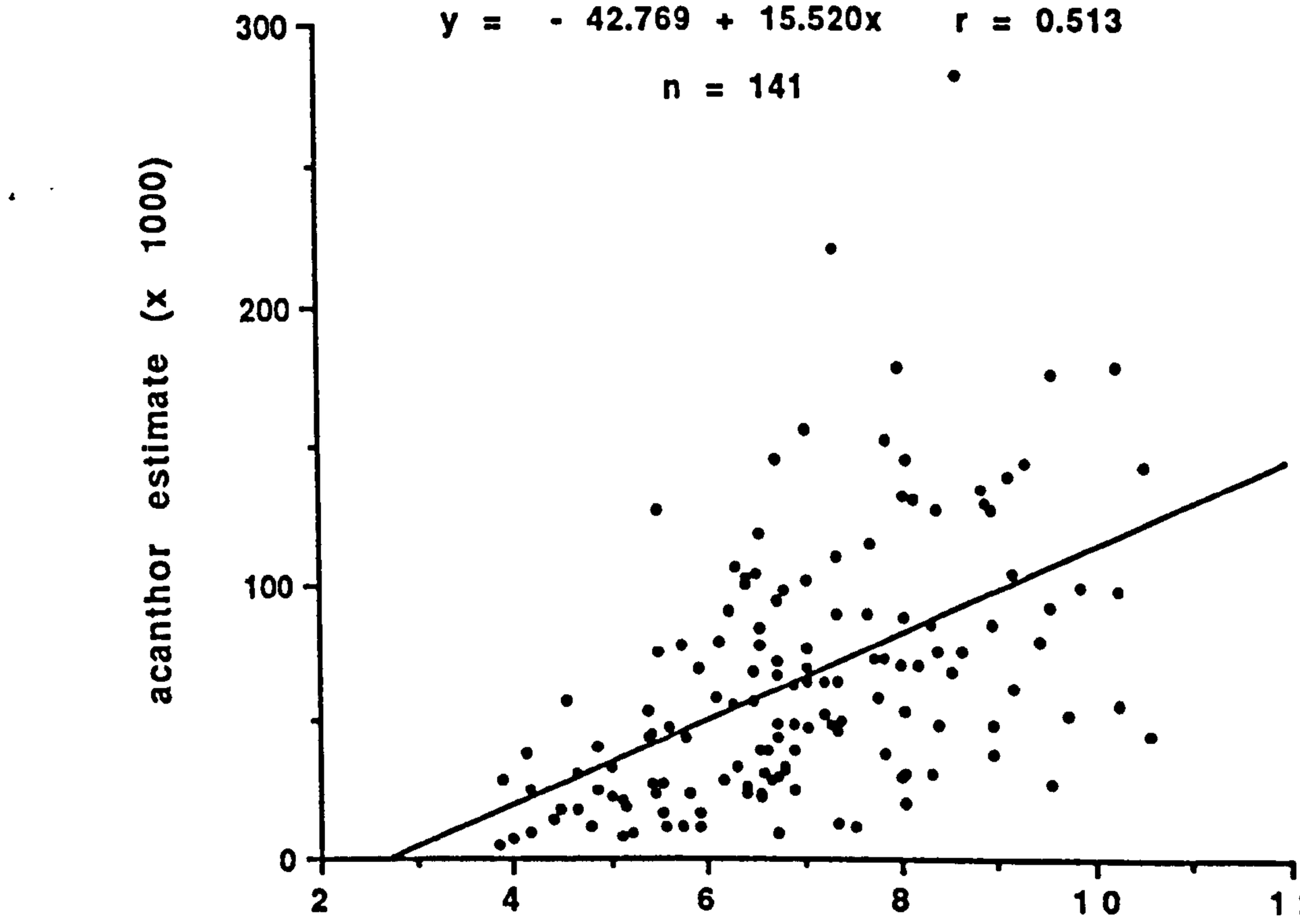


Fig 6.3 Relationship between trunk length and acanthor counts for *Neoechinorhynchus rutili* collected from Loch Maragan brown trout

with respect to gut regional distribution (all heavily infected hosts) indicated that there were no significant differences in mean total acanthor content values (ileum = 71050, rectum = 72250, t= -0.417, P=0.6786) although there were for the trunk lengths (ileum = 6031, rectum = 7008, t= -2.668, P=0.01). Despite the worms in the rectum being longer, the proportion of shelled acanthors in worms from the two gut regions were not statistically different (ileum = 27.6%, rectum = 26.5%, X^2 = 0.58, P>0.05).

6.3.3.2. *Effect of intensity of infection on free ovary and total acanthor content*

The Su worm sample was divided into two groups according to whether they had come from heavily or lightly infected hosts (<20<). Comparisons of the total acanthor estimates between gut regions, within these groups indicated that there was no significant difference between the mean total acanthor content values (I = ileum, R = rectum. High intensity: I = 47140, R = 6771, t= -0.622, P= 0.54; Low intensity: I = 64625, R = 67880, t= 0.19, P= 0.851). Similarly, differences between worms from the same gut region but from hosts harbouring few or many worms were also insignificant (I: low = 64625, high = 64975 :t= 0.693, P= 0.497) (R: low = 67900, high = 68400, t = 0.179, P = 0.859). Therefore no effect of host intensity of infection was found in total acanthor content for either gut region but a significant difference in the proportion of shelled acanthors was found. In the ileal samples, worms from lightly infected hosts had a greater proportion shelled acanthors in the body cavity (X^2 = 26.76, P<0.01, low = 47.23%, high = 30.30%). The reverse pattern was seen in the rectal samples with worms from heavily infected hosts having a statistically significant higher proportion of shelled acanthors than lightly infected hosts (X^2 = 4.77, P<0.05, low = 38.91%, high =

42.56). No significant differences between free ovary counts between gut regions were found in lightly or heavily infected hosts, nor was there any significant difference between these host groups in all gut regions (t - 0.933, P = 0.35).

6.3.3.3. *Seasonal effects upon acanthor content*

Details of the seasonal values for worm trunk length, estimated total acanthor content and shelled acanthor proportions are given in Table 6.2. Since no statistically significant differences were found between the total acanthor counts for worms from the ileal and rectal gut regions in either Sp or Su (all of the A samples came from the rectum), even when worms were derived from hosts with different intensities of infection, the data sets for each season were considered together. Pairwise comparisons of total acanthor counts between seasons indicated that there was no significant difference between Sp and Su (t= -0.328, P= 0.74) or Sp and A (t= 1.398, P= 0.167) but was significant between Sp and A (t= 2.00, P= 0.044). Comparisons of the trunk lengths between seasons (c.f. randomly selected samples) indicated that there were significant differences between Sp and Su (t = -2.65, P= 0.009) and Sp and A (t= -3.44, P= 0.001) but not between Su and A (t= -1.821, P= 0.07). The proportion of shelled acanthors found in worms was statistically significantly different between Sp and Su (X^2 = 195.2, P<0.01) and Sp and A (X^2 = 31.6, P<0.01) but not between Su and A (X^2 = 1.44, P>0.05).

Table 6.2: Seasonal values for reproductive parameters of *Neoechinorhynchus rutili* from Loch Maragan brown trout

Season	n	Mean worm length um	s.e.	% shelled acanthors	Mean acanthor count ($x10^3$)
Spring	53	6437	1371	27.13	71.55
Summer	75	7168	1550	41.58	65.38
Autumn	13	8044	1545	38.88	41.20

Only significant differences are indicated: * = P<0.05, ** = P<0.01

6.3.3.4. *Seasonal effects upon numbers of free ovaries*

Details of the seasonal free ovary counts are given in Table 6.3 The maximum number of free ovaries estimated for an individual worm was 5,500 but there were no significant differences between the estimates for any of the seasonal comparisons.

Table 6.3: Free ovary counts for *Neoechinorhynchus rutili* from Loch Maragan brown trout

Season	n	Number of free ovaries ($x10^2$) Mean	s.e.	range
Spring	63	10.937	1.305	0-55
Summer	19	12.105	2.602	0-55
Autumn	10	11.500	1.675	5-25

6.3.3.5. *Estimate of maximum acanthor production at Loch Maragan*

The estimate for the Su 1987 f3 subpopulation (Table 5.14) was utilized in conjunction with the shelled acanthor estimate for the Su season for worms located in the rectal region to calculate the maximum possible annual production of shelled acanthors by the entire

metapopulation. The estimate for f3 worms in Su 1987 was 5,566 and the mean +/- s.e. for total acanthor counts was 67.89×10^3 +/- 10.93×10^3. The proportion of shelled acanthors in these worms was 0.4257. Thus the total Su shelled acanthor estimate was calculated as follows:

$$\text{Total shelled acanthor estimate} = 67 \times 10^3 \times 5{,}566 \times 0.4257$$
$$= 1.609 \times 10^8 \text{ +/- } 5.0 \times 10^7$$

From this the maximum value was calculated to be 2.117×10^8. If this number of acanthors produces an equal number of reproductive females in the following season, which seems likely given the apparent quantitative seasonal stability of the system, then the percentage success rate of reproduction is:

$$\frac{2.117 \times 10^8}{5{,}566} \times 100 = 3.5 \times 10^{-3} \%$$

This is equivalent to 1 in 38,030 shelled acanthors becoming a reproductively active female worm in the next generation.

6.4. DISCUSSION

6.4.1. Seasonal maturation patterns of *Neoechinorhynchus rutili* in its Scottish definitive hosts

Despite the somewhat crude classification of worm maturity, an apparent seasonal cycle of maturation was observed in the majority of hosts in their various Scottish localities. On the whole maturation, culminating in the production of acanthors, occurred during the warmer months of the year. At Loch Maragan this closely followed water temperature changes and f3 worms were recovered from brown trout hosts for between 6 to 9 months of the year. No acanthor production occurred during winter in these hosts and recruitment, indicated by the presence of f1 and f2 worms, occurred throughout the year. The apparent rapid commencement of reproductive activity when the water

temperatures rose in spring may in part be explained by the precocious nature of the *N.rutili* developmental stages from *Sialis lutaria* larvae. Peura *et al.* (1986) examined this property in *Echinorhynchus gadi*, which has a precocious cystacanth stage and speculated that the biological significance of this phenomenon might be to ensure a short prepatent period in the definitive host. They concluded that this strategy would only be suitable for acanthocephalans with a wide range of host specificity. *Neoechinorhynchus rutili* certainly fulfils this criterion and this precocious trait may be seen as adaptive.

Acanthor production in *N.rutili* was restricted to the warmer months of the year with no gravid females present in winter, a pattern which has been observed elsewhere. This probably indicates that the life span of worms in these hosts in less than 1 year, gravid females being expelled at the end of summer or beginning of autumn. For example, Tesarcik (1970) found acanthors in either the gut samples or faecal samples of carp from the Macha Lake Pond system in Czechoslovakia, from 4th March to 29th July 1966. This pattern repeated itself in the years between 1966 and 1969. By contrast, Scholz (1987) working at the same locality found no gravid *N.rutili* in perch caught in March or May of the sampling year. Eure (1976) found gravid *N.cylindratus* during June, July and August in his worm samples from largemouth bass (*Micropterus salmoides*) from Parr Pond in South Carolina, U.S.A. Halvorsen (1972), although he did not look at acanthor production, found the longest female *N.rutili* in their roach hosts from the River Glomma, Norway, in August.

At other locations several other patterns of acanthor production by *N.rutili* have been observed. Walkey (1967) found gravid females, in his samples of three-spined sticklebacks from Monkton Pond, County Durham, England, throughout the year but the greatest percentage occurred in spring and early summer reaching values of up to 30% of

the worm population at this time. Bwathondi (1976) reported finding mature acanthors within worms in September only, during his year-long study of infections of brown trout from Strathbeg Loch, Grampian. Devine (1988) provided limited seasonal data for a number of host species from Drumore Loch, Tayside. He found gravid female *N.rutili* in both rainbow and brown trout caught in October 1987 but not in any of the 60 three-spined sticklebacks he caught at the same time. Overall the percentage representation of the gravid worms in the female metapopulations from the former 2 host species were 3.57 and 1.87% respectively (based on my own calculations from his raw data). Devine (1988) also reported that both trout species also harboured gravid worms in June 1986, their percentage in the female populations being 46.5 and 54.6% for rainbow and brown trout respectively. Devine (1988) interpreted these results as indicating a peak in maturation in summer. By contrast, my own collections of three-spined sticklebacks from Drumore Loch included gravid female *N.rutili* in the samples from October, November and December 1987. This may have been a genuine observation or could have been the result of post-capture breeding by the worms since their fish hosts were kept in aquarial rooms set at 12^{o}C. Moravec (1984a) found gravid *N.rutili* in April and May only, in carp from the Macha Lake Pond system in Czechoslovakia, a somewhat different pattern to that observed by Tesarcik (1970) some years before at the same site. Steinstrasser (1936) found gravid worms in February and March in his rainbow trout hosts from a trout farm in Germany.

No records of acanthor production by *N.rutili* were given by Bibby (1972) in its minnow hosts in Wales or by Robertson (1953) for brown trout hosts in Dunalastair Reservoir, Tayside. No maturation at all was noted by Chappell (1969c) in a population of three-spined

stickleback hosts in Yorkshire or by Devine (1988) in the same host species from Drumore Loch, Scotland. Valtonen (1979) also reported the same phenomenon for worms recovered from whitefish (*Coregonus nasus*) from the Bothnian Bay. Chappell (1969c) thought that the lack of maturity of *N.rutili* in his samples was because he did not sample older sticklebacks from the whole pond area. He did not comment upon the possible infection and maturity of any infection of brown trout which were present at the site. Both Valtonen (1979) and Devine (1988) described their respective fish hosts as accidental and Devine (1988) attributed the lack of maturity of worms in three-spined sticklebacks to the unsuitable physico-chemical properties of the host.

Chubb (1964) suggested that a definite seasonal cycle of maturation would occur at sites which froze over during winter and Loch Maragan definitely fits this criterion. For example, Amin (1975) found this to be true in a population of *Acanthocephalus dirus* in the Pike River which freezes over in winter and Tedla and Fernando (1969) found the same pattern in *Echinorhynchus salmonis* populations inhabiting yellow perch in Lake Ontario. However, the finding at Drumore Loch, which also freezes in winter (Devine, 1988: p.o.), of gravid worms between October and December in several different host species contradicts this theory. This may have been an artifact due to the post-capture maintenance conditions of the hosts. By contrast, Moravec (1984a) thought that it was the very high summer temperatures (20 to 25^{o}C) at his Czechoslovakian sampling site that brought about a marked seasonal maturation cycle. Considering both viewpoints, the unifying feature is that of the thermal regime at the locality which is claimed to bring about these patterns. The thermal regime can have direct and indirect effects upon this via recruitment, speed of development and loss of parasites from the host (Kennedy, 1975). The possibility that the host endocrine balance influences worm maturation

patterns seems unlikely because worms were found to mature in immature fish hosts.

At Loch Maragan the temperature regime appears to have an important influence upon the maturation cycle of *N.rutili* in its poikilothermic brown trout hosts resulting in a definite period of shelled acanthor production and a seasonal change in the ratio of uninseminated to inseminated female worms. No freezing occurred at Walkey's (1967) site and this may in part account for his observation of continuous acanthor production. The definite period of acanthor production, coupled with the fact that acanthors are not likely to remain viable for more than 6 months (Merritt & Pratt, 1964; p.o.) in the external environment will mean that the following generation of worms in the intermediate hosts will probably be derived from the present year's acanthor production. The seasonality in acanthor production will also tend to induce seasonality in the occurrence in intermediate hosts. An indication of such a pattern was observed in Scottish *Sialis lutaria* larvae infected with *N.rutili* (Chapter 7). Within individual hosts the numbers, sex ratio and intestinal position of worms will effect the onset of mating activity. This will directly depend upon the feeding activity of the host and the nature of the *N.rutili* infection in its prey items.

At other Scottish sites the period of acanthor production is similar to that of Loch Maragan, but there are subtle differences in the ratios of female to male worms and the percentage of shelled acanthors present at any one time. This must be indicative of local differences in the biotic and abiotic factors as stressed by Chubb (1982), despite these sites being in the same climatic region.

In future studies it would be more appropriate to increase the resolution of estimates of reproductive status. Recently, Amin &

Vignieri (1986 a & b) have used the changes in body wall giant nucleus structure in the neoechinorhynchids *Neoechinorhynchus cylindratus*, *N.prolixoides* and *N.robertbaueri* to examine this. Specimens of *N.rutili* from the present study have been sent to Omar Amin for similar analysis and may result in a more precise measure of reproductive activity in both sexes of worm than was attempted here.

6.4.1.1. *The significance of copulatory caps*

The lack of any observation of worms *in copula*, which has been rarely seen for any acanthocephalan species (Crompton, 1985), made it impossible to assess the frequency of copulatory cap production in relation to mating frequency in *N.rutili*. The general view is that the presence of copulatory caps on females is a useful indicator that copulation and probably insemination must have occurred (Crompton, 1985), but this should be checked by examining the free ovaries for the presence of zygotes (Parshad & Crompton, 1981). At Loch Maragan in 1987, copulatory caps were apparently good indicators of the onset of reproductive activity, but not in 1988. The infrequency of copulatory cap occurrence made their use as insemination indicators inappropriate, an observation which Brown (1989) also made in his experimental infections of *Leuciscus cephalus* with *Pomphorhynchus laevis*. Nevertheless, the occasional appearance on worms during the warmer months probably indicates that copulation was occurring throughout this period of the year and/or that caps remained on females for some time after copulation. The possible advantages of a period of attachment of the copulatory cap on a female worm include prevention of sperm loss for the male that made the cap and a temporary obstruction for other males ready to deposit sperm. Although copulatory caps occurred rather infrequently, there was an apparent correlation between their presence and the intensity of worm

infection. For example in Spring 1987, in worms from 2 year old trout hosts, 3.7% of the female worms had c.c. in heavily infected hosts (>50), but none had caps in the lightly infected hosts. Similarly, in his infection experiments with brown trout, Awachie (1966) found that in heavy infections of *Echinorhynchus truttae* there was 120% increase in the number of female worms with c.c in the first 2 weeks of infection. The value for percentage occurrence of c.c. on the Loch Maragan worms is similar to that given by Amin (1986) for *N.cylindratus* in a number of different hosts in spring in Wisconsin, U.S.A. By contrast, Valtonen (1980b) found most of the female specimens of *Metechinorhynchus salmonis* which she collected from white fish in the Bothnian Bay to have c.c. The proportion of males in both groups of host from Loch Maragan during this season were not significantly different and this suggests that in higher intensity infections mating is definitely more frequent and/or the production of copulatory caps is increased to reduce competition between males for females.

Another mechanism for reducing competition between males, is for males to cap each other, thereby preventing their reproductive activity for a period. This so called 'homosexual rape' (Abele & Gilchrist, 1977) has been observed in other fish acanthocephalan species e.g. *Echinorhynchus truttae* by Awachie (1966) and *Acanthocephalus parksidei* by Amin (1975). Both authors thought that the presence of copulatory caps on male worms to be the result of poor sex recognition and hence indiscriminate copulation. If this is the case in *N.rutili*, one would expect 2 patterns of c.c occurrence Firstly, the proportion of capped males in heavily and lightly infected hosts should be equal indicating that no anti-intrasexual competition mechanisms were operating. Examination of the limited data from both 1987 and 1988 from Loch Maragan brown trout hosts showed

that capped males were recovered equally often in both host groups and therefore the actual proportion in heavily infected hosts was lower. However, the fact that all capped males were found in the rectum (n=4) in contrast to capped females, which were found throughout the gut of their hosts may indicate that in this condition they were unable to mate again and hence were passing out of the gut as effectively 'wasted males'. Secondly, the proportion of male and female worms with caps should be equal. This was not the case, females being capped 6 times more often than males and this must indicate that females are positively selected for mating by males. Thus, it appears for *N.rutili* in brown trout, that copulatory caps serve their greatest function in preventing polyandry in females and not in directly reducing intrasexual competition between males.

Both f2 and f3 *N.rutili* were recovered with copulatory caps attached to their posterior ends. The occurrence of capped gravid female *Acanthocephalus parksidei* in their hosts was explained as possibly the result of female worms being commonly fertilized more than once (Amin, 1975) although it could equally mean that the caps remain on the worms for some time after copulation.

The general impression of copulatory cap occurrence is that although infrequent, it probably serves to reduce polyandry and sperm loss in females, especially in heavily infected hosts and may even be the cause of premature loss of so handicapped males from the host gut to the advantage of other competing males.

6.4.2. Acanthor production in *Neoechinorhynchus rutili* from Loch Maragan brown trout

The positive relationship between worm length and the estimate for total acanthor content was not unexpected. Growth of female acanthocephalans has been seen to be accompanied by an increase in

acanthor numbers (e.g. in *Moniliformis moniliformis* in laboratory rats, Crompton *et al.*, 1988). Munro (p.c.) also found a positive correlation between worm length and mean acanthor count, as estimated by a dilution technique, in *Pomphorhynchus laevis* from flounders. The wide spread about the regression line must represent the fact that worms were in different states of maturity and therefore also differed in their stage of acanthor production. This variability must also, in part, be associated with that of the genetic makeup of worms within the population (Dobson, 1986).

The agreement between the observed minimum length of f3 worms and the prediction of the regression line is useful. Walkey (1967) utilized length as a 'quick' method for assessing the state of maturity of the *N.rutili* population from three-spined stickleback hosts and he set the size range for gravid worms at >3.5mm long. For the *N.rutili* metapopulation in Loch Maragan brown trout, it would be necessary to utilize a somewhat shorter cut-off point. This example emphasizes the possible problems associated with extrapolating actual values for use on other parasite populations. Interestingly, the regression line predicted that a worm 10mm long should contain 112430 acanthors and this is similar to the value of 80000 for *Pomphorhynchus laevis* of the same length (Munro, p.c.). Perhaps this numerical similarity provides evidence for similar reproductive strategies in these 2 species.

Analysis of the temporal changes in acanthocephalan reproduction have been carried out before utilizing 'snap-shot' counts of female worms contents in experimental infection (e.g. Crompton *et al.*, 1988). Despite the unknown age of the *N.rutili* infections from Loch Maragan brown trout, which is typical for any field collections (Ashley & Nickol, 1989) it was considered the most appropriate way of analysing

acanthor production. The merit of this technique is that it allows for consideration of individual worms.

At most Scottish sites acanthor production occurred during the warmer months of the year and this must be, in part linked, to the rate of the worms physiological processes as mediated by temperature. However, the more detailed examination of acanthor production has revealed its more subtle elements.

A posterior migration of female worms (f1,2 and f3, Table 5.10) was observed in summer and autumn in Loch Maragan trout. This type of migration has been observed in *Echinorhynchus truttae* in brown trout (Awachie, 1965; p.o. Gladhouse Reservoir). Does this migration have any functional significance for reproduction of *N.rutili*? The spring data indicated that since worms from the ileum and rectum had similar numbers of shelled acanthors, gut position had no effect upon fecundity (all hosts heavily infected). The total numbers of acanthors were also similar between gut regions and hosts of differing levels of infection intensity in summer. Gut regional differences were only manifested in terms of the percentage of shelled acanthors present with ileal worms from lightly infected hosts having significantly more shelled acanthors in their body cavities. How can this phenomenon be explained? Considering the high intensity infections first. In this case the higher density of worms in the gut results in a greater mating success as indicated by the higher proportion of copulatory caps and of f3s found in the populations (5.4.1.4). This tends to suggest that successful mating and fertilization of females occurs earlier in these hosts and they reach maturity sooner than in lightly infected hosts, as was proposed by Awachie (1966) for his experimental infections of brown trout with *Echinohynchus truttae*. These f3 worms then move down the gut as they mature presumably to avoid competition with other worms more anterior in the gut and hence produce more

shelled acanthors.

The posterior migration of acanthocephalans with maturity has been seen in other species e.g Amin & Burroughs (1977) in *Echinorhynchus salmonis* in various fish hosts. Secondly, in lightly infected hosts there should be less competition for nutrients in the anterior gut regions so females can develop their ovarian tissue maximally here after establishment. However, because of lower density of worms present, mating frequency may be reduced. The worms that remain in the ileum may increase their mating success or may stay there until they are mated. If it is necessary for several matings to inseminate all available ova then it would pay the female to remain in this gut region until sufficient sperm has been transferred. Thus, worms with more shelled acanthors would be encountered in this region than further down the gut in these hosts. Overall there appears to be no density dependent effect on fecundity as evidence by the 'snap-shot' results. Perhaps some form of rate analysis in laboratory infections of fish may show otherwise. The correlations between worm length and acanthor estimate and the known smaller size of f3 worms in heavily infected hosts points to the fact that perhaps the Bradley Type III form of population regulation takes place. A similar observation in a population of *Pomphorhynchus laevis* by Kennedy *et al.* (1976) was taken to suggest such a form of regulation but it was stressed that the trend required experimental verification.

The observation that intensity of infection had no effect upon free ovary estimates is difficult to explain. A similar phenomenon was reported by Crompton *et al.* (1988) in infections of laboratory rats with *Moniliformis moniliformis*. In this case they thought that infection intensity only affected establishment in a density dependent way. By contrast, fecundity was thought to be mainly influenced by

host diet. In the present study it was not possible to assess the dietary intake of individual trout.

These hypotheses appear to explain the results, but are in opposition to the observation that in heavier infections worms tend to move forwards in the gut. Perhaps in this case it is the more anterior establishment of new worms that results in this apparently more anterior overall position although the gravid females actually move posteriorly. This needs to be examined further.

The seasonal pattern of changes in mean total acanthor counts is difficult to interpret entirely on the basis of the 'egg package' form of egg release. Large numbers of acanthors were present in f3 worms spring and summer with a significant decrease in autumn. With reference to the 'egg-package' hypothesis this could indicate that in spring and summer maximum acanthor production by the initally established worms occurs these being lost in summer when an apparent plateau of production was reached. The lower value in autumn could be explained by the fact that these are from worms established later in the year and therefore mated later and perhaps less frequently and due to physiological contraints of decreasing water temperatures, are not able to produce so many eggs. If significant egg release via the uterine bell occurs then this could also explain the observed decrease in autumn. Munro (p.c.) found seasonal stability in the proportion of shelled acanthors present in *Pomphorhynchus laevis* from flounders and cited this phenomenon as evidence for uterine bell function. Whitfield (p.c.) suggested that a seasonal flip between egg release in whole worms and single egg release via the uterine bell might be an adaptation to ensure infection of all potential intermediate hosts. In the case of *N.rutili* the former type for *Sialis lutaria* larvae and the latter for ostracods. This seasonal flip in release strategy could also take advantage in seasonality of occurrence of these

invertebrates.

In terms of percentage of shelled acanthors occurring in worms during the year the pattern is much clearer, with a build-up of the mature forms following the increasing water temperatures in the locality, reaching an apparent plateau in autumn when worms would be lost from the host fish.

The lack of seasonality in free ovary counts can be interpreted in different ways. Firstly, it could indicate that the influx of new recruits throughout the year keeps the average value high despite a reduction in counts in more mature individuals. Secondly, it could indicate the continued stability of free ovary counts in all seasons. The latter seems somewhat more likely because Crompton *et al.* 1988 found a build up of ovary numbers over the course of a *Moniliformis moniliformis* infection in laboratory rats. In all cases the estimates of free ovary populations were much greater than those for f2 worms recovered from *Sialis lutaria* larvae (up to 30 times more) and this must indicate that free ovary population increases in size while in the definitive host. Similar increases in terms of order of magnitude have been noted for *Moniliformis moniliformis* in laboratory rats (Crompton *et al.*, 1976).

6.4.3. Reproductive success of the *Neoechinorhynchus rutili* metapopulation inhabiting brown trout at Loch Maragan

The estimate that only 1 in 38,030 shelled acanthors would be likely to become a reproducing female in the next generation indicates that in this host-parasite system severe population regulation is taking place during the life cycle. Additionally the apparent high fecundity of *N.rutili* females indicates that this species should be regarded as being at the r end of th r-k selection spectrum. An r selectionist can be described as a species for which

the optimum strategy is to place 100% of its matter and energy into reproduction, with a minimum into each individual offspring so as to produce the maximum number of progeny (Esch *et al*, 1977). This high fecundity has been seen as an adaptation to the parasitic mode of life. The question in the context of *N.rutili* is what brings about this population regulation and hence forces *N.rutili* to be an r selectionist? Esch *et al*. (1977) described aspects of climate, survivorship, mortality, population size, competition, development time, body size and length of life in parasite systems as being determinants of for the r strategy. How might these factors act upon the apparently large number of acanthors produced by gravid *N.rutili* to regulate the population? Factors include: egg viability, egg loss, transmission to the intermediate hosts, development period in intermediate hosts, transmission to trout, establishment in trout, mating etc. These need to be estimated and filled into the framework to allow for comparison with Dobson & Keymer's (1985) model.

This estimate of reproductive success may even be exaggerated if the viability of the shelled acanthors is equivalent to that of *Moniliformis moniliformis* as described by Arnold & Crompton (1987) with acanthors remaining viable for up to 120 weeks. However, Panton's (1987) finding that *Echinorhynchus truttae* acanthors rot in water quite rapidly and my own finding of *N.rutili* acanthors also rotting after being kept in the refridgerator for a few weeks suggests that no build-up from year to year could occur. The observation of Tesarcik (1970) that eggs only occurred in the Macha Lake pond sediments in Czechoslovakia, where carp were infected with *N.rutili* in some months of the year suggests that no build up of acanthors between years occurs in nature.

6.5. SUMMARY

6.5.1. At Loch Maragan shelled acanthor production by *N.rutili* was observed to be seasonal, being restricted to the months between March and November in the 3 sampling years.

6.5.2. As the proportion of f3s in the female subpopulation from the Loch Maragan brown trout increased, there was a corresponding decrease in the f1,2 subpopulation.

6.5.3. Recruitment of new worms apparently occurred throughout the year.

6.5.4. It was speculated that the well defined seasonal cycle of maturation of *N.rutili* in brown trout at Loch Maragan was initiated by the temperature regime at the locality and reinforced by seasonality in trout host feeding behaviour and availability of *N.rutili* in prey items.

6.5.5. The timing of maturation in *N.rutili* appears similar in all the Scottish sites examined. Quantitative differences were attributed to local differences in abiotic and biotic factors influencing the host-parasite system.

6.5.6. The presence of copulatory caps on *N.rutili* from brown trout collected at Loch Maragan was infequent, 0.23% and 1.37% of male and female worms were capped respectively.

6.5.7. First observation of copulatory caps was not a reliable indicator of the onset of reproductive activity.

6.5.8. The significance of capped males is discussed in terms of intrasexual competition.

6.5.9. Copulatory caps occurred on f2 and f3 worms and may indicate that they remain on females for some time after copulation.

6.5.10. There was a positive relationship between numbers of shelled acanthors in the body cavity and female worm length. The wide

scatter around the regression line was accounted for by the differing states of maturity or age of the worms in the sample and their individual genetic makeup.

6.5.11. For a quick estimation of the state of maturity of female worms from brown trout from Loch Maragan without microscopical examination a cut-off length between 2 and 2.5mm for gravid and non-gravid worms would be appropriate.

6.5.12. There was no effect of gut position on the estimated total acanthor content or proportion of shelled acanthors in worms within seasons.

6.5.13. In summer-collected worms, there was no difference in the total acanthor content of worms from heavily and lightly infected hosts.

6.5.14. Differences in shelled acanthor contents of worms from heavily and lightly infected hosts were found. Ileal worms had a greater proportion of shelled acanthors in lightly infected hosts and rectal worms showed the reverse pattern.

6.5.15. No differences in free ovary counts were found between seasons, gut regions or between hosts with different intensities of infection.

6.5.16. Mean acanthor counts decreased between summer and autumn and the percentage of shelled acanthors increased between spring and summer.

6.5.17. The maximum number of free ovaries estimated for an individual female worm was 5500.

6.5.18. The estimated mean total acanthor production by individual female worms in Summer 1987 in Loch Maragan brown trout was 67.89 $\times 10^3$ +/- 10.93 $\times 10^3$ (s.e.).

6.5.19. The proportion of shelled acanthors was 42.57% in summer 1987 which represents a total of 1.6 $\times 10^8$ shelled acanthors

produced by a population of 5566 female (f3) worms.

6.5.20. The success rate of reproduction of *N.rutili* inhabiting Loch Maragan brown trout was calculated as $3.5 \times 10^{-3}\%$ which is equivalent of 1 in 38030 shelled acanthors becoming a reproductively active female in the next parasite generation.

CHAPTER SEVEN

THE ROLE OF *SIALIS LUTARIA* (MEGALOPTERA) LARVAE IN THE LIFE CYCLE OF *NEOECHINORHYNCHUS RUTILI*

7.1. INTRODUCTION

Knowledge about the life cycle of *Neoechinorhynchus rutili* suggests that it involves ostracod intermediate hosts only (Merritt & Pratt, 1964; Walkey, 1967; Valtonen, 1979) and a diverse range of fish definitive host species. There are at least 8 separate reports from this century describing 6 species of ostracod acting as intermediate hosts for *N.rutili* (see Table 4.7, Chapter 4). However, in many localities where the definitive fish hosts are reported to be infected with *N.rutili*, the intermediate hosts are not known. *Neoechinorhynchus rutili* appears to be a typical eoacanthocephalan, in that its intermediate host is a crustacean species (Bullock 1969, Buron & Golvan, 1986). The contrary reports of Robin (1871) and Villot (1885) which describe the leech *Erpobdella octoculata* and the alder fly larva *Sialis niger=Sialis lutaria* as hosts for *N.rutili* respectively, seem to have been more or less ignored. Villot (1885) described the larval *N.rutili* which he found in the fat body of *Sialis lutaria* larvae, as being encapsulated and it may therefore have not been considered as a likely host. Despite this, Schmidt (1985) in his collation of information about the intermediate hosts of the Acanthocephala obviously considers these reports to be important and describes these 2 species as either the facultative second intermediate or the paratenic host (Nickol, 1985b) of *N.rutili*. The detailed studies of the life cycle of *N.rutili* by Walkey (1967) and Merritt & Pratt (1964) made comments on these alternative intermediate hosts. Walkey (1967) dismissed both species as being intermediate hosts for *N.rutili* and suggested that they are probably only paratenic hosts which become infected as a result of feeding upon infected ostracods. Indeed, in his study population none of the fish fed upon leeches, while feeding upon *Sialis* larvae was extremely restricted in number and season (only

recorded in the diet in February, March and April). He was also unable to infect the latter experimentally with *N.rutili*. Merritt & Pratt (1964) reported that they were investigating the role of *Sialis lutaria* in the life cycle of *N.rutili* at their site, Suttle Lake, Oregon, and intended to publish their results but no such report appears in the citation list. No further consideration of the role of these organisms in the life cycle of *N.rutili* appears to have been made until the present study on *S.lutaria* larvae, the main details of which have been published by Lassiere (1988a).

7.1.1. Observations of the life cycle of *Neoechinorhynchus rutili* in Scottish populations

My main interest in the intermediate host species of *N.rutili* arose for two reasons. Firstly, in the Loch Maragan population of brown trout which is infected with *N.rutili* and contributes much to this thesis, I never found ostracods as part of the trout diet (see Chapter 4) and benthic faunal surveys at Loch Maragan indicated that ostracods were not common. Therefore what was acting as the transmission host for *N.rutili* to trout at this site? Robertson's (1953) report of finding a single alder fly larva harbouring a larval *N.rutili* in Lochan an Daim, Scotland (Site 17) where the brown trout were also infected, led to the consideration of this insect larva's having a role in the life cycle at other Scottish sites including Loch Maragan.

Secondly, an interesting piece of field evidence came to light: a brown trout caught from Loch Monzievaird, Scotland (Site 30) which was harbouring *N.rutili* in its gut was also found to be feeding on *Sialis lutaria* larvae. Routine dissection of these dietary elements revealed an acanthocephalan parasite lying free and unencapsulated in the haemocoel of the insect. Collection of further larvae from the same

site indicated that 7% were infected with the same acanthocephalan. Therefore this site provided the ideal material for the experimental examination of this possible transmission route. The subsequent finding of *S.lutaria* larvae infected with encapsulated *N.rutili* larvae at the West of Scotland trout farm, Bridge of Weir, Scotland (Site 6) provided an opportunity to examine whether encapsulation, similar to that described by Villot (1885), had a qualitative effect on transmission. The finding of 2 further sites, Drumore Loch (Site 11) and Powder Works Dam Lochan (Site 35) where *N.rutili* was infecting both trout and *S.lutaria* larvae further justified the examination of this aspect of the life history of *N.rutili*.

Thus the work described in this chapter aimed to extend the isolated and preliminary reports of Villot (1885) and Robertson (1953) and my own field observations at a number of sites in Scotland by an experimental investigation of the role of *Sialis lutaria* as a transmission host of *N.rutili* to trout. These experiments were complemented by an attempt to discover how these insects might themselves have become infected. Also attention was given to the significance of infections of *S.lutaria* at a number of Scottish sites.

7.2. MATERIALS AND METHODS

7.2.1. Light and electron microscopy

Live worms were photographed with a Leitz 22 EB microscope using ASA 32 Panatomic black and white print film. Specimens for scanning electron microscopy were fixed in 5% formalin, dehydrated in an ethanol series, critical point dried in CO_2 using a Polaron 3000, and sputter coated using a Polaron E5000. Specimens were viewed at 3kV on a Philips 500 scanning electron microscope (Brown, Chubb & Veltkamp, 1986).

Specimens of *Sialis lutaria* infected with *N.rutili* (n=3) were

fixed in Carnoy's fluid for 3 h, dehydrated in absolute alcohol for 2 h and cleared in Histoclear overnight. Specimens were transferred to molten wax for 2 periods of 2 h each and then embedded in paraffin wax. Serial sections of 7 um thickness were cut through the insect larvae in both transverse and longitudinal planes and were stained with Mallory's triple stain. Sections were photographed as described above.

The identity of the acanthocephalan from the *S.lutaria* larvae was established through the measurement of several body characters of whole mounted specimens (Chappell, 1969c; Bullock, 1969) and from examination of scanning electron micrographs of the proboscis compared with the proboscis morphology of specimens of known identity. The nature and form of the encapsulating layer around some *N.rutili* larvae from Bridge of Weir (Site 6) *S.lutaria* specimens was examined using the S.E.M. and in serial wax sections prepared as described above. Observations of encapsulated specimens were also made on fresh specimens of *N.rutili* from *Sialis lutaria* larvae collected from Drumore Loch, Loch Maragan and Bridge of Weir, and of preserved specimens from Powder Works Dam Lochan.

7.2.2. Source and maintenance of rainbow trout

The rainbow trout (*Salmo gairdneri*) used for the two experimental transmission experiments were obtained from the River Doon Trout Company, Cassilis Mill, Ayr, Scotland. For each experiment (designated 1 and 2) 21 trout were purchased, having an average wet weight of 230 and 262g respectively. Fish were kept in 60 litre tanks with secure lids in well-aerated copper-free tap water in a constant temperature room set at 12°C with from 1 to 3 fish per tank. The trout were left to settle for 2 days before any experimental manipulation. All experiments were conducted with the necessary approval of the Home

Office.

7.2.3. Source and maintenance of alder fly larvae for transmission experiments

For experiment 1 the alder fly larvae were collected from the benthic material along the southern shore of Loch Monzievaird near Crieff, Perthshire (Site 30) during April and May 1987. The life cycle of *S.lutaria* lasts 2 years and the larvae undergo 10 instars prior to pupation in the soil (Elliot, 1977a; Fitter & Manuel, 1986). Since *S.lutaria* exhibits an allometric growth pattern, their relative sizes were compared by measurement of the head width using an ocular micrometer (Giani & Laville, 1973).

For experiment 2 the insect larvae were collected from the narrow, shallow drainage channels at West of Scotland trout farm, Bridge of Weir (Site 6) on 26.2.88. and 11.3.88 with a hand net and were kept at 10°C in a refrigerator in copper-free tap water prior to use.

7.2.4. Collections of alder fly larvae from Scottish sites

During the course of the present research, samples of *Sialis lutaria* larvae were collected from a number of sites and the distribution of *N.rutili* infection was examined in specimens of various origin (Table 7.1). For each specimen the head was removed and a mid-dorsal incision was made of the larva and the whole body was examined for the presence of parasites under a binocular microscope. The head widths of the *Sialis lutaria* larvae were measured and a frequency distribution graph was plotted showing both infected and non-infected individuals. For each sample the mean intensity of infection, variance and also the k value were calculated in order to assess the degree of overdispersion of *N.rutili*. The extent of agreement with the negative binomial distribution was calculated using

the method described by Elliot (1977b). The frequency distribution of *N.rutili* within *S.lutaria* larvae was plotted for each site giving the expected values from the negative binomial distribution. Utilizing the data of Giani & Laville (1973) for the range of head widths for each larval instar, the percentage prevalence and mean intensity of infection of each instar was calculated. In some cases individual worms were sexed and their fresh trunk length measured. The mean values for the trunk lengths of immature females, females with free ovaries and males were calculated for some samples. When worms were observed to be encapsulated, comparisons between the lengths of encapsulated and unencapsulated individuals were made using a Mann-Whitney U test. The number of free ovaries present in the body cavity of a sample of worms from Loch Maragan was determined by direct counting. The structure of the populations of *N.rutili* in the *S.lutaria* samples was also examined in terms of sex ratio and the distribution, according to sex, of worms in individual insect larvae. These analyses were undertaken to gain an impression of the dynamics of this host-parasite relationship at a number of field sites in Scotland where *N.rutili* is known to infect some of the resident fish species.

Table 7.1: Details of *Sialis lutaria* collections from various Scottish sites

Site	Sample origin	Date	No. collected	No. dissected
Bridge of Weir (site 6)	drainage channels Expt.	26.2.88.	98	50
		11.3.88.	299	29
		both dates	-	154
		All	397	233
Drumore Loch (Site 11)	loch edge detritus	31.10.87.	9	9
		11.11.87.	19	19
		10.12.87.	26	26
		All	54	54
Loch Maragan (Site 29)	loch edge detritus	27.7.88.	30	30
		20.8.88.	15	15
		All	45	45
	trout diets	10.86.-6.88.[a]	328	328
Loch Monzievaird (Site 30)	loch edge detritus	2.4.87.	235	135
		28.4.87.	82	31
		mixed[b]	-	40
		28.7.87.	308	126
		31.10.87.	378	50
		All	1003	382
Powder Works Dam Lochan (Site 29)	trout diets	22.6.86.	32	7

a = see Chapter 4 for details of trout diets; b = includes larvae collected in April and 5 May 1987.

7.2.5. Transmission experiments

Several transmission experiments were carried out to examine aspects of of the role of *Sialis lutaria* in the life cycle of *N.rutili*.

7.2.5.1. *Consumption of ostracods by* Sialis lutaria *larvae*

Several trials were conducted to see *S.lutaria* larvae are capable of feeding upon ostracods. Individual *S.lutaria* larvae of a range of sizes were placed in crystallizing dishes (vol. 100ml) containing copper-free tap water, filter paper to provide shelter and 10 ostracods. Weekly counts of the numbers of ostracods in each dish were made to assess whether feeding had taken place. Ostracods of the species *Herpetocypris reptans* collected from the shores of Drumore Loch (Site 11) by sieving loch detritus were utilized in this experiment.

7.2.5.2. *Experiment to infect* Sialis lutaria *larvae with* Neoechinorhynchus rutili *acanthors*

7.2.5.2.1. <u>*Rationale*</u>

Since experiments to see whether *Sialis lutaria* larvae would feed on ostracods were unsuccessful (see section 7.3.5.1) this experiment aimed to determine whether the insect larvae could become infected directly by feeding upon *N.rutili* acanthors within the body cavity of female *N.rutili*.

7.2.5.2.2. <u>*Method*</u>

Fifty randomly selected *S.lutaria* larva from a sample of 308, collected from Loch Monzievaird on 28.7.87., were dissected and found to be free of any *N.rutili* infection. The remaining insect larvae were arranged into 3 groups according to size, so that size-related differences in resistance to infection would be detected. The head widths of 10 randomly selected larvae were measured from each size class. Each size group was divided equally and placed in a 5 litre tank lined with filter paper and filled up to a depth of 30 mm with copper-free tap water in a constant temperature room set at 12°C (Table 7.2).

Table 7.2: Numbers of *Sialis lutaria* larvae per tank

	Size class		
Treatment	1	2	3
Experiment	38	40	8
Control	38	39	8

(Mean head widths of size classes: 1 = 1763um (Instar 8-9); 2 = 2222um (Instar 10); 3 = 2493um (Instar 10))

Thirty five gravid *N.rutili* were each cut in half and divided into 3 groups. Each group was added to each of the 3 experimental tanks and 57 days later the surviving *S.lutaria* larvae were dissected and examined for evidence of any *N.rutili* infection. The duration of the experiment was designed to correspond to the development time quoted by Merritt & Pratt (1964).

7.2.5.3. *Experimental infection of ostracods with* Neoechinorhynchus rutili

7.2.5.3.1. *Introduction*

The ostracod species *Herpetocypris reptans* was found in large numbers at two sites of *N.rutili* infection of trout in Scotland: Loch Monzievaird, Site 30 and Drumore Loch, Site 11. *Herpetocypris reptans* (Baird, 1835) is a detrital feeder which spends all of its time on the bottom or climbing on vegetation (Hounsome, p.c.; Tressler, 1959) and would be a potentially suitable intermediate host of *N.rutili*. Walkey (1967) conducted a number of experiments in which he tried to infect several species of ostracod with *N.rutili* acanthors. He experimented with *Candona candida*, *Cypria ophthalmica*, *Cyclocypris serena* and *Herpetocypris reptans*. *C.candida* and *C.ophthalmica* were the only

species to acquire successful infections. These 2 ostracod species were also found to be acting as functional intermediate hosts for *N.rutili* in a natural system involving three-spined sticklebacks (*Gasterosteus aculeatus*) (Walkey, 1967). Although *N.rutili* has been reported from 8 fish species in 41 locations around Scotland, the intermediate hosts have not been identified at any of these sites. Despite Walkey's lack of success at infecting *H.reptans*, the value the attempt to infect this species was justified because of its high population densities at sites of *N.rutili* infection and its known benthic habit. Successful infection would also allow the acanthellae to be compared with specimens recovered from *Sialis lutaria* larvae.

7.2.4.3.2. *Method*

Five live, gravid female *N.rutili* were placed into 500ul tapwater in a watch glass. The body of each worm was teased apart with fine forceps and seekers in order to release the body contents. The suspension was thoroughly agitated prior to a drop being removed and placed onto a Neubauer haemocytometer. Two counts were made and the approximate numbers of shelled acanthors and immature eggs were calculated for the total suspension. The volume of solution that contained approximately 600 shelled acanthors was calculated to be 20ul.

This dose of shelled acanthors was sel ted to ensure that each ostracod would be exposed to 60 acanthors n contrast to Walkey's (1967) experiment in which up to 20 shelled canthors were offered to each ostracod. Twenty microlitres of 'acanthor' suspension was added to each of 5 large solid watch glasses (55mm x 55mm x 20mm) containing 10 randomly selected *Herpetocypris reptans* and these were maintained at around 15°C for 26 days (in a constant temperature room). To prevent evaporation, each watch glass was covered with a greased glass

lid. Five similar control sets of 10 ostracods were set up. Twenty six days after the introduction of the shelled acanthors, the number of surviving ostracods was counted and each living individual was dissected and examined microscopically. The control ostracods were also examined. This time period was 2 days longer than in Walkey's (1967) successful experiments because the temperature was 3°C lower on average.

7.2.5.4. *Experimental transmission of* Neoechinorhynchus rutili *to rainbow trout via feeding with infected* Sialis lutaria *larvae*

The same basic experiment was conducted on 2 occasions, the main difference being the source of the infected *S.lutaria* larvae (see section 7.2.3).

7.2.4.4.1. *Experiment 1*

A batch of 160 *S.lutaria* larvae from Loch Monzievaird were randomly divided into 4 groups of 40 larvae each. One of these groups was dissected and the prevalence of *N.rutili* infection was found to be 7.5% and each of the 3 infected larvae in the group harboured a single *N.rutili*. Twenty-one rainbow trout (see section 7.2.2) were randomly divided into 2 groups, 1 group of 3 fish for the experiment and 1 group of 18 fish for assessment of gut parasite status. On the basis of the results of the dissection of the control group of *S.lutaria* larvae, by exposing each of the 3 experimental trout to 40 larvae the trout should have had the possibility of acquiring 3 *N.rutili*. Each experimental trout was housed in a separate tank and exposed to 40 larvae and 7 days later, the trout were killed and the guts were examined for *N.rutili*. Any worms found in any of the 21 trout were measured fresh, photographed, their sex and state of maturity was assessed and their position in the gut was recorded according to the

regions described by Burnstock (1959). Worms were subsequently prepared as whole mounts and measured.

7.2.5.4.2. *Experiment 2*

A batch of 267 *S.lutaria* larvae, collected on 11.3.88. and 48 collected on 26.2.88. from Bridge of Weir (Site 6), were randomly arranged in 7 groups of 45 larvae each. The prevalence of infection in one of these groups was 2.22%, which represents one infected larva in 45. Twenty-one rainbow trout were randomly divided into 2 groups, one of 6 for experimental feeding with *S.lutaria* larvae and one of 15 for immediate dissection for the assessment of gut parasite status. The 6 trout were exposed to the groups of *S.lutaria* larvae individually. After 1 day, 3 experimental trout died and these were dissected and the number and state of digestion of consumed larvae was noted. Any undigested or unconsumed *S.lutaria* were dissected and their parasite status assessed. Nine days post-exposure to the insect larvae the 3 remaining experimental trout were killed and examined for the presence of an *N.rutili* infection as described in the previous section. In addition, the position of attachment with respect to the entire post-pylorus length was recorded.

7.3. RESULTS

7.3.1. Identity of the acanthocephalan from *Sialis lutaria* larvae

The acanthocephalans found in the larvae unquestionably belonged to the class Eoacanthocephala as defined by Bullock (1969). The parasites from the alder fly larvae possessed characters listed by Yamaguti (1963) of the genus *Neoechinorhynchus*, including a small cylindrical body with 4-5 giant hypodermic nuclei on the dorsal side and 1-2 on the ventral side, a short globular proboscis bearing 18 hooks arranged in 6 spiral rows of 3 hooks each, anterior proboscis

hooks of considerably larger size than the more posterior ones, long and digitiform lemnisci and testes positioned in the mid-region or posterior half of the body. Furthermore, the worms had a syncytial cement gland with a rounded cement reservoir. These acanthocephalans from the larval alder flies were identified as *N.rutili* according to Van Cleave & Lynch (1950). The male and female *N.rutili* from alder fly larvae showed close similarities to *N.rutili* taken from brown trout (Fig. 7.1). The similarity in proboscis morphology is revealed in the scanning electron micrographs of the proboscides of *N.rutili* from alder flies and roach (Fig. 7.2). The measurements of various body characteristics are compared with similar measurements of worms from a number of other sources (Table 7.3). Although there are obvious differences in body length measurements the hook and proboscis measurements of worms from *S.lutaria* larvae in the present study are similar to worms from other invertebrate hosts and definitive hosts. In general, the specimens from *S.lutaria* larvae were larger in terms of body length than those described by Villot (1885) from *Sialis niger* and by Merritt & Pratt (1964) from ostracods (*Cypria turneri*), however differences in specimen preparation may partly account for this.

7.3.2. Observations of *Sialis lutaria* larvae infected with *Neoechinorhynchus rutili*

7.3.2.1. *Size distribution of* Sialis lutaria *populations and their* Neoechinorhynchus rutili *infections*

Collections of benthic *S.lutaria* larvae and of those consumed by trout were made at 5 sites (Table 7.1). The numbers of larvae consumed by trout at Powder Works Dam Lochan were insufficient for any graphical display of the distribution of infection and are described in the text below. Evidence of 32 (30 heads measured) *S.lutaria* larvae from instars 5 to 10 consumed by 3 brown trout caught at Powder Works

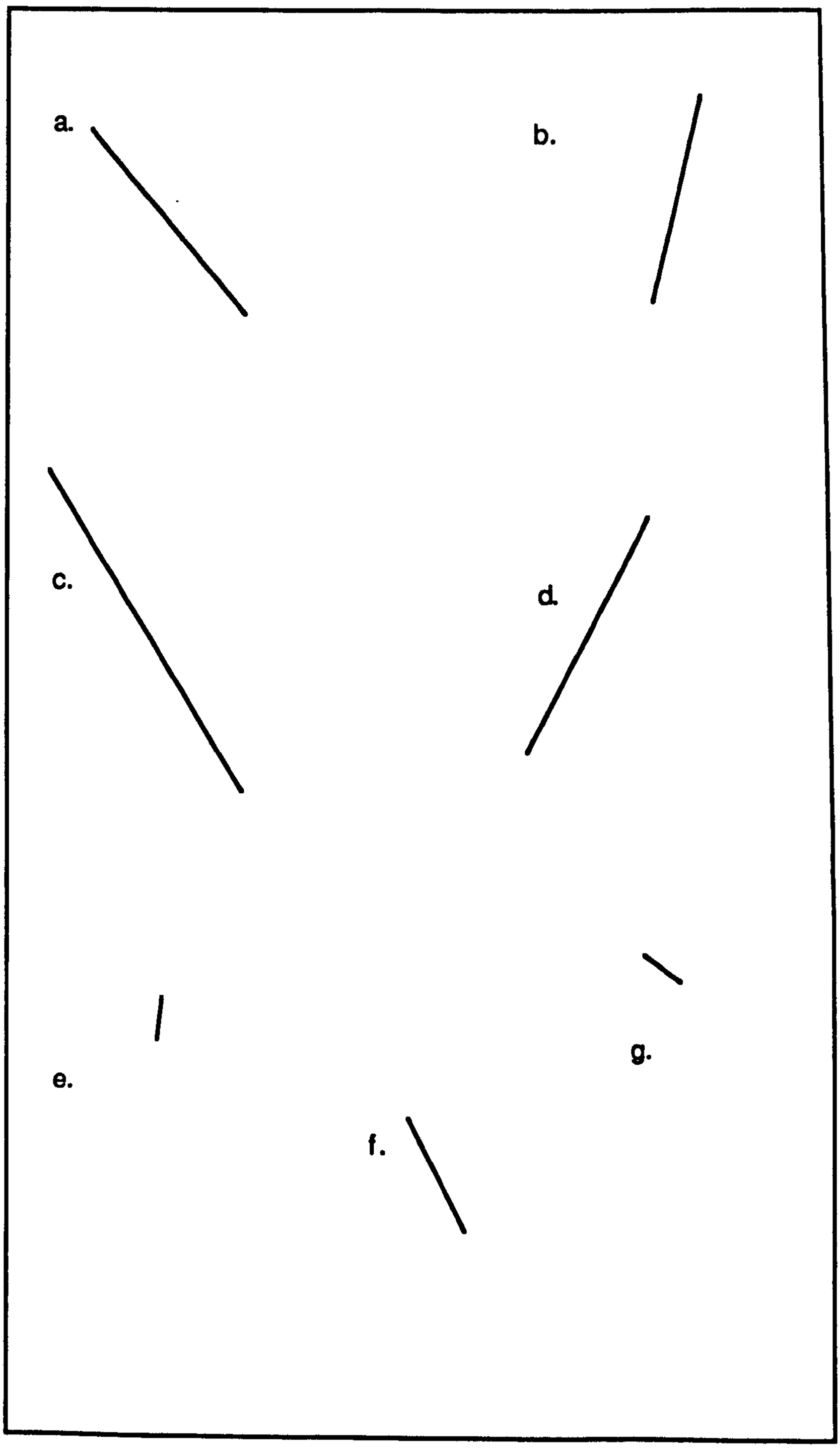
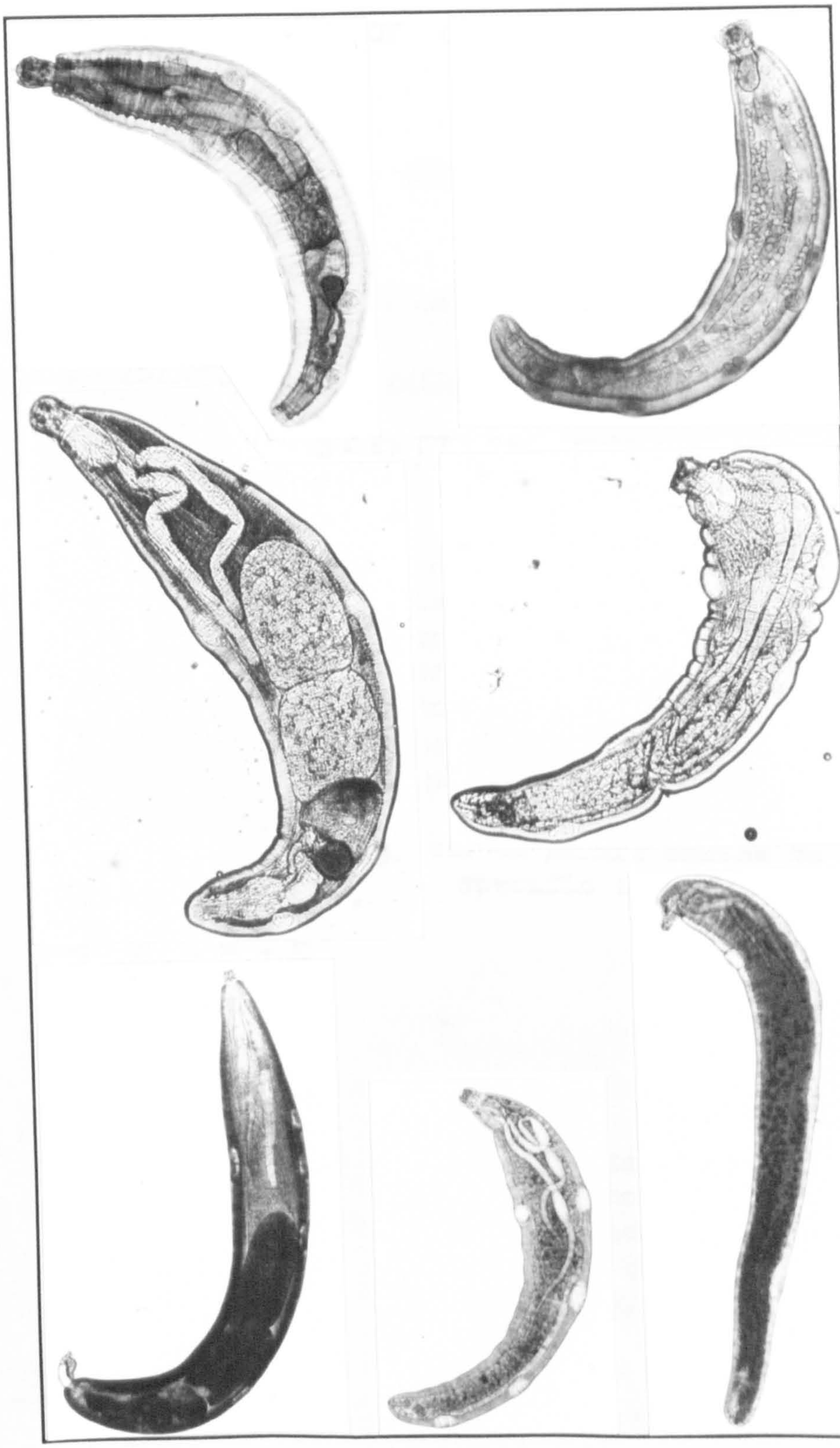


Fig. 7.1 **Comparison between live unstained specimens of *Neoechinorhynchus rutili* collected from larval alder flies (*Sialis lutaria*) (a & b), experimentally infected rainbow trout (*Salmo gairdneri*) (c & d) and brown trout (*Salmo trutta*) (e, f & g). (All bars represent 1 mm)**

Specimens a, c & e are male, note the prominent testes and copulatory bursa (e). Specimens b, d, f & g are females. g. has been inseminated and contains large numbers of acanthors. Note the size difference between specimens from different hosts and also the large subcuticular nuclei which are characteristic of the genus.



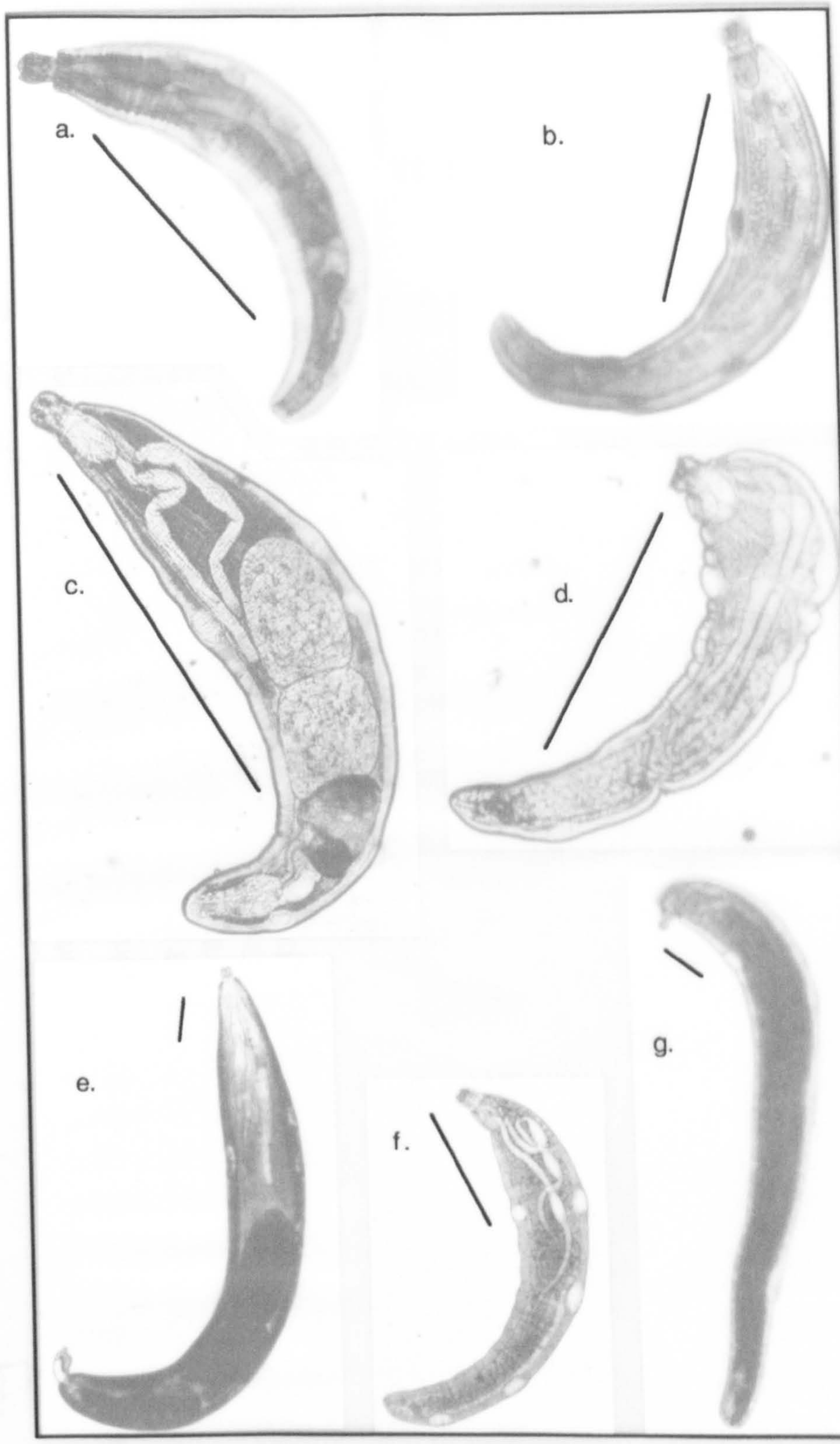


Fig. 7.1 Comparison between live unstained specimens of *Neoechinorhynchus rutili* collected from larval alder flies (*Sialis lutaria)* (a & b), experimentally infected rainbow trout (*Salmo gairdneri*) (c & d) and brown trout (*Salmo trutta*) (e, f & g). (All bars represent 1 mm)

Specimens a, c & e are male, note the prominent testes and copulatory bursa (e). Specimens b, d, f & g are females. g. has been inseminated and contains large numbers of acanthors. Note the size difference between specimens from different hosts and also the large subcuticular nuclei which are characteristic of the genus.

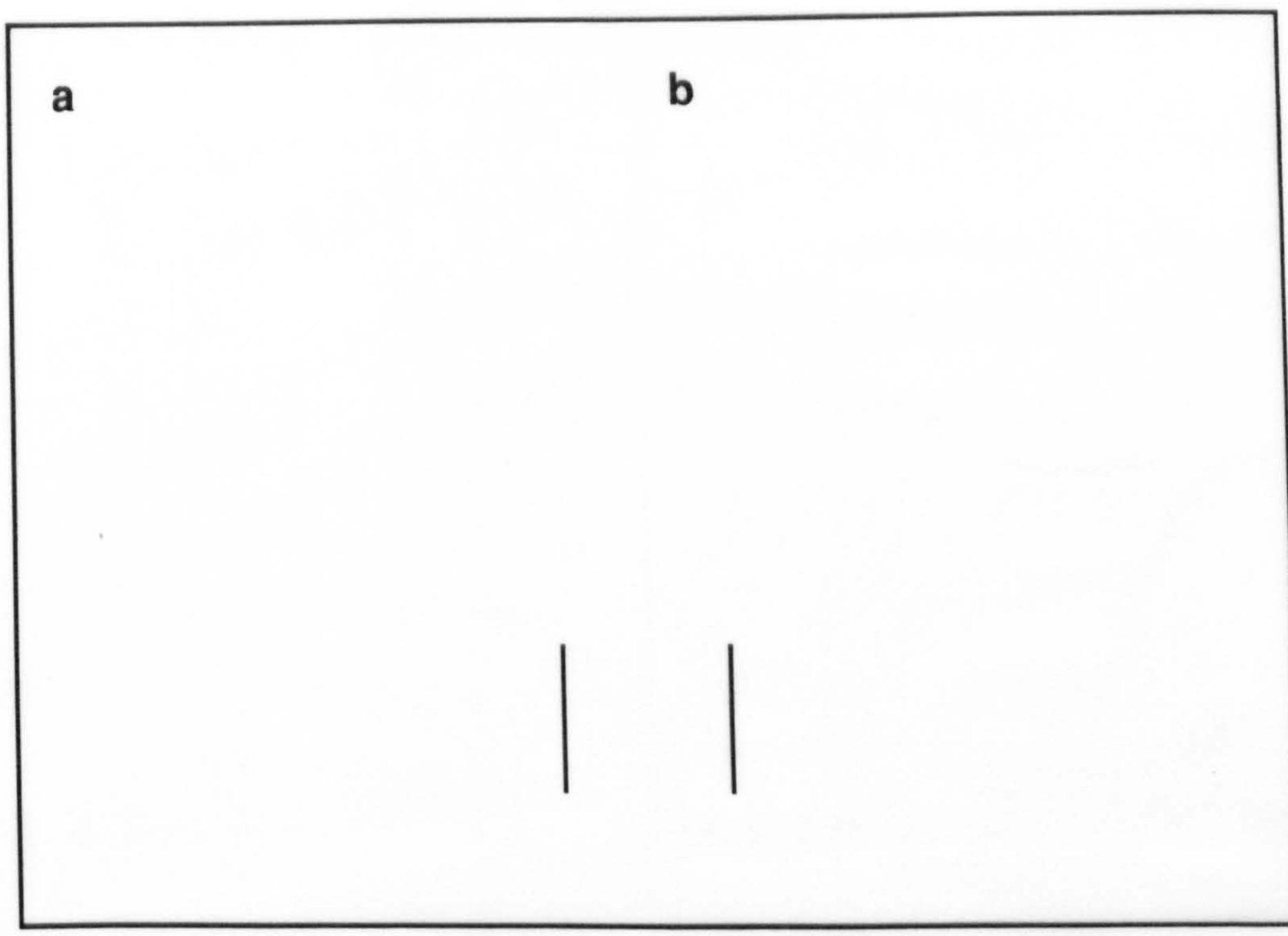
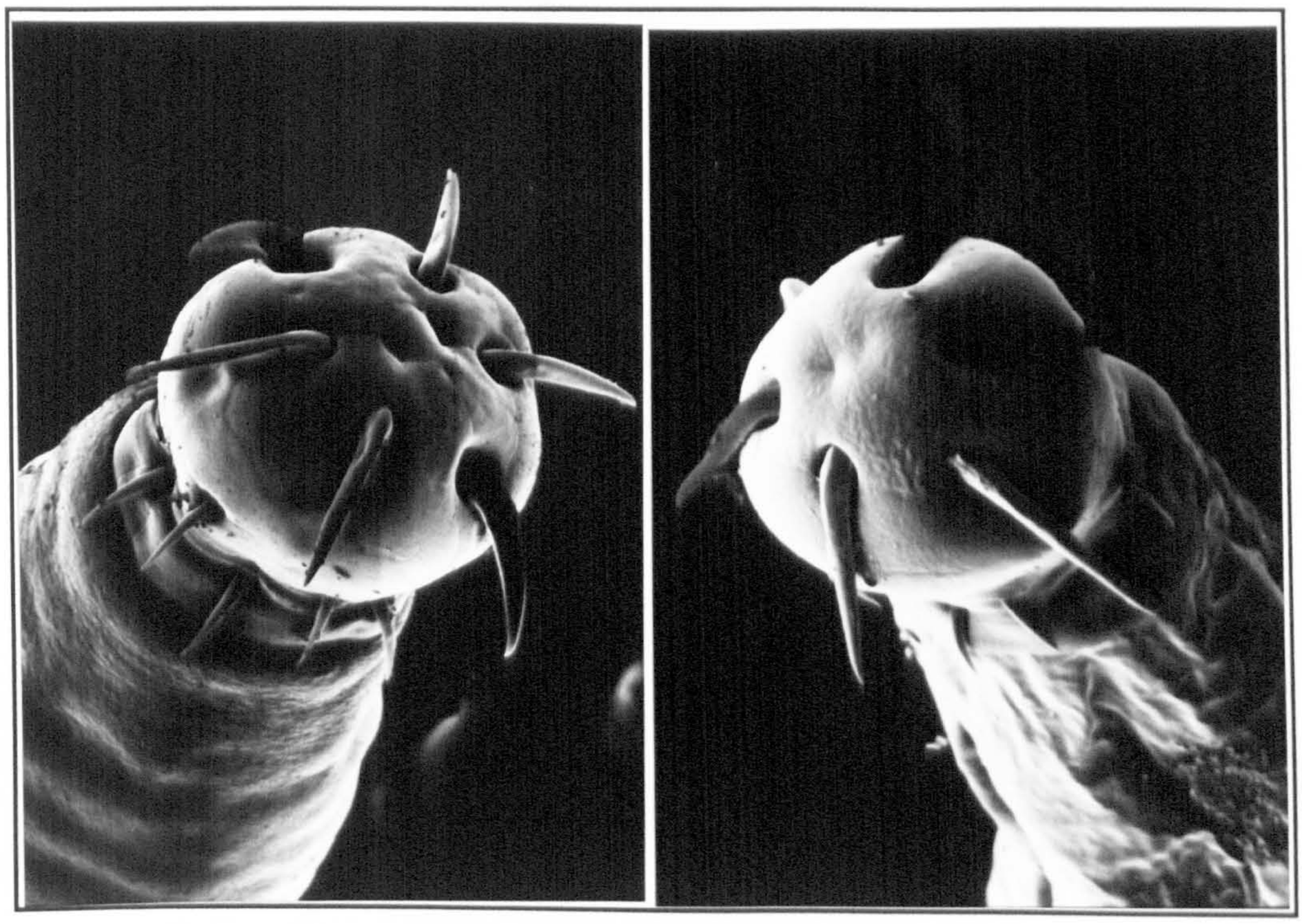


Fig. 7.2 Scanning electron micrographs of the proboscides of *Neoechinorhynchus rutili* specimens collected from a. an alder fly (*Sialis lutaria*) and b. a roach (*Rutilus rutilus*) respectively. Bars represent 30 μm. (The specimen from *Rutilus rutilus* was kindly provided by Dr E.T. Valtonen).



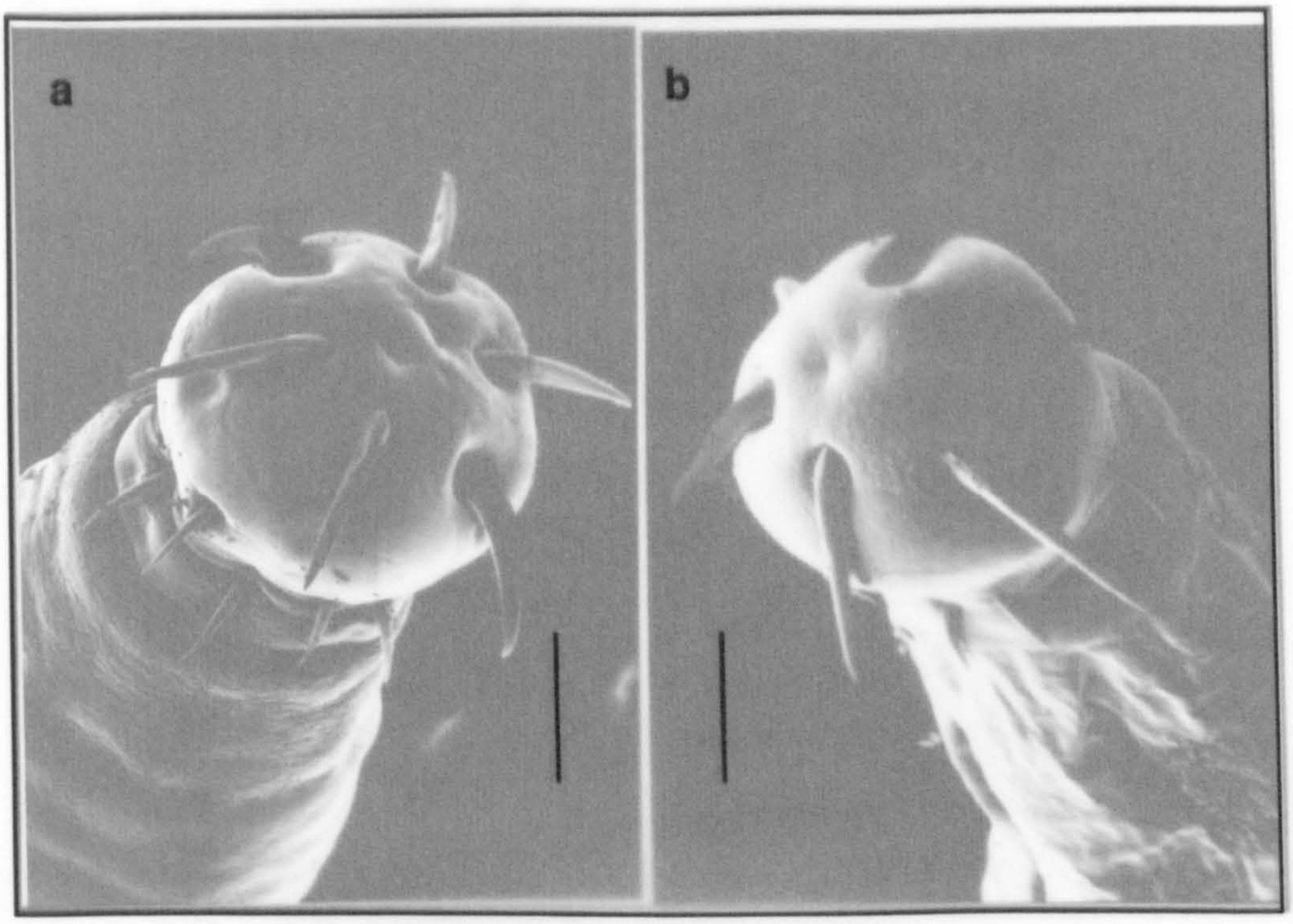


Fig. 7.2 Scanning electron micrographs of the proboscides of *Neoechinorhynchus rutili* specimens collected from a. an alder fly (*Sialis lutaria*) and b. a roach (*Rutilus rutilus*) respectively. Bars represent 30 μm. (The specimen from *Rutilus rutilus* was kindly provided by Dr E.T. Valtonen).

Table 7.3 Dimensions of Neoechinorhynchus rutili (Acanthocephala) from a number of different hosts

Origin	Sex	Body length	Body width	Lemniscus length	Proboscis length	Proboscis width	Hook lengths anterior	Hook lengths median	Hook lengths posterior	Testes	Cement gland l
Loch Monzievaird *Sialis lutaria* larvae	f	1592 (4) 1216-2016	96-288 (4)	573-1006 (3)	151 (1)	114 (1)	74.8 (4) 69-79	39.5 (2) 38-41	28.3 (2) 25-31.5		
	m	1272 (4) 832-1600	102-288 (4)	344-672 (3)	115 (3) 107-120	101 (3) 95-110	59.1 (4) 50.4-66	34.6 (3) 25-41	28.3 (3) 22-31.5	102-224 191-280	76-480 (4)
	All	1432 (8) 832-2016	96-288 (8)	344-1006 (6)	124.5 (4) 107-151	104.3 (4) 95-114	66.9 (8) 50.4-79	36.4 (5) 25-41	28.3 (5) 22-31.5		
Loch Maragan *Sialis lutaria* larvae	f	1520 (2) 1024-2016	96-224 (2)	509 (1)	120 (2) 117-123	116.5 (2) 104-129	59.8 (2) 53.6-66	33.0 (2) 31.5	25.2 (1) 34.6		
	m	1290 (3) 992-1696	128-384 (2)	382-407 (2)	112 (2) 104-120	105.5 (2) 104-107	56.6 (3) 47-63	31.5 (3) 25.2-37.8	21.9 (3) 18.9-25	102-191 178-573	102-242 (3)
	All	1382 (5) 992-2016	96-384 (5)	382-509 (3)	116 (4) 104-123	111 (4) 104-129	57.9 (5) 47-66	32.1 (5) 25.2-37.8	22.7 (4) 18.9-25.2		
Grenoble France *Sialis lutaria* larvae Villot (1885)	All	480-640	160 (max)								
Ostracods Merritt & Pratt (1964)	All	400-1000									

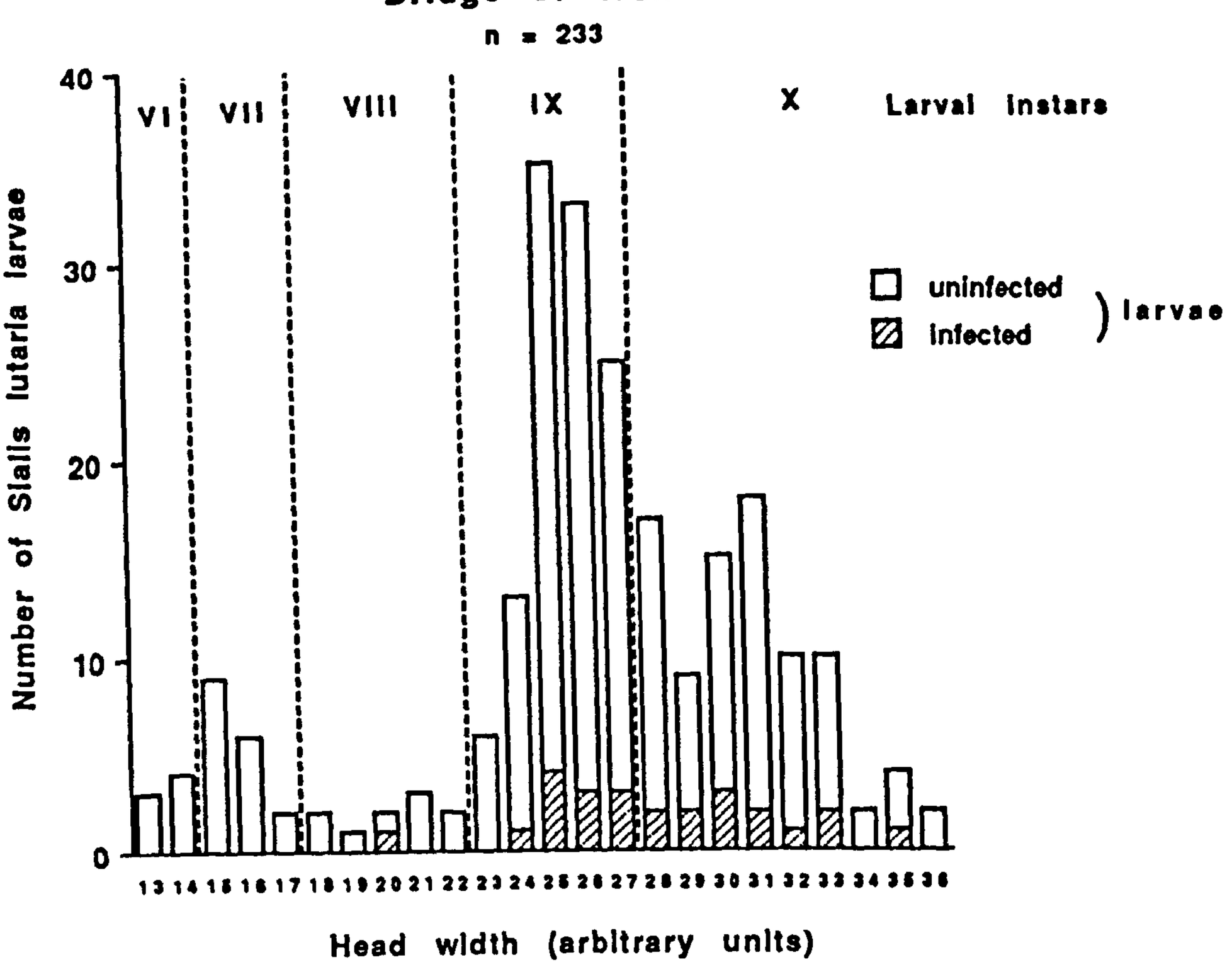
Origin	Sex	Body length	Body width	Lemniscus length	Proboscis length	Proboscis width	Hook lengths anterior	Hook lengths median	Hook lengths posterior	Testes	Cement gland
Various def. hosts Van Cleave & Lynch (1950)	m	1500-6000	250-630	-	79-120	79-120	53-82	29-44	22-34	-	-
Transmssion experiment	f	1568 (1)	128-288 (1)	1019 (1)	-	-	79 (1)	38 (1)	22 (1)		
Sialis lutaria larvae eaten by rainbow trout	m	1248 (2) 1152-1344	160-256 (2)	726-815 (2)	-	-	60 (2) 57-63	36.3 (2) 31.5-41	28.3 (2) 25-31.5	102-153 204-280	127-178 (2)
Salmo gairdneri	All	1354 (3) 1152-1568	128-288 (3)	726-1019 (3)	-	-	66.3 (3) 57-79	36.8 (3) 31.5-41	26.2 (3) 22-31.5		
Loch Monzievaird brown trout	m	1844 (8) 800-3040	128-512 (8)	484-1024 (8)	107 (1)	94 (1)	63.8 (4) 63-66	32.3 (4) 28.4-37.2	25 (4) 22-28	96-256 152-480	140-672 (8)
Loch Maragan brown trout	f	2024 (4) 1948-2048	128-480 (4)	1019-1440 (2)	-	-	66.2 (3) 60-72.5	34.7 (2) 31.5-37.8	26.8 (2) 25.2-28.3		
	m	1842 (7) 1408-2688-	128-416 (7)	704-1088 (5)	113 (1)	101 (1)	61.8 (5) 60-66	34.6 (3) 31.5-37.8	24.1 (3) 18.9-31.5	140-216 254-522	153-382 (7)
	All	1908 (11) 1408-2688	128-480 (11)	704-1440 (7)	113 (1)	101 (1)	63.4 (8) 60-72.5	34.6 (5) 31.5-37.8	25.2 (5) 18.9-31.5		
Kola Peninsula U.S.S.R.	f	5900-9400 (10)	-	1260-3360 (10)	130-150	-	73-86	34-43	21-34		
Thymallus thymallus Skryabina (1978)	m	4200-5700 (10)	-	1470-2830 (10)	120-130	-	82-86	34-43	21-30	520-840	590-840

f = female, m = male. In all cases values are in microns. Mean values and ranges are given with numbers of worms measured given in parenthe:

Dam Lochan on 22.6.86. was obtained. Only 7 of these larvae were in a suitable condition for dissection. Fifteen worms were recovered from these whole *S.lutaria* larvae and a further 2 from a piece of *S.lutaria* abdomen found in one of the trout guts. Between 1 and 5 *N.rutili* were recovered from individual larvae which included immature females, females with free ovaries and males. Only individuals of the largest 2 instars (9 & 10) were found to be infected, the mean intensity of infection being 2.33 (n=3) and 4.00 (n=2) respectively. Subsequent to this date, *S.lutaria* had appeared in very small numbers in the trout diet and none was found to be infected.

The frequency distributions of *N.rutili* infection in the *S.lutaria* samples from Bridge of Weir (Site 6), Drumore Loch (Site 11) and Loch Maragan (Site 29) (consumed and benthic larvae) and Loch Monzievaird (Site 30) in terms of larval size, expressed as head width, are shown in Figs. 7.3 a,b,c,d & e respectively. Although these samples are of differing origins in terms of season (see Table 7.1 for dates) only larvae of instars 6 to 10 are represented. Elliot (1977a) has described the 2 year life cycle of *S.lutaria* and how the prevalent instars vary during the year. Data from the study of Giani & Laville (1973) of a *S.lutaria* population in the Pyrenees is also shown in Table 7.4.

a



b

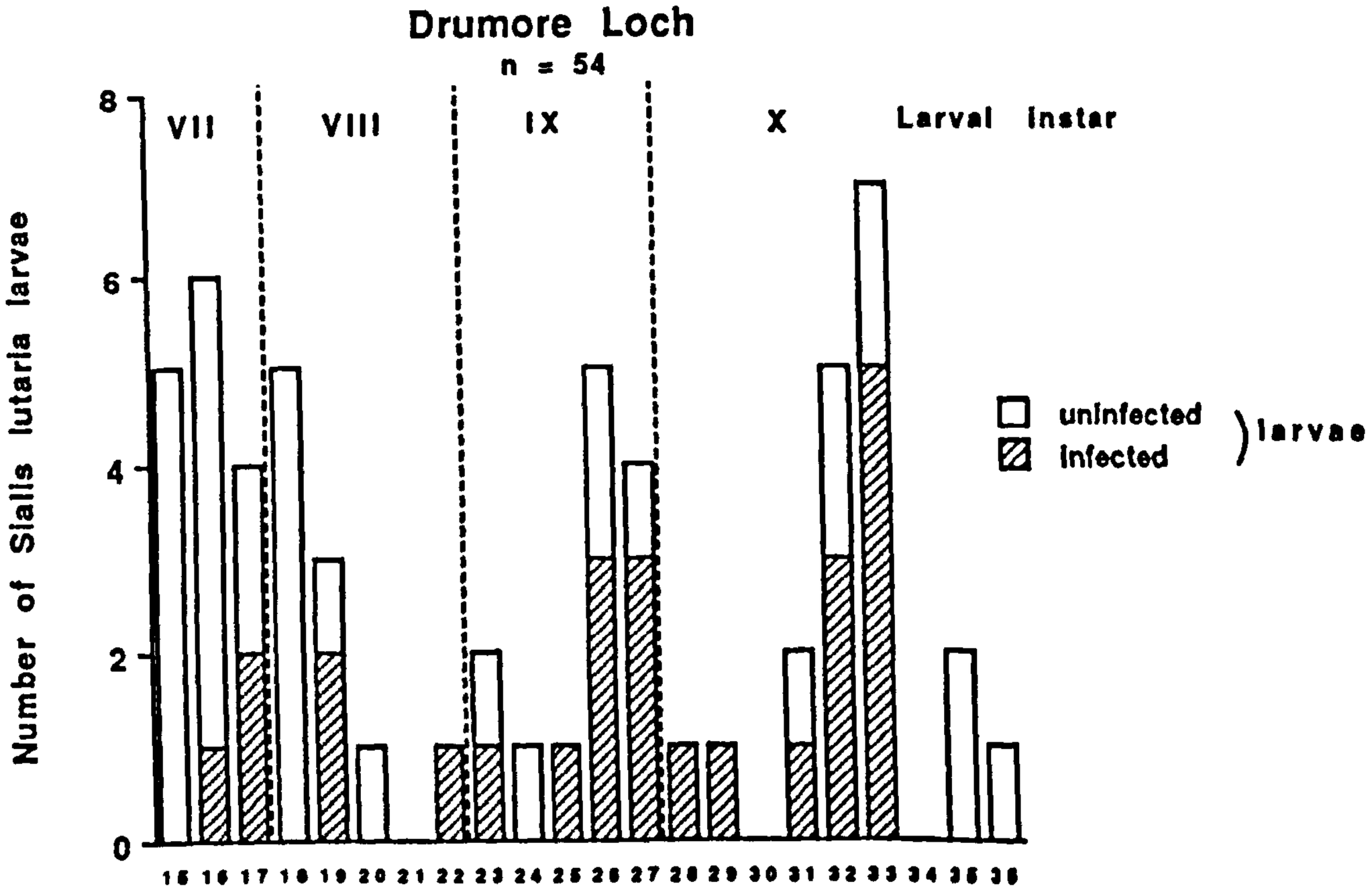
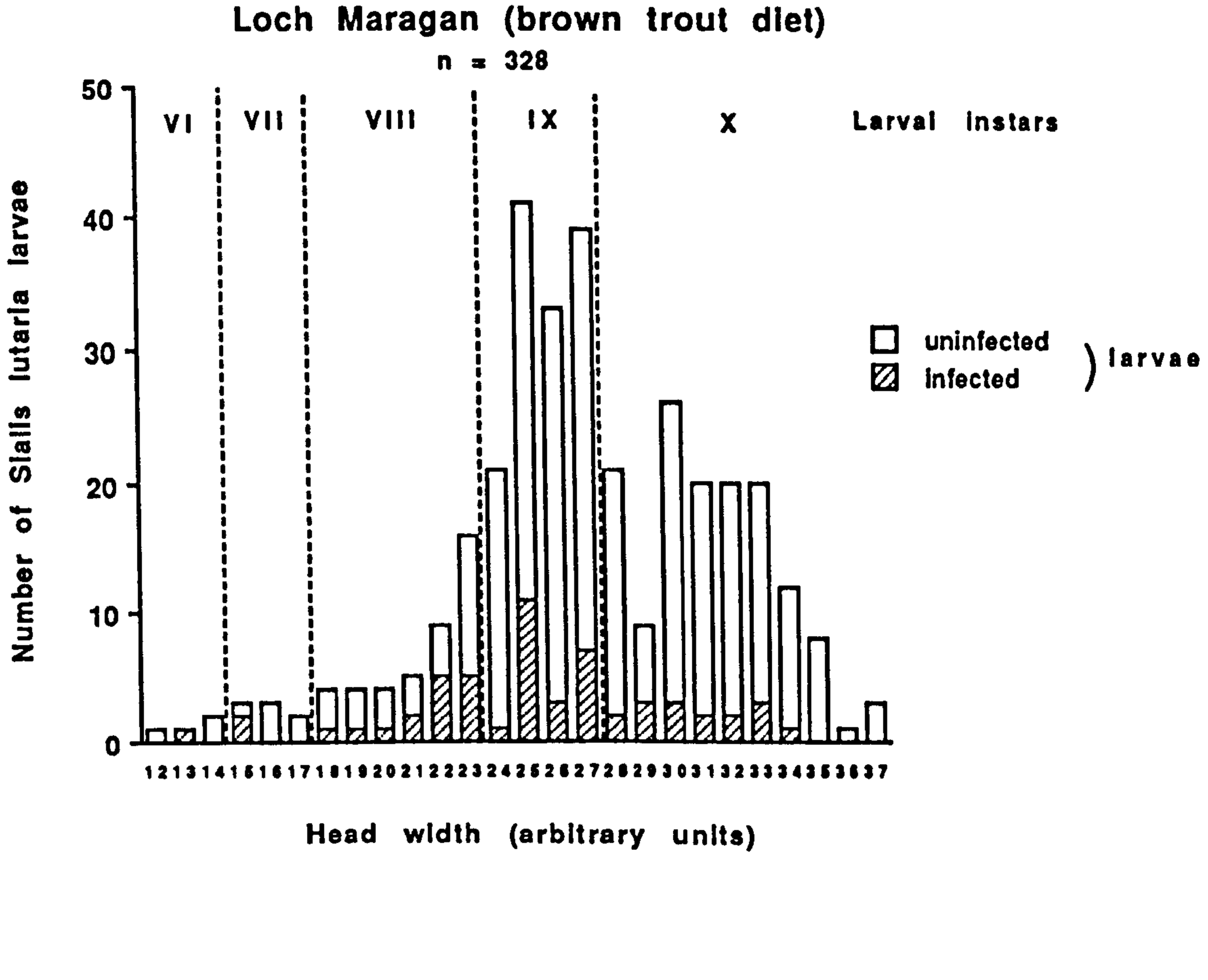
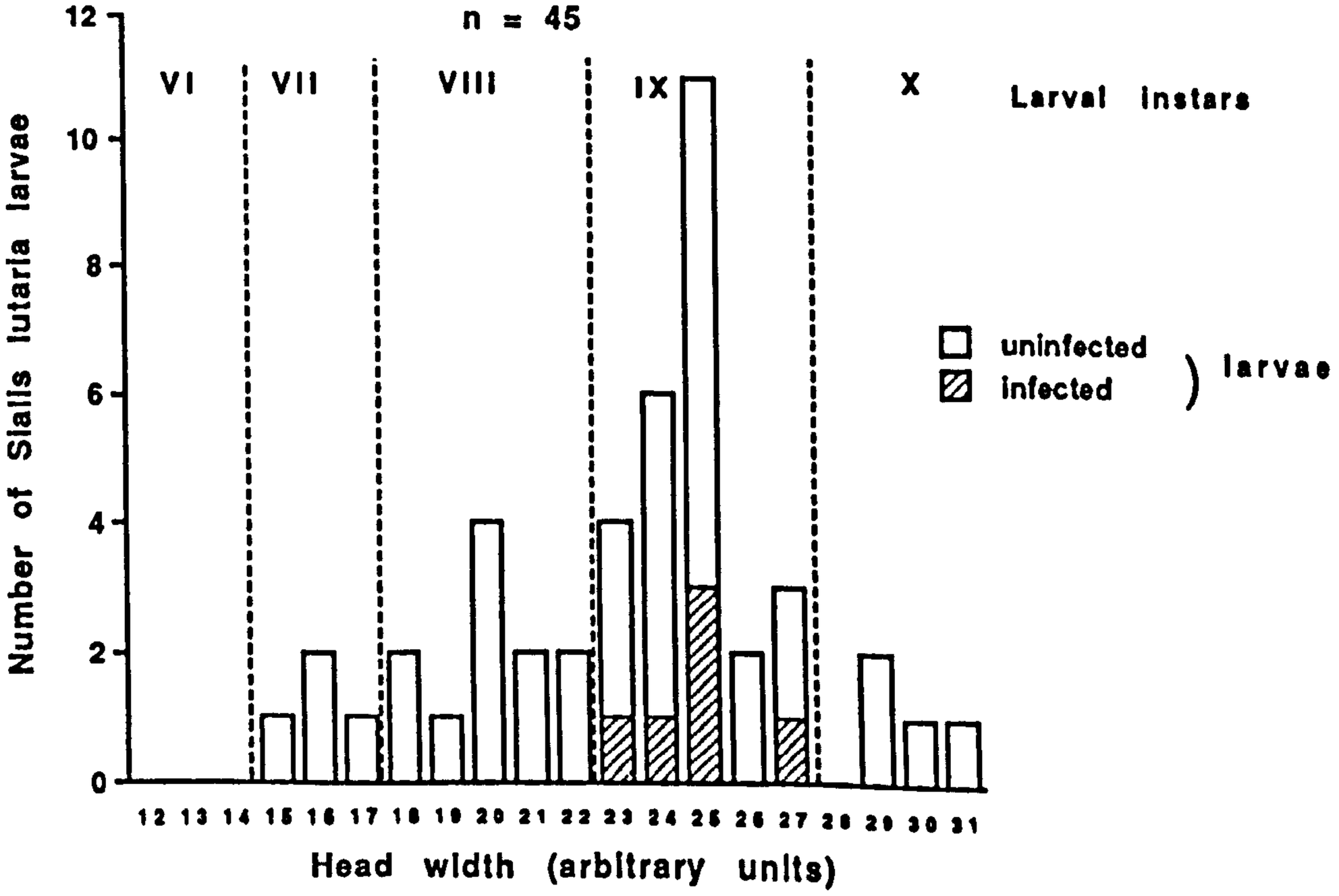


Fig. 7.3 Distribution of *Neoechinorhynchus rutili* infection in various instars of *Sialis lutaria* larvae from 4 Scottish sites: a. Bridge of Weir, b. Drumore Loch, c. Loch Maragan (brown trout diet), d. Loch Maragan (benthic larvae), e. Loch Monzievaird

c


Loch Maragan (brown trout diet)
n = 328
Number of Slalis lutaria larvae
50
40
30
20
10
0
VI
VII
VIII
IX
X
Larval instars
uninfected
infected
larvae
1 2 1 3 1 4 1 5 1 6 1 7 1 8 1 9 2 0 2 1 2 2 2 3 2 4 2 5 2 6 2 7 2 8 2 9 3 0 3 1 3 2 3 3 3 4 3 5 3 6 3 7
Head width (arbitrary units)

d


Loch Maragan (benthic larvae)
n = 45
Number of Slalis lutaria larvae
12
10
8
6
4
2
0
VI
VII
VIII
IX
X
Larval instars
uninfected
infected
larvae
12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Head width (arbitrary units)

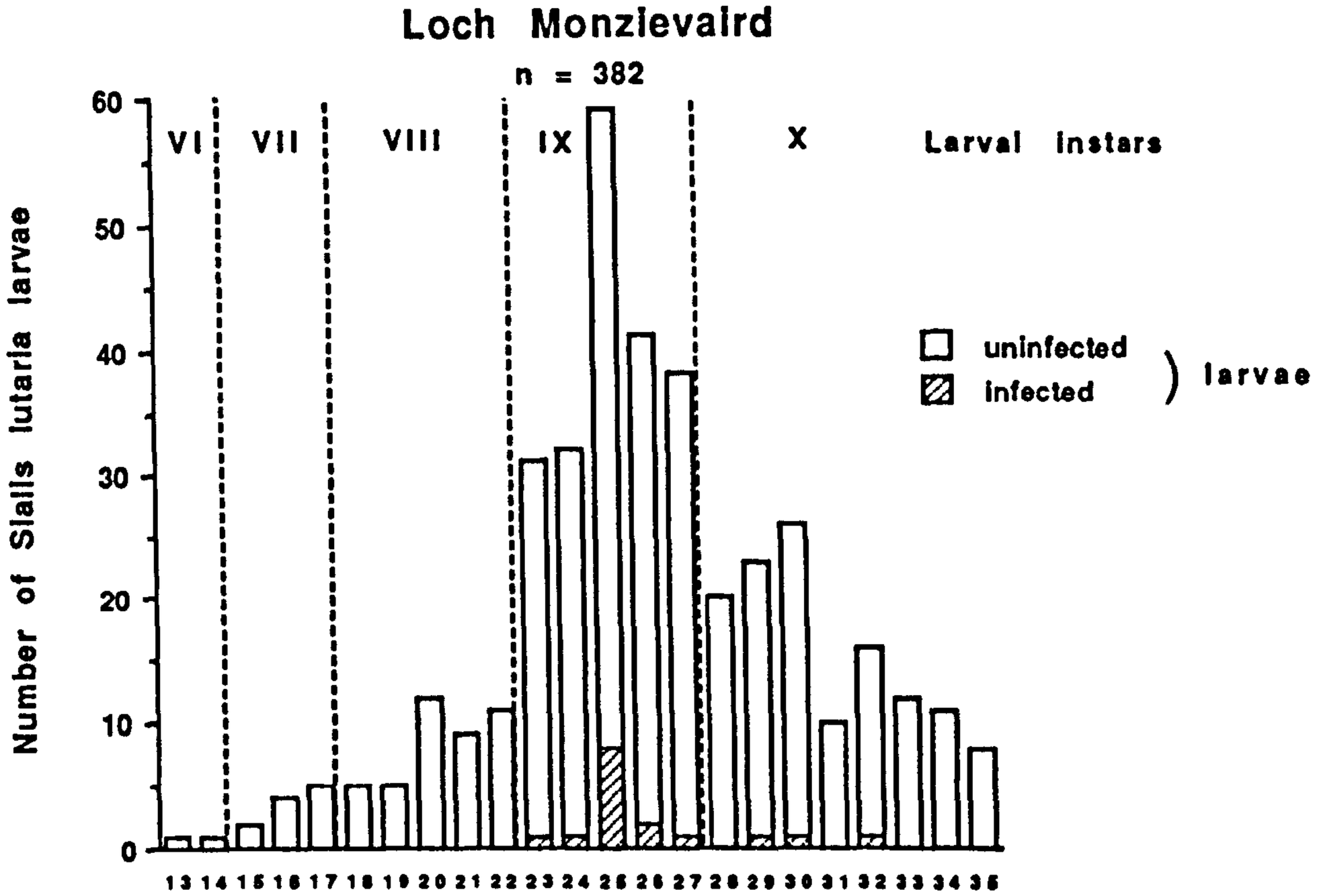

Loch Monzievaird
n = 382
Number of Slalls lutaria larvae
60
50
40
30
20
10
0
VI
VII
VIII
IX
X
Larval instars
uninfected
infected
larvae
13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Head width (arbitrary units)

Table 7.4: Seasonal changes in the occurrence of *Sialis lutaria* instars

Month	Instars present		
	Present year larvae (Elliot, 1977a)	Previous year larvae	Giani & Laville[a] (1973)
Jan	6 7	10	4 5 6 7 9 10
Feb	7 8	10	
Mar	7 8	10	
Apr	7 8	10	
May	1 2	8 9 10	
Jun	1 2 3 4	8 9	
Jul	1 2 3 4 5	9	4 5 6 7 8 9 10
Aug	4 5 6	9 10	1-10
Sep	5 6 7	9 10	1-10
Oct	6 7	9 10	2-10
Nov	6 7	10	
Dec	6 7	10	

a only data for some months of the year given.

According to the observations of Elliot (1977a) more small larvae should have occurred in the summer samples. Elliot (1977a) describes the first instars as being planktonic and subsequent instars as being negatively phototactic with a habit of burrowing in the mud. As the larvae get older their maximum numbers are found at progressively greater depths. The smaller larvae in their first year are therefore most abundant in the littoral, whereas the larger larvae in their second year are most abundant in the sub-littoral or profundal zones, except when they migrate inshore before pupation in spring (Elliot, 1977a). The samples of larvae correspond approximately to Elliot's scheme. For example, the June sample from Powder Works Dam Lochan is dominated by smaller instars (5-8) as expected at this time of year and the winter collections from Drumore Loch had large proportions of instars 7 aswell as 9 and 10 which is similar to Elliot's description of instars 6, 7 and 10 being dominant in this period. The summer collections of larvae from Loch Maragan benthic substrates were

dominated by instars 8 and 9, the time when Elliot describes large numbers of small larvae being present and this must reflect difficulties in the capture of these smaller individuals.

7.3.2.2. *Prevalence and intensity of* Neoechinorhynchus rutili *infection in larval instars*

Details of the prevalence and mean intensity in various *S.lutaria* larval instars from various Scottish sites are given in Table 7.5. These data are displayed graphically in Figs. 7.4a, b, c and d for individual sites (excluding Loch Maragan benthic and Powder Works Dam Lochan) and prevalence and mean intensity of infection for all sites together in Figs. 7.5 a & b respectively. The distribution of prevalence of infection with instar in the Drumore Loch, Loch Maragan (dietary larvae) and Loch Monzievaird samples show a similar pattern with smaller and larger instars exhibiting lower values and the intermediate instars (8 & 9) showing the highest values. Although the forms of the graphs are similar the values are very different ranging, for example, for instar 9 between 6.47% and 61.5%. At Bridge of Weir a different pattern is seen in which there is a trend of increased prevalence of infection with instar with the overall values fairly low. Interestingly, at the sites where the prevalences were low the infection was restricted to the larger instars in the samples (8, 9 & 10). In terms of mean intensity of infection, 3 patterns were observed: at Bridge of Weir there was a trend of increased values with advanced instar, in the Loch Maragan sample of larvae consumed by trout the values were fairly similar for all instars and in the samples from Drumore Loch and Loch Monzievaird smaller and larger instars exhibited lower values than intermediate-sized instars. The mean intensity values had similar distributions to the prevalence values, with the highest values for the former being recorded in

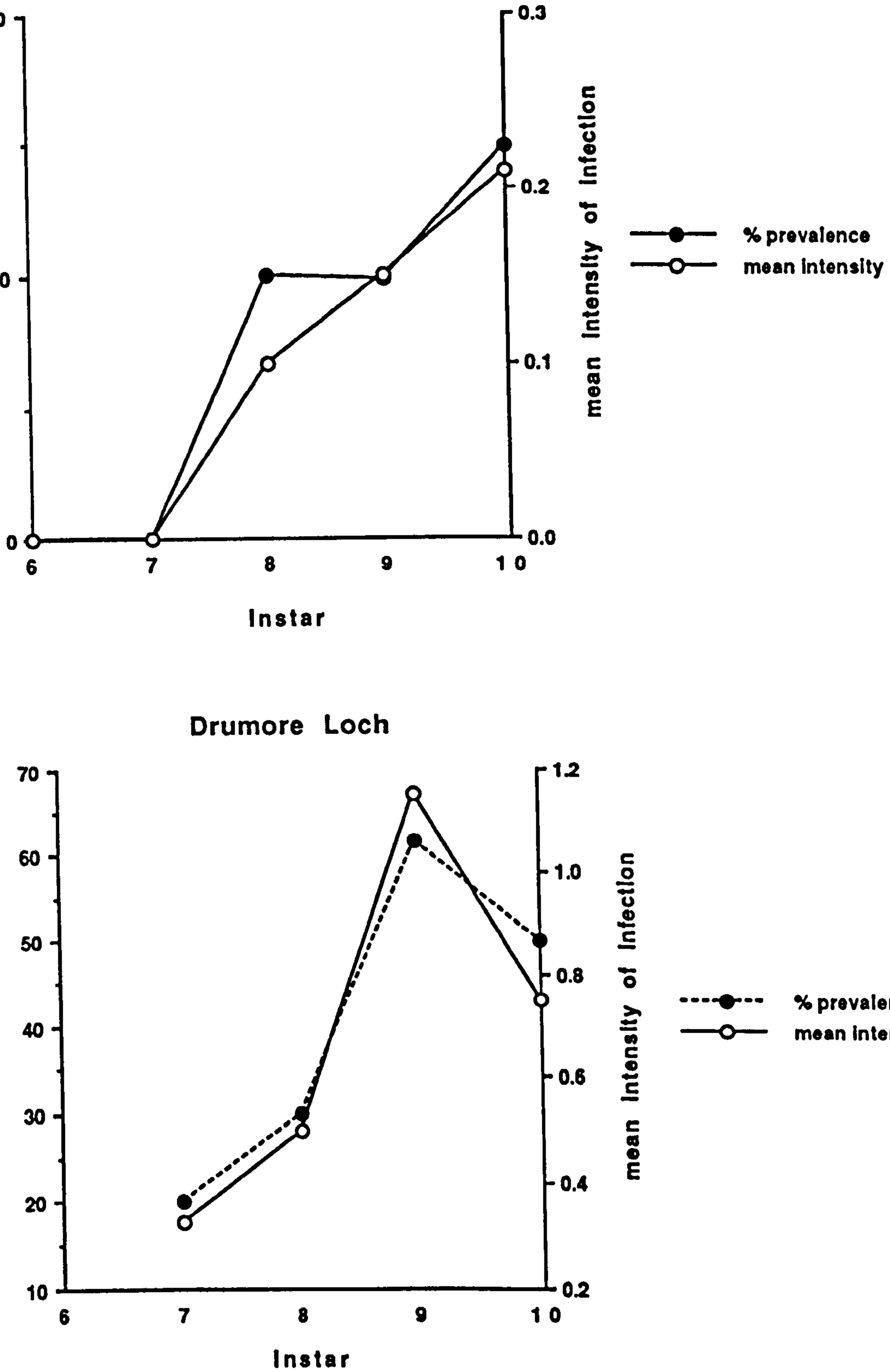
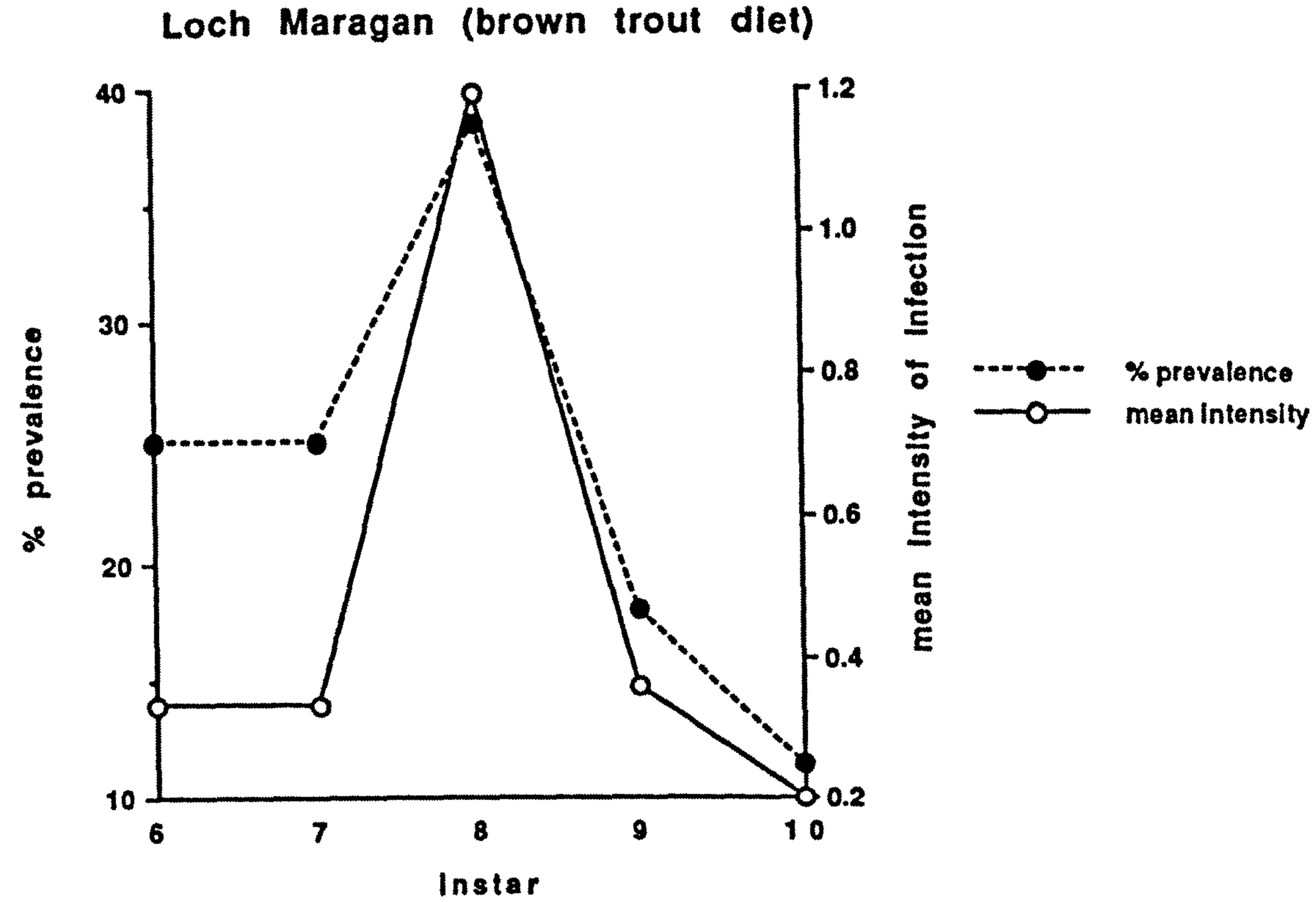
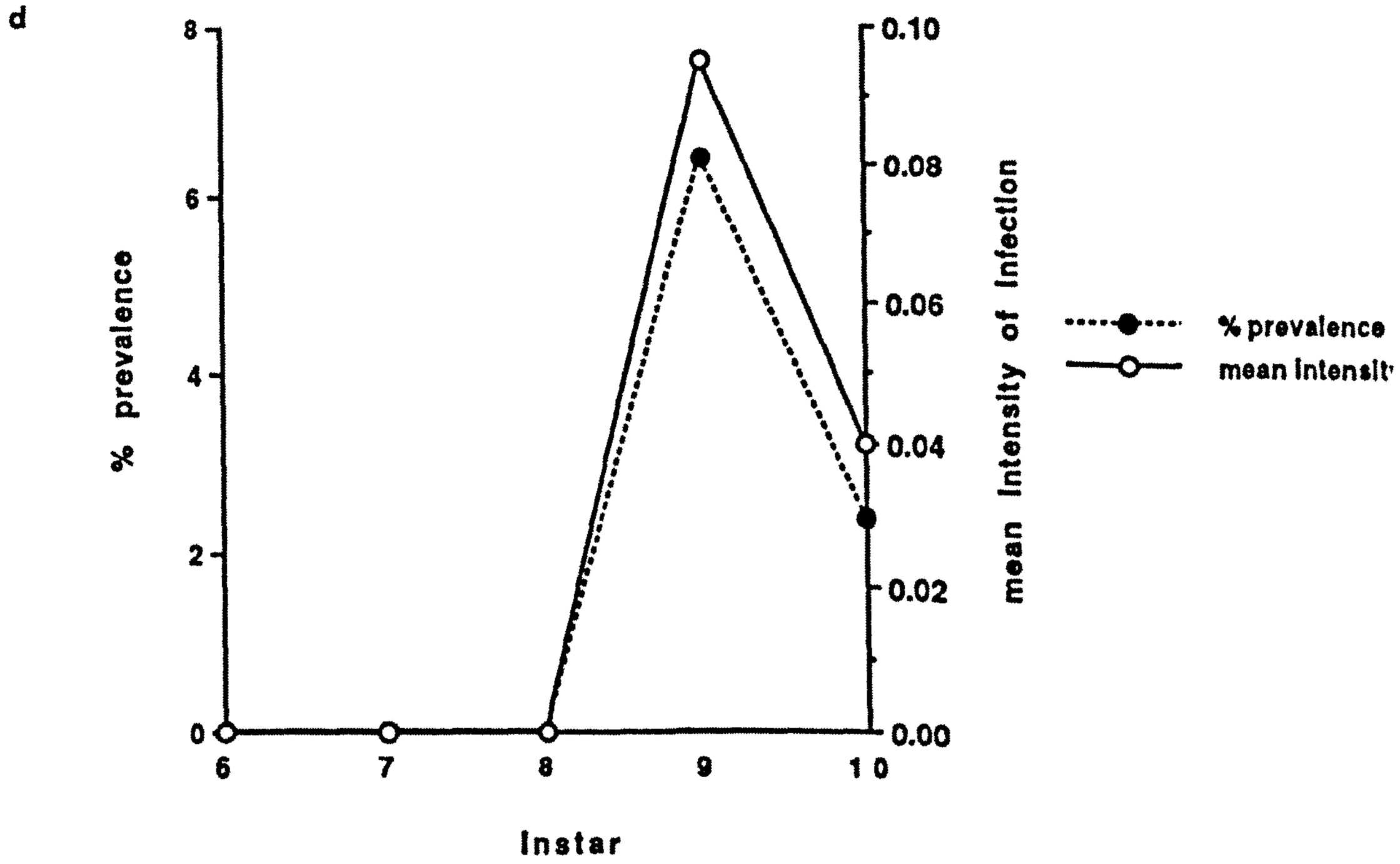
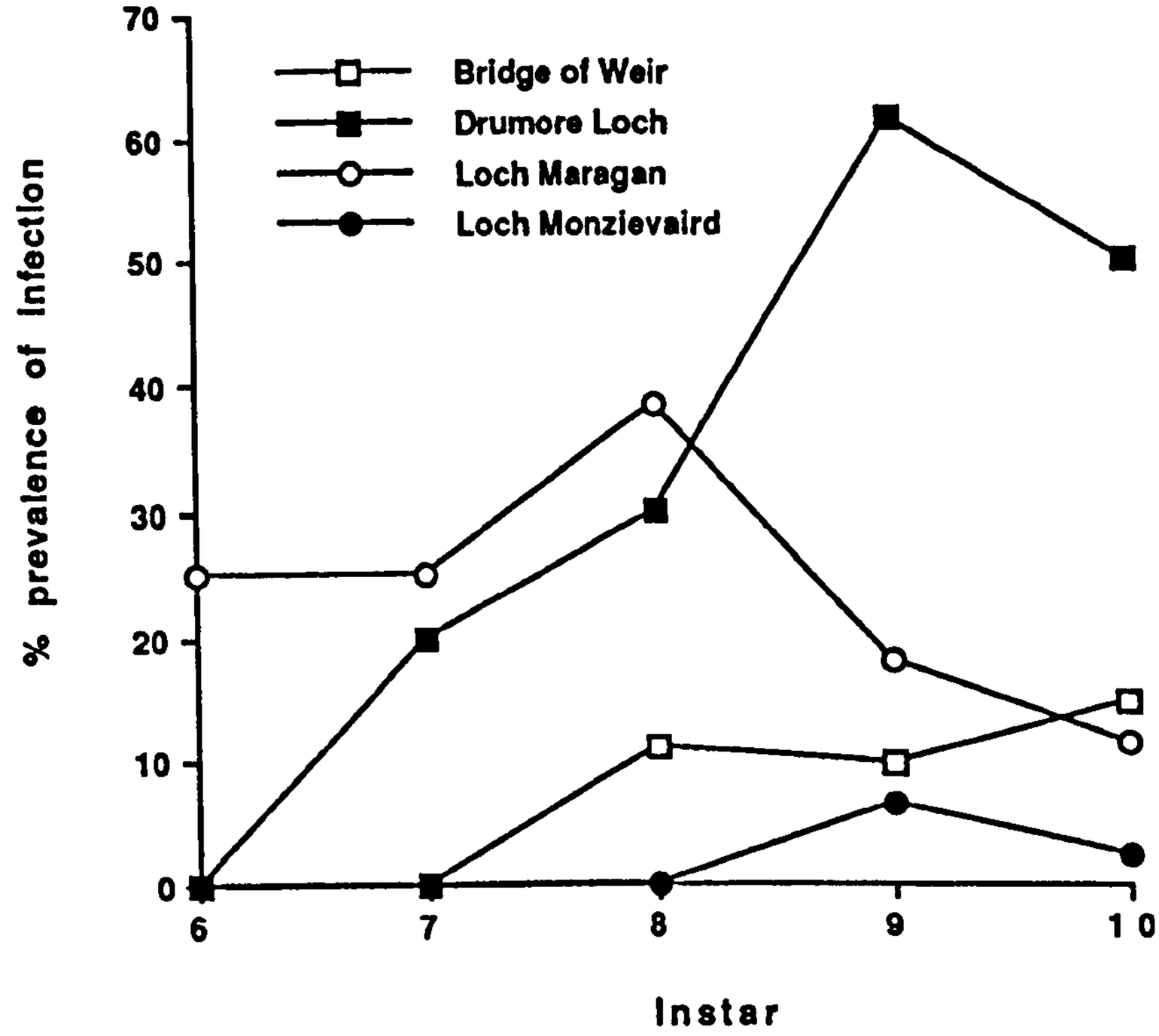


Fig. 7.4 Percentage prevalence and mean intensity of *Neoechinorhynchus rutilli* infection in *Sialis lutaria* larvae: a. Bridge of Wier, b. Drumore Loch, c. Loch Maragan (brown trout diets), d. Loch Monzievaird

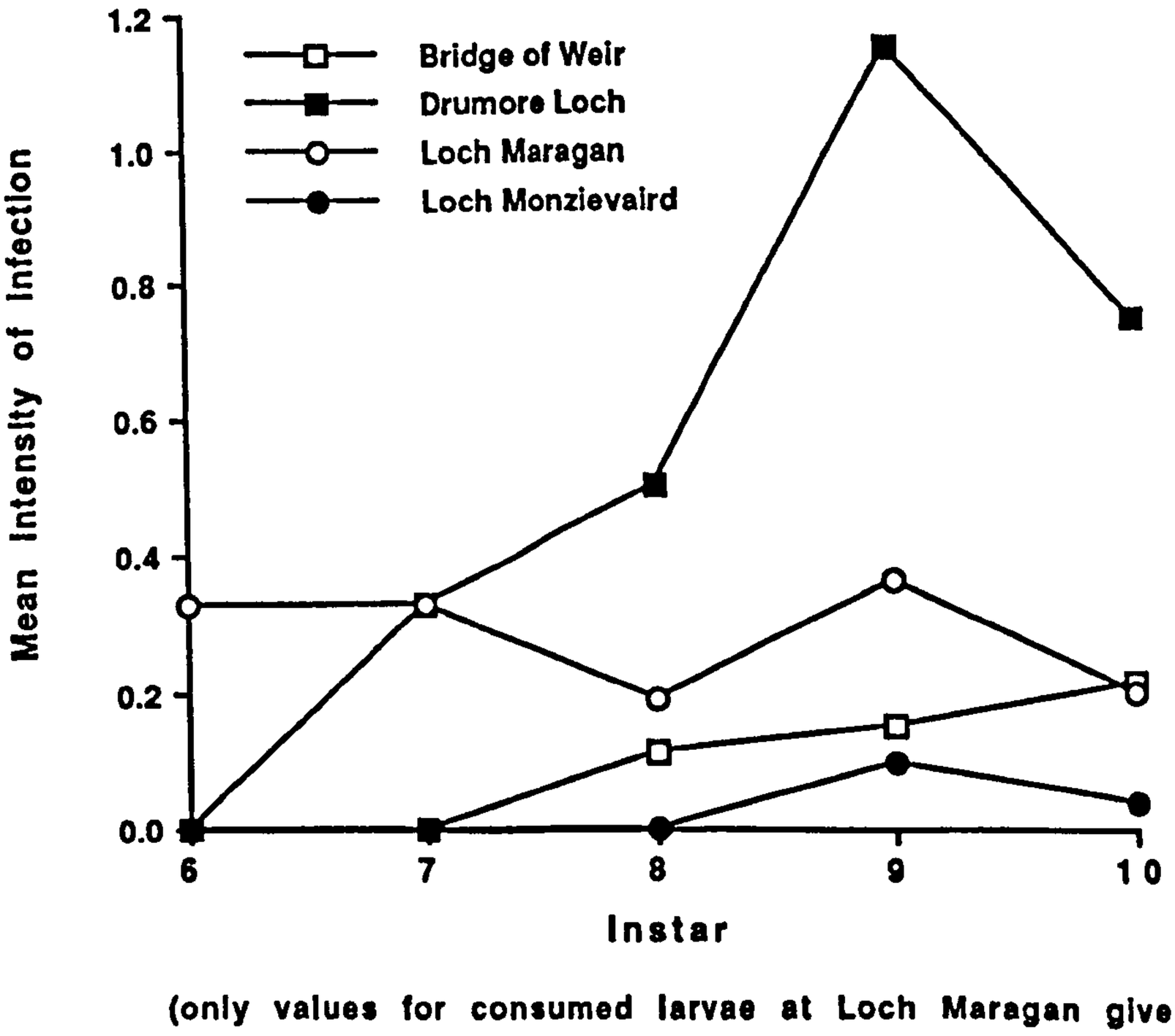

Loch Maragan (brown trout diet)
% prevalence
mean intensity of infection
40
30
20
10
1.2
1.0
0.8
0.6
0.4
0.2
6
7
8
9
10
Instar
% prevalence
mean intensity


d
Loch Monzievaird
% prevalence
mean intensity of infection
8
6
4
2
0
0.10
0.08
0.06
0.04
0.02
0.00
6
7
8
9
10
Instar
% prevalence
mean intensity

a



b



(only values for consumed larvae at Loch Maragan given)

Fig. 7.5 Infection of various instars of *Sialis lutaria* with *Neoechinorhynchus rutili* from 4 Scottish field sites: a. Percentage prevalence, b. Mean intensity

instars exhibiting the highest prevalence values. The range of intensity values was quite large with the Loch Monzievaird samples exhibiting the lowest values. There were also differences in the instars showing infection at different sites: at Bridge of Weir instars 8-10 were infected, at Drumore Loch instars 7-10, at Loch Maragan the consumed larvae instars 6-10 and benthic sample only instar 9 and at Powder Works Dam Lochan only instars 9 and 10 were infected with *N.rutili*.

Table 7.5: Prevalence and intensity of *Neoechinorhynchus rutili* infection in *Sialis lutaria* larval instars from various sites

Site	Instar	n	% Prevalence of infection	Mean intensity of infection
Bridge of Weir	6	7	0.00	0.00
	7	17	0.00	0.00
	8	10	10.00	0.10
	9	112	9.82	0.15
	10	87	14.94	0.21
	Total	233	10.73	0.16
Drumore Loch	7	15	20.00	0.33
	8	10	30.00	0.50
	9	13	61.50	1.15
	10	16	50.00	0.75
	Total	54	40.74	0.67
Loch Maragan :trout diet	6	4	25.00	0.33
	7	8	25.00	0.33
	8	26	38.50	1.19
	9	150	18.00	0.36
	10	140	11.40	0.20
	Total	328	17.07	0.28
benthic	7	4	0.00	0.00
	8	11	0.00	0.00
	9	26	23.08	0.23
	10	4	0.00	0.00
	Total	45	13.33	0.13
Loch Monzievaird	6	2	0.00	0.00
	7	11	0.00	0.00
	8	41	0.00	0.00
	9	201	6.47	0.09
	10	126	2.38	0.04
	Total	382	4.19	0.06
Powder Works Dam Lochan	5	1	-	0.00
	6	9	-	0.00
	7	6	-	0.00
	8	7	-	0.00
	9	3	-	2.33
	10	4	-	4.00
	Total	30		

7.3.2.2.1. *Temporal changes in* Neoechinorhynchus *rutili infection of* Sialis lutaria *larvae*

Temporal data about changes in *N.rutili* infection of *S.lutaria* larvae was only available for 2 sites, Drumore Loch and Loch Monzievaird (Table 7.6).

Table 7.6: Temporal changes in *Neoechinorhynchus rutili* infection of *Sialis lutaria* larvae from 2 Scottish sites

Site	Date	n	mean intensity of infection	% prevalence of infection	sex ratio f:m
Drumore Loch	31.10.87.	9	0.78	22.2	0.75:1
	11.11.87.	19	0.42	31.6	0.33:1
	10.12.87.	26	0.81	53.8	1.00:1
	All				0.75:1
Loch Monzie-vaird	2.4.87.	135	0.11	6.7	0.40:1
	28.4.87.	31	0.16	9.7	2.00:1
	28.7.87.	26	0.008	0.08	0:1
	31.10.87.	50	0.00	0.00	-
					0.50:1

Two contrasting patterns were detected at these 2 sites, one of a rapidly increasing prevalence of infection at Drumore Loch over the winter months and one of rapidly decreasing prevalence of infection at Loch Monzievaird in the period between late spring and early autumn. The pattern of intensity of infection at Drumore Loch is less clear but appears to be fairly stable at this time in contrast to Loch Monzievaird where the initially low intensities drop off to very low levels by mid-summer.

7.3.2.2.3. *Distribution of* Neoechinorhynchus rutili *amongst* Sialis lutaria *larvae*

In all cases (except the benthic Loch Maragan sample) the distribution of *N.rutili* in the *S.lutaria* larvae was overdispersed.

Between 1 and 6 worms per host were found in the samples. The distribution for each site is shown in Figs. 7.6a, b, c and d respectively including values for mean and variance of intensity of infection and the calculated k value as a measure of overdispersion. The expected distribution as described by the negative binomial distribution is also shown and in all cases the X^2 values indicated good agreement with this theoretical distribution.

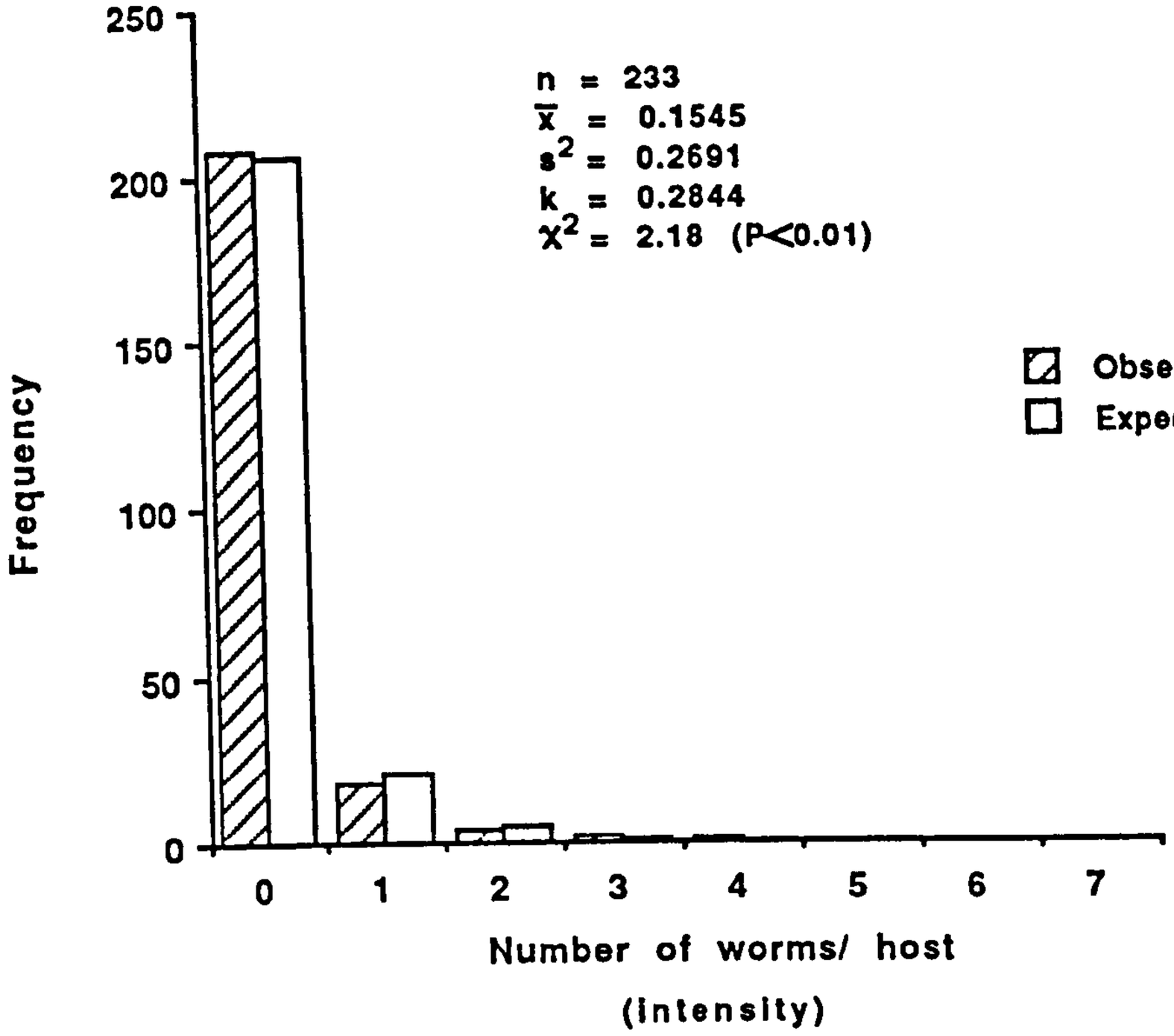
7.3.2.4. *Sex of worms recovered from* Sialis lutaria *larvae*

Both single sex and mixed infections were observed at all 4 sampling sites. Females with unfragmented ovarian tissue (f1), females with ovarian balls (f2) and males were found in the insect larvae. Some apparently monorchic males were found in Drumore Loch *S.lutaria* larvae. The sex ratio data is rather sparse (see Table 7.6), but appears to indicate a ratio biased towards males at Drumore Loch and Loch Monzievaird. Further data must be obtained to confirm this trend.

7.3.2.4.1. *Numbers of free ovaries in female* Neoechinorhynchus rutili *from* Sialis lutaria *larvae*

Females of 2 stages, those with unfragmented ovarian tissue and those with free ovaries present in the body cavity, were recognised. Merritt & Pratt (1964) indicated that the ovarian fragmentation occurs as maturity approaches and for this reason the numbers of free ovaries in 7 individuals collected from Loch Maragan trout diets in May 1988 were counted (Table 7.7).

a



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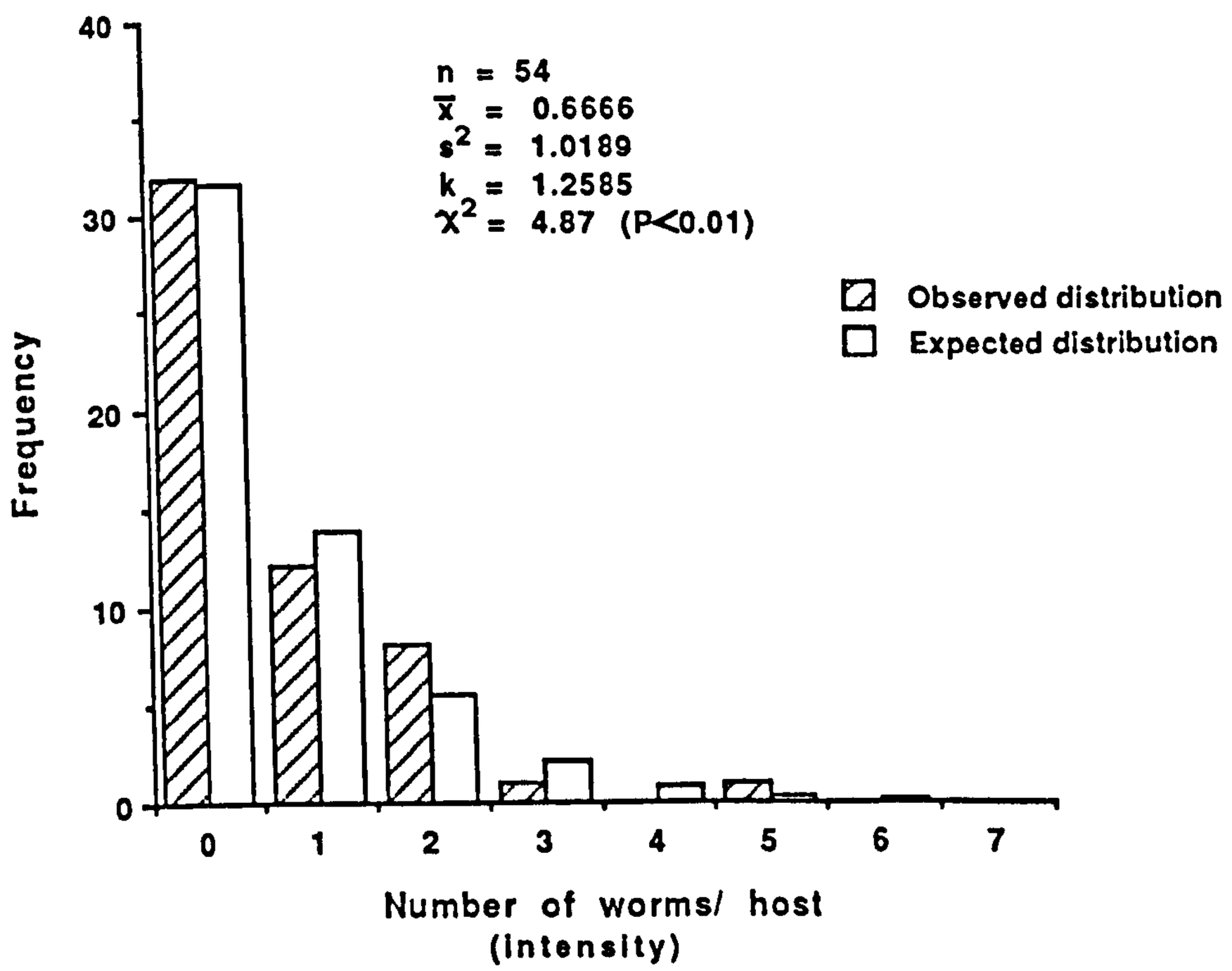
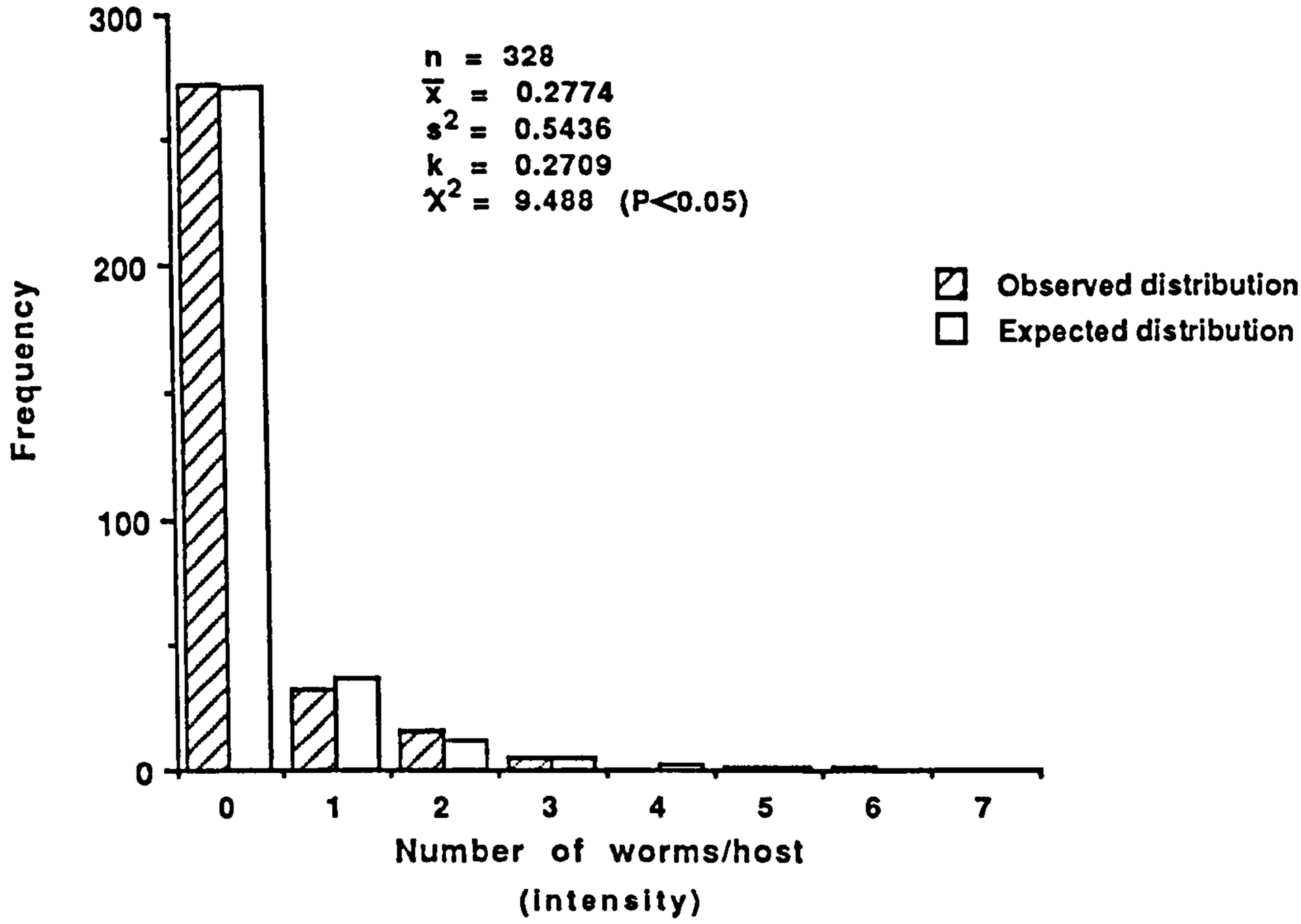


Fig. 7.6 Frequency distribution of numbers of *Neoechinorhynchus rutili* in *Sialis lutaria* larvae from 4 Scottish sites: a. Bridge of Weir, b. Drumore Loch, c.Loch Maragan (trout diets), d. Loch Monzievaird

c

Loch Maragan (trout diets)



d

Loch Monzievaird

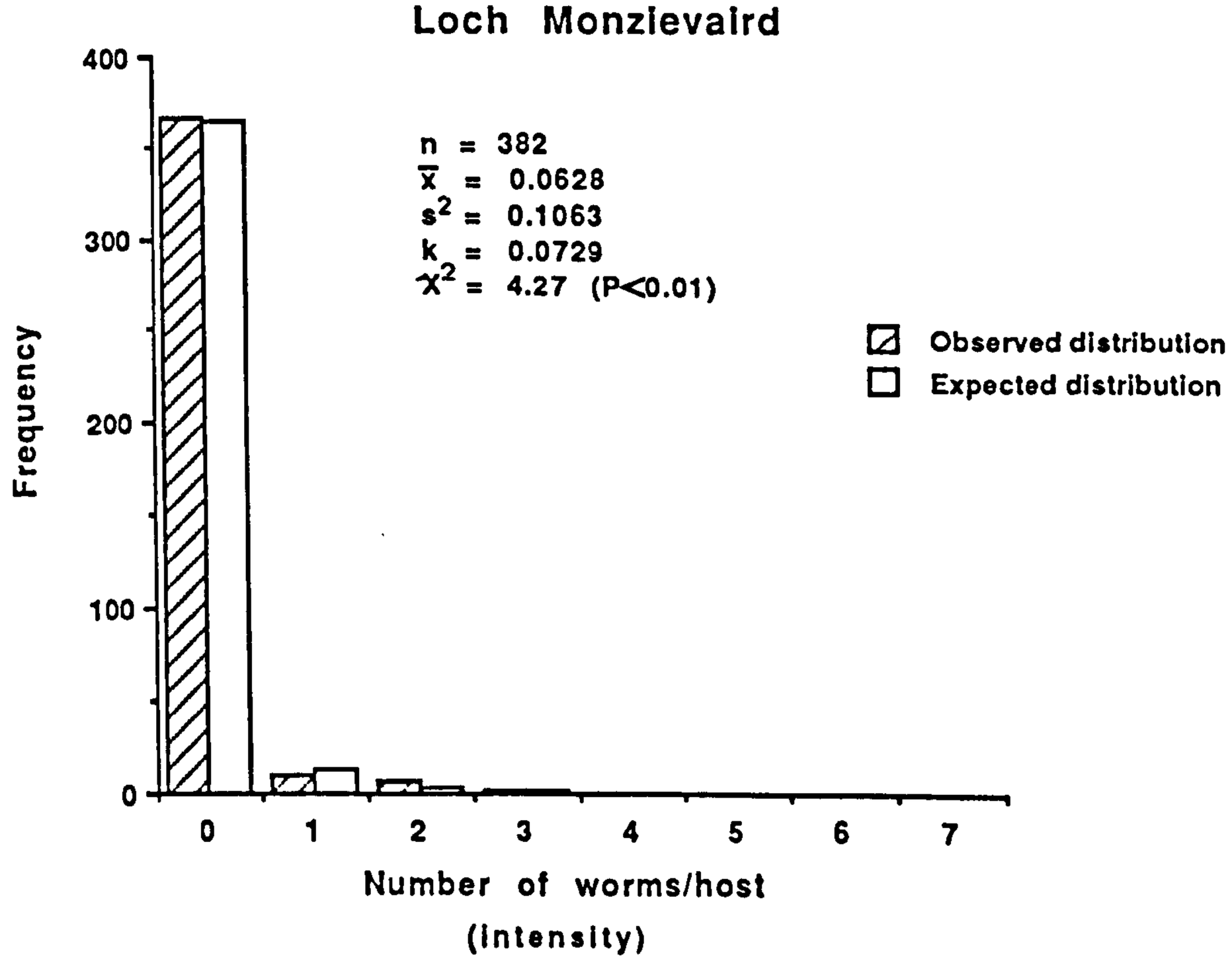


Table 7 7: Numbers of free ovaries in female *Neoechinorhynchus rutili* from *Sialis lutaria* larvae

Length um	No. of free ovaries
586	94
701	85
713	157
739	60
752	166
866	179
917	105

7.3.2.5. *Size of* Neoechinorhynchus rutili *in* Sialis lutaria *larvae*

Besides worms that were prepared as whole mounts for identification purposes, a number of body length measurements of fresh and fixed *N.rutili* were made (Table 7.8).

Table 7.8: Body length measurements of a selection of *Neoechinorhynchus rutili* from *Sialis lutaria* larvae

Site	Body length measurements um								
	f1			f2			m		
	n	mean	range	n	mean	range	n	mean	range
1	9	595	382- 815	6	824	611- 955	19	614	306- 892
2	24	618	280- 879	13	780	573-1210	28	592	255- 866
3	2	1962	1949-1975	4	1380	994-1860	9	1169	866-1592

Sites: 1 = Drumore Loch (fresh), 2 = Loch Maragan (dietary and benthic samples, fresh), 3 = Powder Works Dam Lochan (formalin fixed)

f1 = females with unfragmented ovarian tissue, f2 = females with free ovaries, m = male worms.

A general trend of f1 females being shorter than f2 females is

evident with the females being longer than males. The values from sites 1 and 2 are comparable for all worm groups. The values for the fixed specimens from site 3 were much greater than those of the fresh specimens from the other 2 sites so the fixation process must influence the observed lengths of these worms and those measured as whole mounts. Perhaps the greater length of the f2 worms compared with the f1 indicates that the former were older.

7.3.2.6. *Observations of encapsulated* Neoechinorhynchus rutili *from* Sialis lutaria *larvae*

Encapsulation of some form was observed in specimens from 4 of the sampling sites. No encapsulation was observed in specimens from Loch Monzievaird *S.lutaria* larvae.

7.3.2.6.1. *Bridge of Weir*

In a sample of 50 *S.lutaria* larvae collected from Bridge of Weir (Site 11), 7 individuals were found harbouring between 1 and 3 *N.rutili* larvae. Ten of the 11 worms recovered were either partially or fully encapsulated with a transparent envelope. In addition some individuals were partially covered with a brown exudate. These observations are similar to those of Villot (1885). A number of these individuals were prepared as wax sections (Fig. 7.7) and an S.E.M. was also prepared (Fig. 7.8). In the opinion of Dr. A.M. Lackie, this was not full encapsulation, as would have been expected from a protective response mounted by an insect, and the *N.rutili* larvae did not appear to be adversely affected by the envelope. When individuals were freed from their envelopes, they moved freely, rapidly inverting and everting their proboscides. In the entire dissected sample of 233 larvae from this site, 36 *N.rutili* were recovered from 25 larvae, of which 29 (80.6%) showed evidence of encapsulation. Encapsulation of both male and female (f2) worms was observed.

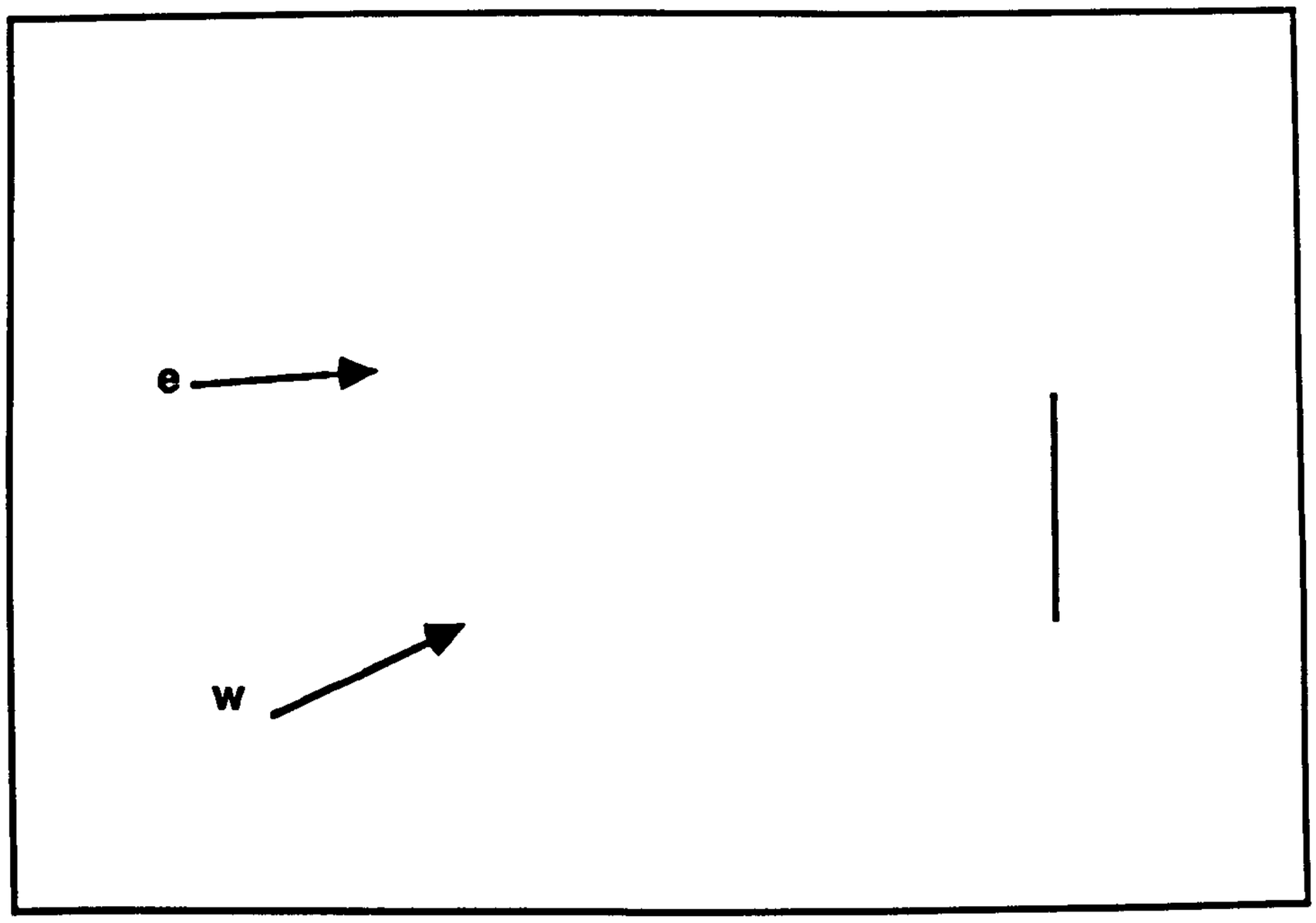


Fig. 7.7 **Transverse section of an encapsulated specimen of *Neoechinorhynchus rutili* from a *Sialis lutaria* larva.** Bar represents 500 μm.

Note: Mallory's triple stained wax section. w = worm, e = encapsulating layer.

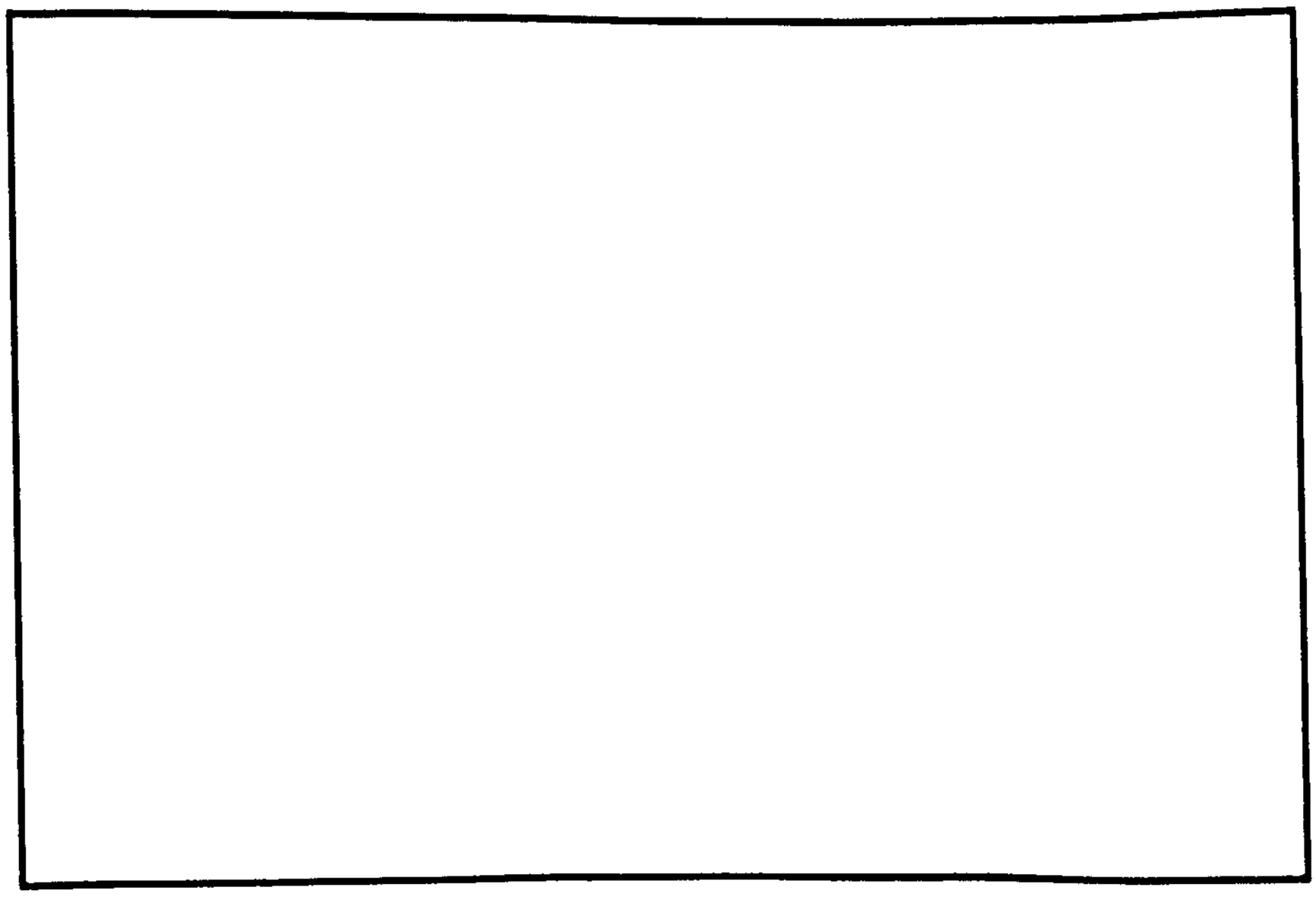
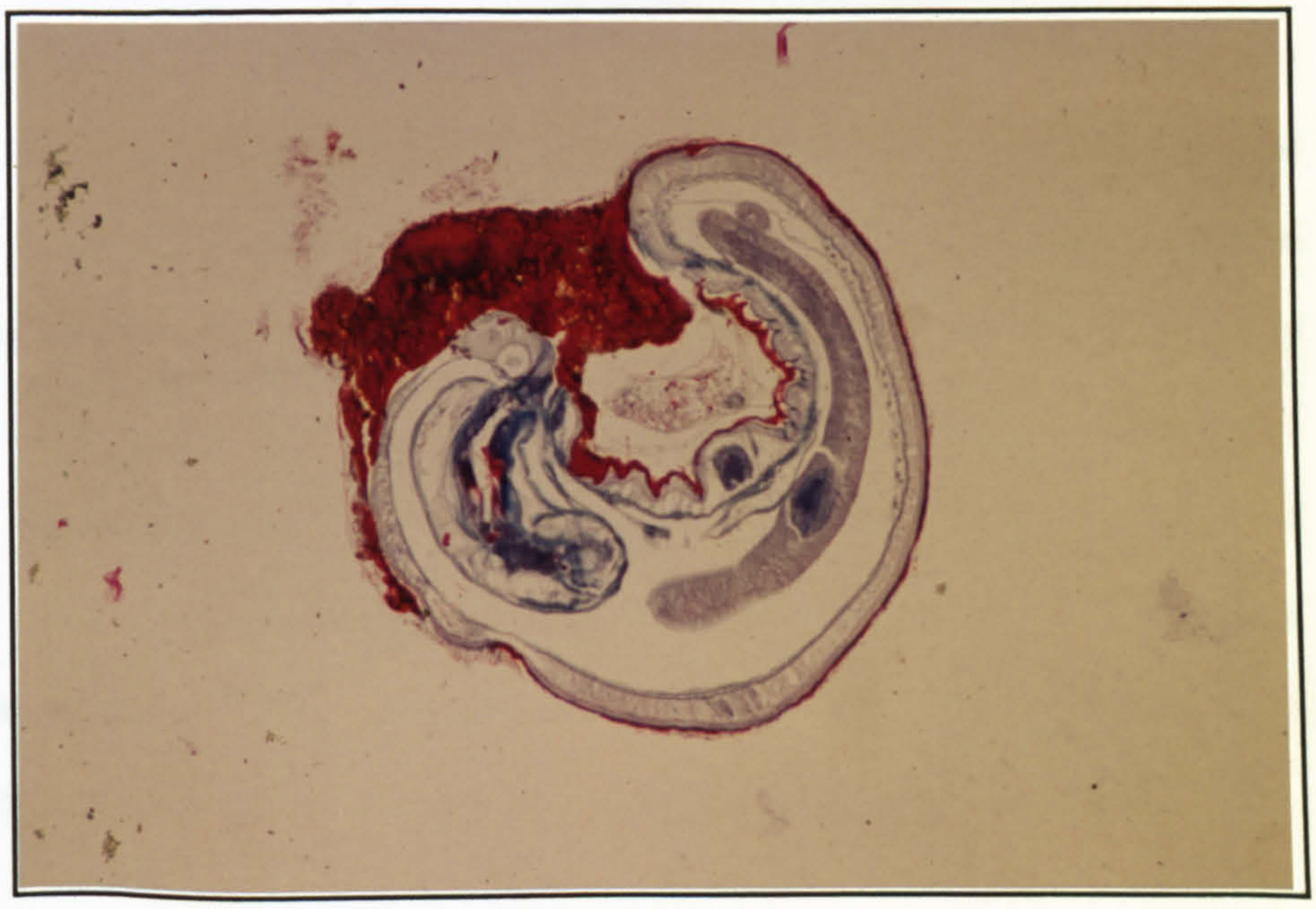
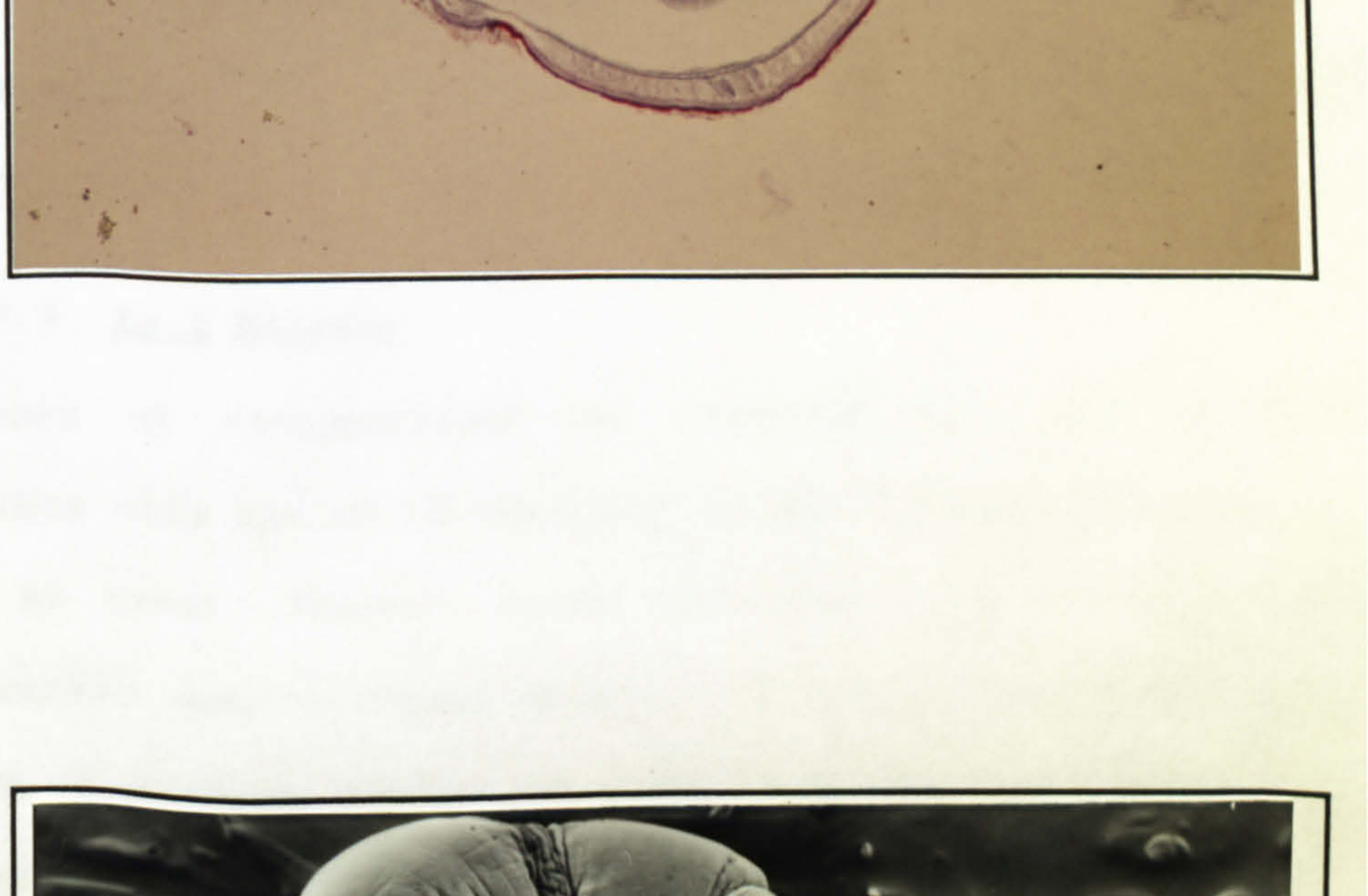


Fig. 7.8 **Scanning electron micrograph of an encapsulated *Neoechinorhynchus rutili* from a *Sialis lutaria* larva.** Magnification x 200.





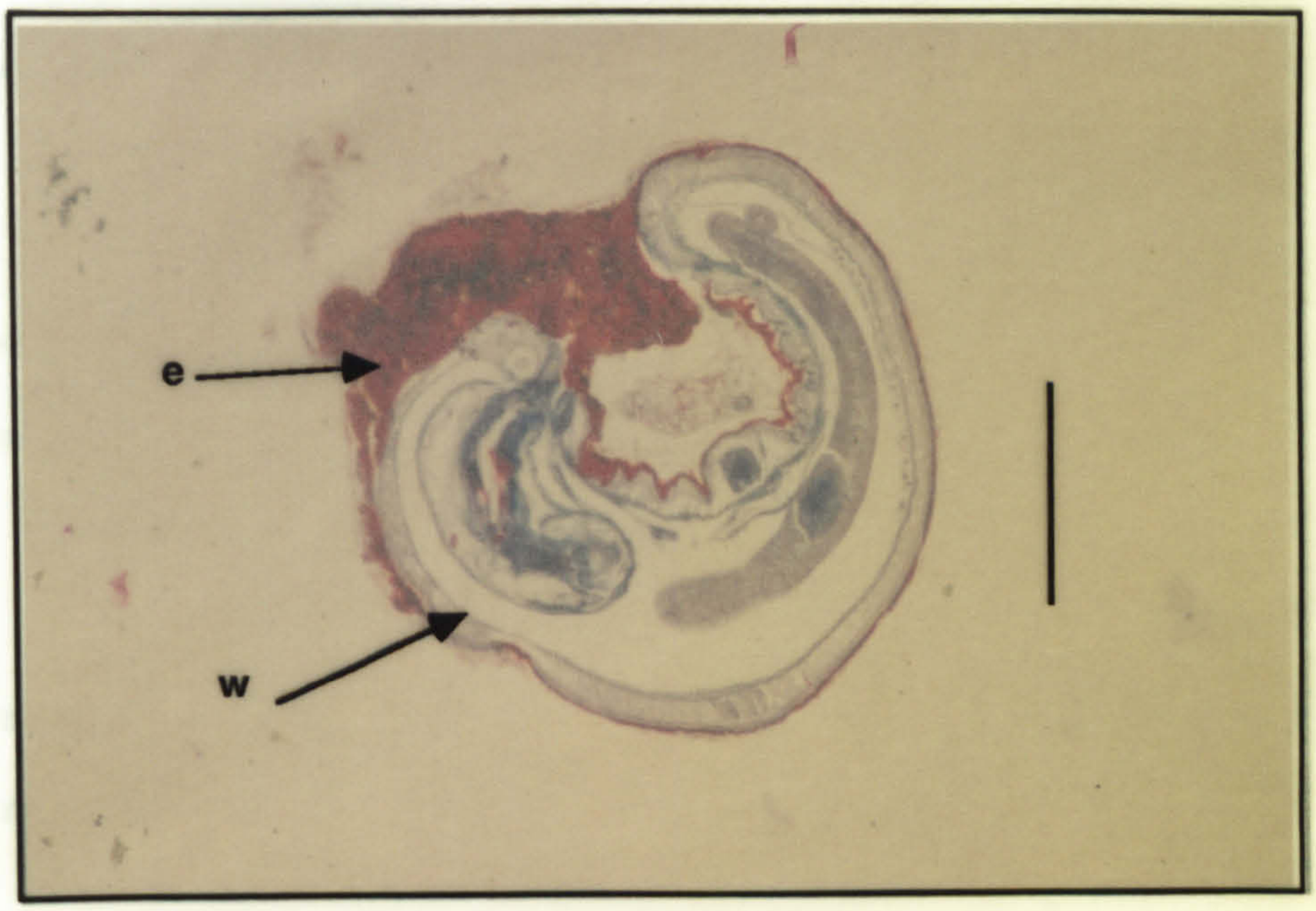


Fig. 7.7 **Transverse section of an encapsulated specimen of *Neoechinorhynchus rutili* from a *Sialis lutaria* larva.** Bar represents 500 μm.

Note: Mallory's triple stained wax section. w = worm, e = encapsulating layer.

Fig. 7.8 **Scanning electron micrograph of an encapsulated *Neoechinorhynchus rutili* from a *Sialis lutaria* larva.** Magnification x 200.

7.3.2.6.2. *Drumore Loch*

Encapsulation was observed around immature female (f1) and male *N.rutili* and it ranged from point encapsulation, for example around the proboscis, to full body encapsulation with evidence of a brown deposit present on the envelope. In 1 case, involving a heavily encapsulated male worm the gonad only represented 6.6% of the total body length. Mann-Whitney U-tests comparing median body length of encapsulated and unencapsulated worms only showed a significant difference for immature female worms ($U = 35.5$, $P < 0.05$). This effect merits further study.

7.3.2.6.2. *Loch Maragan*

No evidence of encapsulation was obtained from the initial samples from this site nor in the sections of whole infected *S.lutaria* larvae eaten by trout. However, worms recovered from the July and August 1988 benthic samples showed evidence of being encapsulated with the deposition of a brown exudate on them. Both immature female and male worms were found to have been encapsulated.

7.3.2.6.3. *Powder Works Dam Lochan*

Eleven of 15 worms (73.3%) examined, showed evidence of some form of having been encapsulated with the deposition of a brown substance. Encapsulation of both mature female and male worms was observed.

7.3.2.7. *Observations of the orientation of* Neoechinorhynchus rutili *in* Sialis lutaria *larvae*

The serial sections of 3 *S.lutaria* larvae infected with *N.rutili* indicated that the worms were usually orientated such that the longitudinal axes of worm and host were parallel. In 2 cases, the proboscis was orientated more anteriorly in the host and in a third case more posteriorly. In larvae 2 and 3, in which 2 *N.rutili*

occurred, both parasites were orientated in the same direction with the body trunks lying in fairly close proximity. In all cases, the *N.rutili* larvae were found exterior to the host gut in the adipose tissue in the haemocoel of the abdomen (see Figs. 7.9 & 7.10).

7.3.3. Transmission experiments

7.3.3.1. Sialis lutaria *larvae and ostracods*

None of the ostracods offered to the *S.lutaria* larvae as food were consumed during a period of 4 weeks without alternative food.

7.3.3.2. Sialis lutaria *larvae and* Neoechinorhynchus rutili *acanthors*

No successful infection with *N.rutili* was observed in any of the experimental groups of insect larvae 57 days post-exposure to *N.rutili* acanthors. A single specimen of *N.rutili* was found in 1 of the larvae from the size 2 (Instar 10) control group. The result is similar to that of Walkey (1967) who was unable to directly infect *S.lutaria* larvae with *N.rutili* acanthors.

7.3.3.3. *Ostracods and* Neoechinorhynchus rutili *acanthors*

There was no evidence of any *N.rutili* infection in the ostracods. Some individuals contained an unidentified cysticercoid (Table 7.9).

Table 7.9: Details of experimental infection of ostracods with *Neoechinorhynchus rutili*

Treatment	Number of Ostracods exposed to *N.rutili* acanthors	Number of Ostracods alive on day 26 p.i.a.	Number of Ostracods with an *N.rutili* infection
Control	50	7	0
Experiment	50	4	0

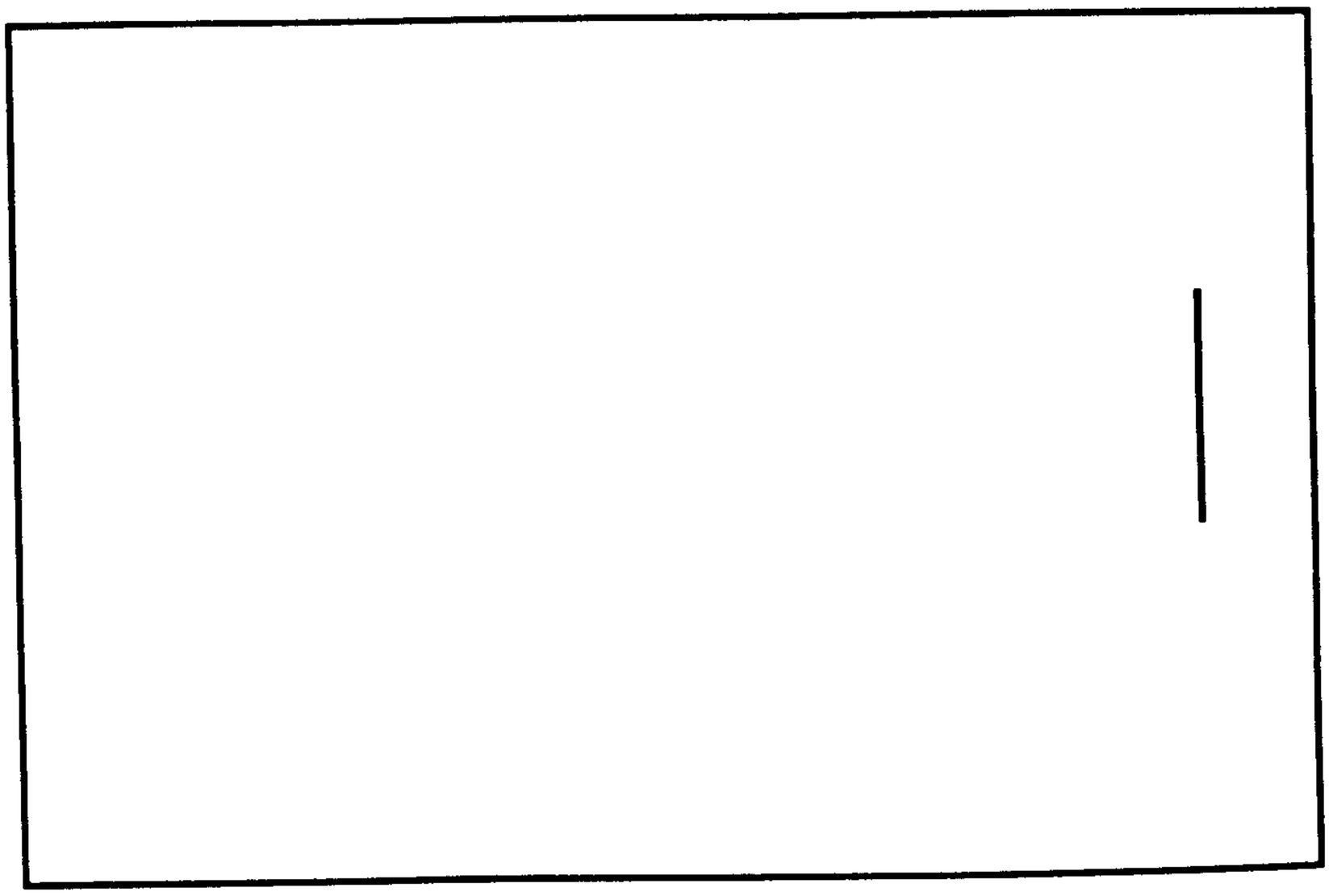


Fig. 7.9 **Longitudinal section through the abdomen of a *Sialis lutaria* larvae containing a female *Neoechinorhynchus rutili*. Bar represents 400 µm. (Mallory's triple stained wax section).**

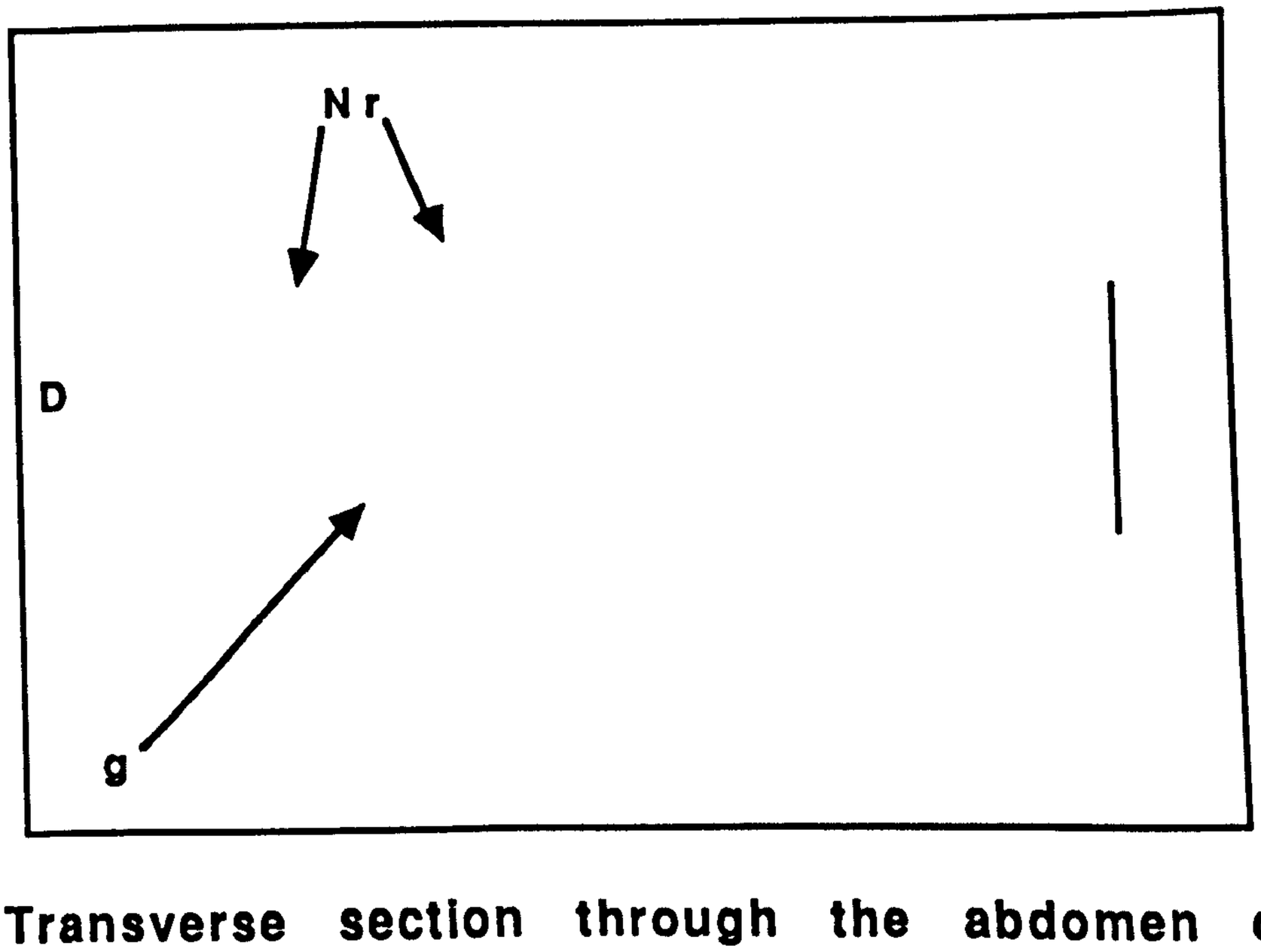
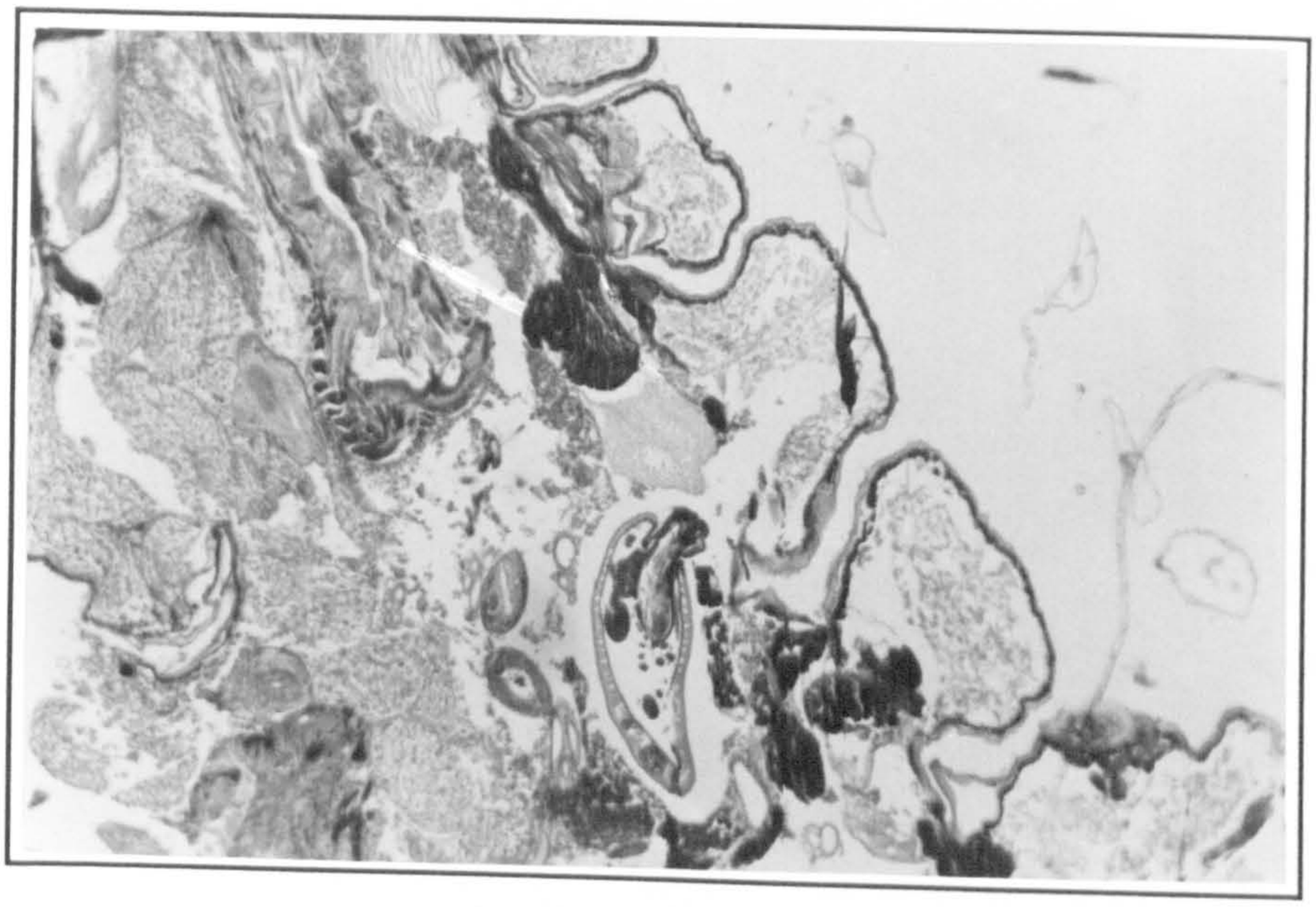


Fig. 7.10 **Transverse section through the abdomen of a *Sialis lutaria* larva containing a male *Neoechinorhynchus rutili* lying close to the gut of the insect. Bar represents 500 µm. Nr = *N.rutili*, g = insect gut, D = dorsal surface of insect. (Mallory's triple stained wax section).**



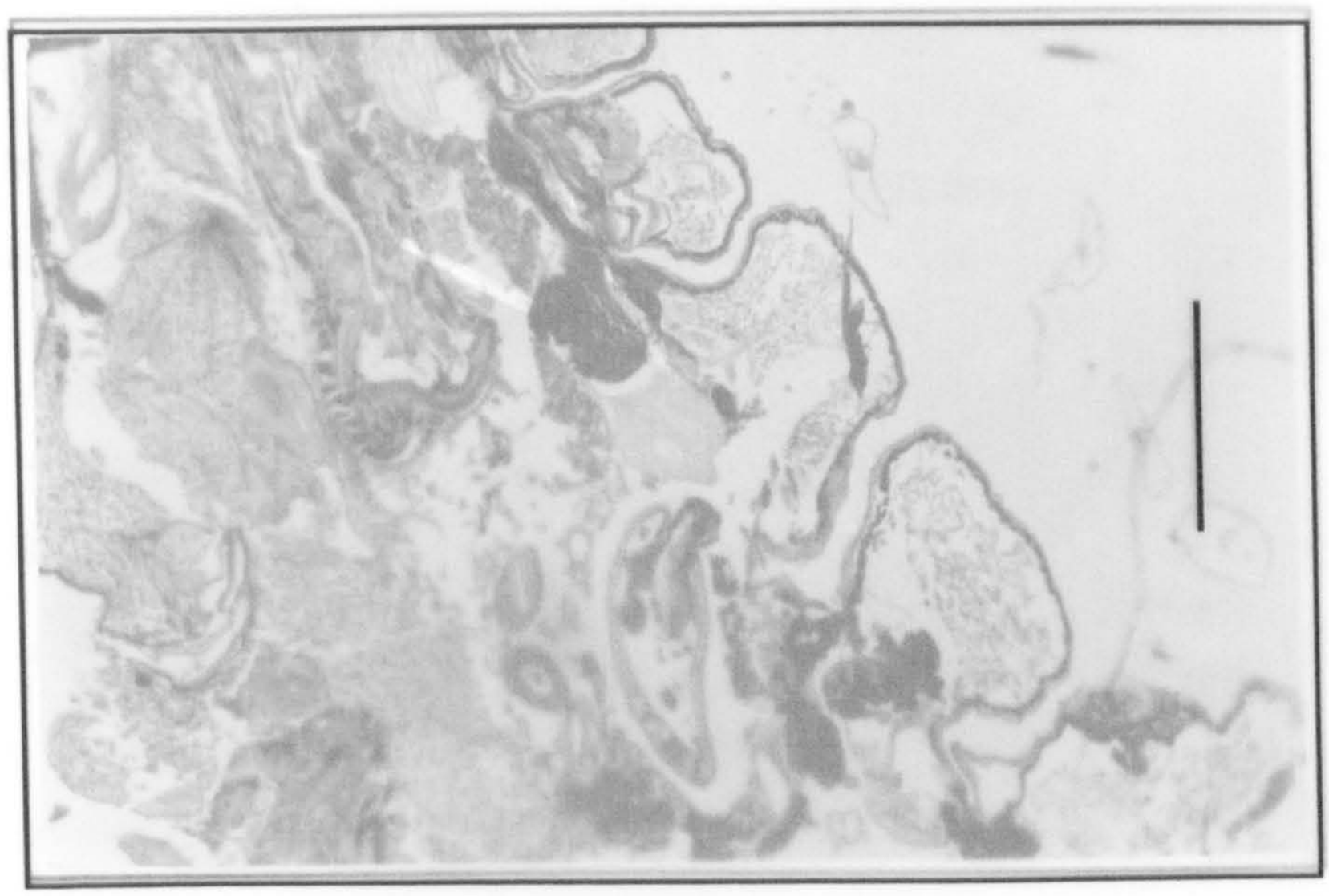


Fig. 7.9 **Longitudinal section through the abdomen of a *Sialis lutaria* larvae containing a female *Neoechinorhynchus rutili*.** Bar represents 400 μm. (Mallory's triple stained wax section).

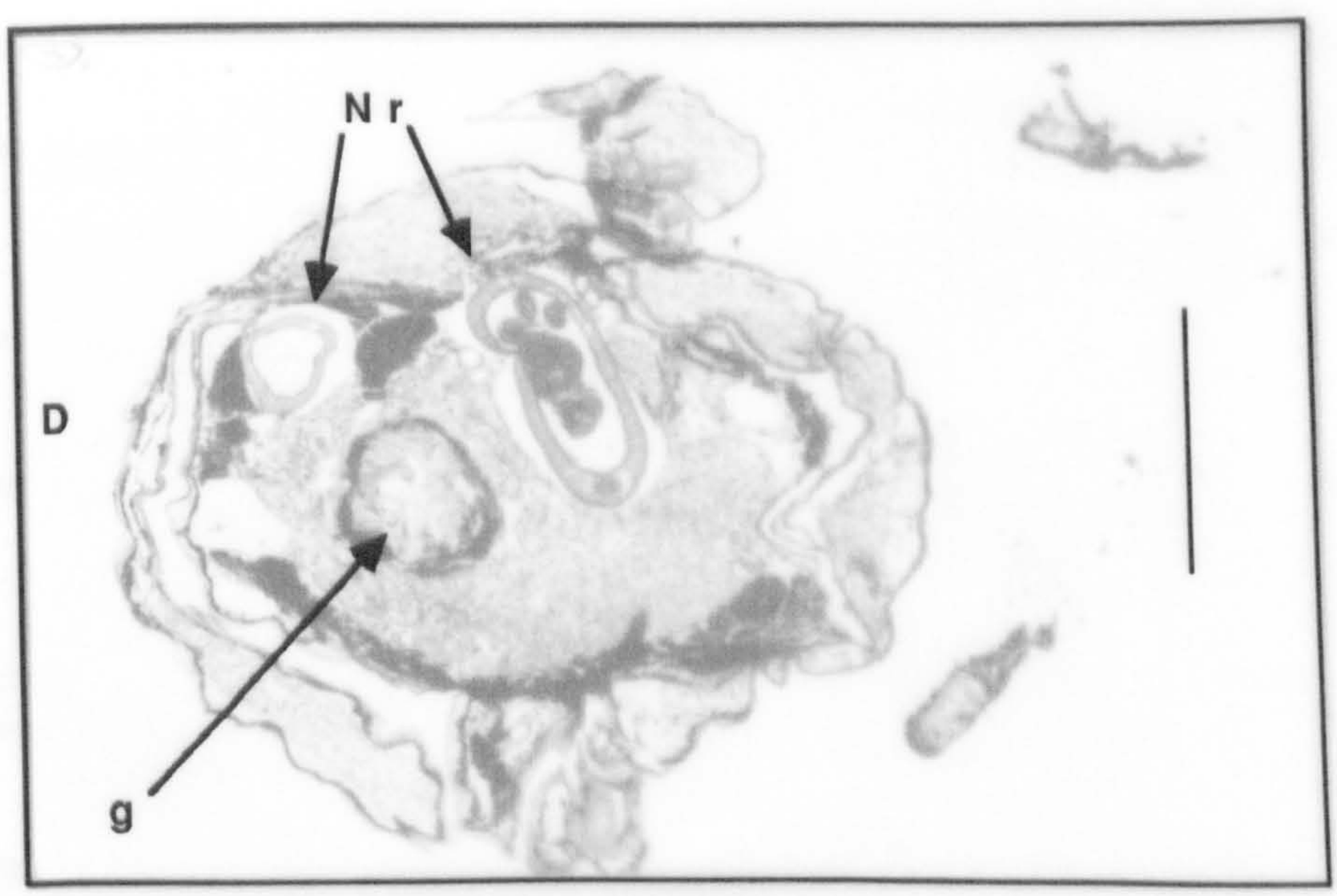


Fig. 7.10 **Transverse section through the abdomen of a *Sialis lutaria* larva containing a male *Neoechinorhynchus rutili* lying close to the gut of the insect.** Bar represents 500 μm. Nr = *N.rutili*, g = insect gut, D = dorsal surface of insect. (Mallory's triple stained wax section).

7.3.3.4. *Rainbow trout and infected* Sialis lutaria *larvae*

7.3.3.4.1. *Experiment 1*

Two of the 3 experimental trout were found to be infected with *N.rutili* attached to the intestinal mucosa 7 days after exposure to the *S.lutaria* larvae. One fish contained an attached female (f2) worm (2.08mm long) and a male (1.82mm long) in the ileum, 1 contained 1 male worm (1.82mm long) attached in the rectum. Therefore, it seems reasonable to assume that the *N.rutili* in the experimental trout were obtained when they fed on the groups of *Sialis lutaria* larvae. A single female *N.rutili* (6.08mm long) was found in the control group of 18 trout dissected to monitor parasite status, some 3 times longer than the female believed to have originated from the *S.lutaria*. Thus the mean intensities of *N.rutili* infection were 1.0 and 0.56 in the experimental and control groups of trout respectively.

7.3.3.4.2. *Experiment 2*

One day post-exposure to *S.lutaria* larvae, 3 of the 6 experimental trout died. Dissection revealed that none were infected with *N.rutili*. Details of the fate of the 45 *S.lutaria* larvae offered to each of these 3 trout is summarized on Table 7.10.

Table 7.10: Fate of *Sialis lutaria* larvae in 'dead trout' tanks on Day 1

Trout No.	Number of *Sialis lutaria* larvae offered to trout	in tank on Day 1	associated with trout: gills	stomach	duodenum	ileum
1	45	9	8	28	0	0
2	45	41	4	0	0	0
3	45	0	0	30	2	13

Only in trout no. 3 had any clear digestion of the larvae taken place. The remaining 109 unconsumed or undigested *S.lutaria* larvae

were dissected to give a more precise assessment of the prevalence and intensity of infection of the *S.lutaria* used in the experiment. The prevalence and mean intensity of infection were 14.7% (16/109) and 0.21 (23/109) respectively. Of the 23 *N.rutili* recovered 19 (82.6%) showed evidence of some form of encapsulation. Including these data with that for the control group the overall prevalence and mean intensity for the sample of larval *S.lutaria* from Bridge of Weir were 11.0% (17/154) and 0.156 (24/154) respectively with 79.2% encapsulation. Thus, the remaining 3 experimental trout should have had the possibility of acquiring 7 *N.rutili* by feeding on their 45 *S.lutaria* larvae. The distribution of the *N.rutili* infection in the *S.lutaria* sample is presented in sections 7.3.2.2 and 7.3.2.3.

The remaining 3 experimental trout, killed 9 days post-exposure to *S.lutaria* larvae, were all found harbouring *N.rutili* in the gut (Table 7.11). The prevalence and mean intensity of infection in the experimental group were 100% and 1.33 respectively. All 3 trout had consumed all 45 insect larvae available to them. No intestinal helminths were recovered from any of the 15 control fish.

Table 7.11: *Neoechinorhynchus rutili* recovered from experimental rainbow trout on day 9 post-exposure to *Sialis lutaria* larvae

Trout No.	Sex, length and position of *N.rutili* in intestine	
	Duodenum	Ileum
4	f2 955um (20.7%)	m 687um (49.6%)
5	m 1095um (21.6%)	
6	m 713um (12.0%)	

(f2 = female with free ovaries; m = male)

Values in parentheses indicate position of attachment expressed as a percentage of the total post-pylorus intestinal length.

7.4. DISCUSSION

7.4.1. Identity of the acanthocephalan from *Sialis lutaria* larvae

Observations of fresh and whole mounted worms and of proboscis S.E.M.s have confirmed the identity of the acanthocephalan from Scottish *Sialis lutaria* larvae as consistent with *Neoechinorhynchus rutili* (Eoacanthocephala). Although only a few specimens were available for measurement (see Table 7.3) the comparisons with *N.rutili* collected from other hosts and localities by myself and other workers indicate, as previously noted by Skryabina (1978) that the proboscis and hook measurements are most consistent characters in this species. The values recorded for these characters in the specimens from *S.lutaria* larvae fell into the ranges described by Van Cleave & Lynch (1950) and Amin (1986).

This identification is further supported by the similarity of the proboscis morphology with that of *N.rutili* from the type host (*Rutilus rutilus*) in Finland, kindly provided and identified by Dr. E.T. Valtonen, University of Jyvaskaya and circumstantially by the fact that *N.rutili* is so far the only species of the genus known from European freshwater fishes (Bullock, 1970).

7.4.2. The relationship between *Neoechinorhynchus rutili* and its *Sialis lutaria* host

7.4.2.1. *Size distribution of* Sialis lutaria *larval populations and their* Neoechinorhynchus rutili *infections*

The size distribution of the samples represents a combination of the real population structure and the sampling bias. At the lower end of the size range difficulties in seeing and capturing small individuals explains their absence or paucity in the samples. At the upper end of the size scale, fewer large individuals represents the normal type of 'population effects' such that cohorts become

increasingly smaller as they get older and has been observed in other *Sialis lutaria* populations (Giani & Laville, 1973). The distribution of *N.rutili* infections of different instars is discussed in sections 7.4.2.6.1 and 7.4.2.6.2.

7.4.2.2. *Prevalence of infection*

An examination of the data, without reference to season, reveals different prevalence values ranging between around 4% to about 41% for *Sialis lutaria* larvae collected from benthic substrates. This finding may be an indication of differences of shelled acanthor densities and/or ostracods at various sites. For example, at Drumore Loch, where the overall prevalence of infection was found to be 40.47%, 3 species of fish (wild populations) were found to be infected with *N.rutili* (see Table 3.2, Chapter 3). These species were *Salmo trutta*, *S.gairdneri* and *Gasterosteus aculeatus* which often had very high intensities of infection, up to 812, 1199 (Devine, 1988) and 130 (Lassiere, unpublished observation) respectively. This site is a man-made eutrophic loch created by the damming of a small stream and has a slow flow-through rate which has resulted in heavy silting (Devine, 1988). Consequently there must be a build-up of large numbers of acanthors during the year in the substrate which would be available to the *Sialis lutaria* larvae either directly or indirectly, depending on how they become infected (see section 7.4.2.4) and hence the observed prevalence is high. Merritt & Pratt (1964) found that *N.rutili* acanthors remain viable for up to 6 months in tap water in the refridgerator so a build-up of viable acanthors from year to year might occur. Drumore Loch also has a large caged population of rainbow trout (a commercial farm) infected with *N.rutili* with intensities of infection up to 8 per fish and these worms must also contribute to the overall shelled acanthor density in the loch substrates.

At Bridge of Weir and Loch Maragan, the prevalence values were lower than those seen at Drumore Loch, being 10.73 and 13.33% respectively. As at Drumore Loch, several fish species were found to be infected: at Bridge of Weir rainbow trout, brown trout and three-spined sticklebacks were infected with maximum intensities of infection of 891, 31 and 4 respectively; at Loch Maragan minnows and brown trout were infected with maximum intensities of infection of 6 and 324 respectively. Considering the physical properties of the sites in turn: Bridge of Weir is a fish farm with several mud-lined main holding tanks for trout supplied by water from the river Gryfe via narrow, shallow water channels. Sticklebacks inhabit both the channels and the main holding tanks. The *S.lutaria* larvae were sampled from the channels, where only sticklebacks would be most likely to be providing an acanthor input and this coupled with the likely acanthor loss due to water flow may explain the lower prevalence of infection observed at this site. In the more static trout holding tanks, housing high densities of infected trout, the acanthor output and build-up must be greater and thus the density should be higher leading to a higher prevalence of infection in the *S.lutaria* larvae. This was not measured. By contrast Loch Maragan is a fairly large, static water body where there is a build-up of a deep layer of benthic material. Here the relatively sparse, often heavily infected population of brown trout and more dense population of minnows, which had a low intensity of infection, is likely to result in a wide dispersal of the acanthors and hence a lower transmission rate of *N.rutili* infection to *S.lutaria*.

At Loch Monzievaird, which is a large loch with wild brown trout and three-spined stickleback populations and introduced rainbow (200 fish introduced per annum, p.c. J.A. Ewald) and brook trout

(*Salvelinus fontinalis*) populations, the maximum observed intensities of infection of brown trout and sticklebacks were 8 and 1 respectively. Thus there would appear to be a limited input of acanthors into the loch resulting in a relatively low prevalence of infection at this site (4.19%). Putting these observations in the context of other studies, Merritt & Pratt (1964) reported a 24% prevalence of infection of the ostracods *Cypria turneri* at Suttle Lake, Oregon, which they described as a small oligotrophic lake. There they found 4 species of fish harbouring *N.rutili* but no intensity values were given. Walkey (1967) found lower intensities of infection in the ostracods *Cypria ophthalmica* and *Candona candida* (3.75 & 3.85% respectively) in Monkton Pond, a small static water body (area = 1.2ha) where *Gasterosteus aculeatus* was the only fish host present. These values were for the entire study period and the original data shows prevalence values of up to 15% (March 1961) (Walkey, 1963). In general these observations show that in static water environments the density of the fish populations, size of the water body and number of infected definitive hosts producing acanthors will influence the observed prevalence and distribution of infection in the intermediate hosts.

An example of this phenomenon for another species *Neoechinorhynchus* is given by Amin (1986), who attributed the difference in density of *Neoechinorhynchus cylindratus* populations in 2 Wisconsin lakes to the physical conditions of the water bodies. In the smaller land-locked Silver Lake (188ha in area) where 11 species of fish had reproductively active infections, the *N.cylindratus* population was much 'denser' than in the larger Fox River-connected Tichigan Lake (458ha in area). In lentic populations of *N.rutili* the prevalence in the fish are much higher than in lotic environments. For example, Bwathondi (1976) found mean intensities of infection of brown

trout from the Burn of Savoch, Scotland only reaching values of 2.27 (value calculated from his raw data) and this must be a least partially a consequence of the fish and potential host densities and the rate of loss of acanthors from the immediate habitat. In a fast flowing stream it would be likely that many acanthors would be washed downstream and no build-up as occurs in lakes would be possible. The study of the *N.rutili* infections of brown trout in Malham Tarn by Kennedy & Burrough (1978) resulted in some contrary conclusions about the most likely origins of the infections. They found that only the native brown trout and none of the stocked brown trout were infected with *N.rutili*. They attributed this difference to the native trout picking up their infection by feeding upon infected ostracods while in the spawning streams, implying that all the acanthors were accumulated there. The mean intensity of infection of these trout was very low (2.0/host) so it could be possible that the stocked trout were also infected in the main lake but the prevalence was too low to be detected in their sample of 28 fish. The infected native fish were all from the 2+ age class which was not represented in the stocked trout sample and this may account for the absence of infection observed in the sample group. The minnows and three-spined sticklebacks inhabiting the lake were not examined for *N.rutili* infection in this study and it may be the case that these are the preferred definitive hosts at this site and they had higher intensities of infection than the trout. In my opinion, it seems highly unlikely that life cycle of *N.rutili* was maintained only in the streams adjoining the lake.

The overall impression is that high prevalences of infection of *S.lutaria* larvae are observed at sites where there are either many fish species infected and/or slow water currents which allow for the build-up of shelled acanthors in the benthic sediments. Consequently

observed mean intensities of infection of definitive hosts are higher in these types of systems as compared to lotic systems were the build-up and consequent transmission success of *N.rutili* must be significantly smaller.

7.4.2.3. *Intensity of infection*

The intensity of infection values for *Sialis lutaria* larvae showed a similar pattern to the prevalence values and must be attributable to the same factors of acanthor input, build-up probability and transmission rates specific to each site.

7.4.2.4. *Prevalence of infection: seasonal*

The limited temporal data from Drumore Loch and Loch Monzievaird can be interpreted as representing part of a seasonal pattern. At Loch Maragan, shelled acanthors are produced and presumably released from their trout or minnow hosts during the summer months (see Chapter 6 for details) and, if a similar pattern exists at Drumore and Monzievaird, then the *S.lutaria* larvae would be exposed to infection at this time. If infection of *S.lutaria* is direct, then an increase in prevalence over the winter months would be expected as the larvae feed on the acanthors during this period. Instars 1 to 7 from the same year are known to be common at this time (Elliot, 1977a) so all susceptible instars should be available for infection. In spring, the observed prevalence pattern will depend on 3 factors: firstly the continued establishment of infection, secondly predation by trout on the larvae and thirdly any density-dependent mortality effects on overinfected larvae.

If infection occurs only through feeding on infected ostracods, there would be a long period between the release of shelled acanthors from fish, the infection of ostracods and the *Sialis lutaria* larvae growing large enough to be able to feed upon infected ostracods and

become infected themselves. Thus, the increase in prevalence would show a lag after the summer months. Once the overwinter increase is observed the spring migration of instar 10 larvae to pupate would also have an influence on the observed prevalence values. Trout tend to eat preferentially migrating instars (Ball, 1961) so there will be an overall reduction in the percentage prevalence of infection of the *S.lutaria* population during these months. This type of pattern was seen at Loch Monzievaird, but no autumnal increase was observed as at Drumore Loch. Thus, the dynamic interaction of infection time lags caused by temperature, different potential routes of infection, the seasonal introduction of young instars of *S.lutaria* and migration and pupation of older instars and the extended period of acanthor release from gravid females from their fish hosts should bring about a specific pattern. This is envisaged as an increased prevalence of infection of larvae over the winter months followed by transmission to the definitive host in the spring months resulting in a reduction in *S.lutaria* prevalence values. The data of Walkey (1963) shows similarities to this pattern with the percentage prevalences of infection of *Cypria ophthalmica* (ostracod) of 7, 15 and 3.3% in November, March and July respectively.

7.4.2.5. *Intensity of infection: seasonal*

The intensity of infection data is not so clear cut but the same underlying trends as described for the prevalence values are apparent.

7.4.2.6. *Size of* Sialis lutaria *and their* Neoechinorhynchus rutili *infections*

7.4.2.6.1. *Prevalence*

At 3 of the 4 sites examined a pattern of lower percentage prevalence of infection in small and large instars and higher values

in intermediate sized instars was found with either instar 8 or 9 showing the highest prevalence values. This is a similar pattern to that observed by Muzzall (1978) of the isopod *Caecidotea communis* infected with *Fessisentis friedi* and in *Asellus aquaticus* infeted with *Acanthocephalus lucii* (Brattey, 1986). Kennedy (1985a) used the former example as evidence for parasite-induced reduction in host growth rate and/or death of older infected hosts resulting in the occurrence of the higher prevalence values in the hosts of medium size. Experimental superinfection of *S.lutaria* larvae would give some indication of whether this effect could operate in natural systems.

A different pattern was observed at Bridge of Weir where the percentage prevalence of infection increased with instar. A possible explanation of this is that all ages or instars of *S.lutaria* are susceptible to infection by *N.rutili* and thus older instars which will have had the opportunity to be exposed to infection for the longest period of time, will be the most heavily infected. This pattern has been observed in other acanthocephalan systems. For example, Liat & Pike (1980) found this pattern in *Profilicollis botulus* infections of *Carcinus maenas*. This pattern could also further be explained by the known feeding habits of the larvae. Older larvae feed upon smaller individuals (Elliot, 1977a) and successful transmission of *N.rutili* by this route could explain the higher percentage prevalence values in larger, older instars.

Thus 2 conflicting patterns with alternative explanations for their generation were observed in the infections of *Sialis lutaria* larvae by *N.rutili*. The question of parasite-induced effects on host growth/ survival could be investigated experimentally. The negative results of the infection experiment with *N.rutili* acanthors (section 7.3.3.2) appear to indicate that the larger instars are not susceptible to direct infection by acanthors and therefore the former

explanation may be most appropriate. However, if infection is only via feeding upon infected ostracods then susceptibility could be size linked, the percentage prevalence of infection merely being a measure of the ability of the larvae to capture and ingest ostracods.

7.4.2.6.2. *Intensity*

The intensity of infection values showed similar patterns to the percentage prevalence values discussed in section 7.4.2.6.1 and could therefore be attributable to similar factors. An exception is that observed for *S.lutaria* larvae consumed by Loch Maragan trout (see Fig. 7.5b) where the mean intensities of infection for instars 6 to 10 were fairly similar. If the benthic samples from the same site are representative of the field situation then it appears that the Loch Maragan trout were selecting *S.lutaria* larvae of greater size (57.9% of all larvae consumed were instars 9 & 10) and a disproportionately large proportion of any smaller instars which they consumed were infected (see Table 4.5, Chapter 4). Therefore there may be some parasite induced effect upon the larvae at the earlier stages which despite their small size make them available to the trout as food. This change could simply be mediated by a greater oxygen requirement thus forcing the larvae out of the main body of the sediment and onto the surface. Another alternative is that their activity cycle might change such that they become day active instead of night active. This seems probable because such effect are known to occur for every acanthocephalan studied in its intermediate host (Moore, 1984).

7.4.2.7. *Frequency distribution of numbers of* Neoechinorhynchus rutili *infections in* Sialis lutaria

At the 4 sites for which the distribution of *N.rutili* infection in *S.lutaria* larvae was compared with the negative binomial

distribution there was good agreement. This is now a well known feature of the association between acanthocephalan cystacanths and their intermediate hosts and has been reported by many authors for many species (e.g *Polymorphus minutus* infecting *Gammarus pulex*; *Leptorhynchoides thecatus* in *Hyalella arctica*; *Moniliformis moniliformis* in *Periplaneta americana* ; *Acanthocephalus dirus* in *Asellus intermedius* and *Neoechinorhynchus emydis* in *Cypria maculata* by Crofton (1971), Uznanski & Nickol (1980), Holland (1983), Camp & Huizinga (1980) and Hopp (1954) respectively). The calculated k values which are a measure of the degree of aggregation in the distribution, ranged from 0.0729 to 1.2585. In comparison to the values given for k for the distibution of other acanthocephalan species in their intermediate hosts by Dobson & Keymer (1985) these values can be considered indicating a high degree of aggregation. Indeed, Dobson & Keymer (1985) go as far as to describe distributions of acanthocephalans in their intermediate hosts as tending 'to be random or only slightly overdispersed' as compared to the highly overdispersed distributions in corresponding definitive hosts. They envisage that at low parasite population densities overdispersion would increase the effective rate of transmission between intermediate and definitive hosts and at higher densities it might tend to destabilize the system by minimizing the impact on the parasite on the population growth of the intermediate host. So the question remains, why are the k values so low in the case of *N.rutili* in *S.lutaria* larvae? This may be a consequence of larvae being exposed to concentrated foci of *N.rutili* as acanthors in female worms or as later developmental stages in infected ostracods which also show an aggregated distribution of infection.

The maximum number of *N.rutili* per individual insect was found to be 6, in contrast to 4 found by Brady (1910) in *Cypria angulata*, 1 by

Walkey (1967) in *Cypria opthalmica* and *Candona candida*, and 3 by Merritt & Pratt (1964) in *Cypria turneri*. A similar observation was made by DeMont & Corkum (1982) for the distribution of *Octospiniferoides chandleri* in *Physocypria pustulosa*. The k values for the latter 2 examples for *N.rutili* are shown as being greater than 5 by Dobson & Keymer (1985). In ostracods the dynamics of infection would be directly influenced by the rate of feeding upon viable *N.rutili* acanthors which would originate either from those released in the faeces of infected fish or contained within whole female worms voided from the host gut, and the length of the ostracod lifetime. The rate of encounter will therefore be dependent upon the movements of the definitive hosts and the ostracods. In this context, as pointed out by Devine (1988), lack of infection of ostracods collected at the edge of Drumore Loch might reflect the spatial distribution of acanthors. He concluded more acanthors would be present at the centre of the loch which was frequented by the trout. However this explanation is questionable since three-spined sticklebacks, also known to be infected at this site frequented the shallow waters at the loch edge. Although no specific experiments examining possible density-dependent effects of infection of ostracods with *N.rutili* acanthors have been done it appears that 4 cystacanths might be the maximum intensity of infection above which the host does not survive. Merritt & Pratt (1964) report that if ostracods (*Cypria turneri*) were allowed to feed for too long on acanthors, overinfection resulted and killed many of them.

The dynamics of *S.lutaria* infection may be affected by 2 factors, assuming that infection can occur via feeding on ostracods and/or acanthors: firstly by the rate of encounter with acanthors, which will be similar to that described for ostracods, although the migration of

larvae, their comparatively large size and longer life-span may increase this rate. Secondly, by the number of infected ostracods consumed by the *S.lutaria* larvae and the success of transmission of *N.rutili* within these and the factors which directly influence this process. The maximum intensity of infection in *S.lutaria* larvae is greater than that reported for ostracods and this can be interpreted as evidence for a density-dependent effect similar to that envisaged for ostracods, the value being higher merely as a function of host size and/or age. *S.lutaria* larvae are known live for up to 3 years (Giani & Laville, 1973) and the simple difference in life span of ostracods and *S.lutaria* and their relative size may explain these observed differences in intensity of infection.

As Kennedy (1985a) explains, the view that density-dependent mortality of intermediate hosts occurs is supported by the agreement with the truncated negative binomial model, as proposed by Crofton (1971) for *Polymorphus minutus* in *Gammarus pulex*, but can also be explained simply in terms of host age (Hirsch, 1980). The example of such a density-dependent effect on mortality of hosts given by Amin, Burns & Redlin (1980) of an *Acanthocephalus dirus* infection of *Caecidotea militaris* supports Crofton's view. Thus an ambiguity exists in the interpretaion of a truncated negative binomial distribution which in the *Sialis* case could be resolved only through experimental infections.

Multiple infections in intermediate hosts can have an influence on the population dynamics of the acanthocephalans in the definitive hosts (Crompton, 1985). Differential longevities of adult male and female worms in their definitive hosts, females living longer, leads to single experimental infections ultimately being dominated by females. After mating, male worms are no longer required and their departure from the host gut results in the female worms experiencing a

more nutrient-rich environment in which to produce acanthors. Thus such multiple, mixed sex infections of intermediate hosts, as observed in *Sialis lutaria* larvae infected with *N.rutili*, could increase the overall reproductive success of the parasite in the definitive host.

7.4.2.8. *Sex of worms infecting* Sialis lutaria *larvae*

Both sexes of worms were found infecting *Sialis lutaria* larvae with both single sex and mixed sex infections observed. Although there appeared to be a bias towards male worms in the larvae this is probably not a genuine phenomenon (see section 7.4.3.4 for explanation). Crompton (1985) suggested that mixed sex infections in intermediate hosts can increase the reproductive success of the adult worms by synchronizing their entry into the definitive host environment (see section 7.4.2.7).

7.4.2.9. *State of maturity of* Neoechinorhynchus rutili *infecting* Sialis lutaria *larvae*

Female worms with unfragmented ovarian tissue were recovered from the *Sialis lutaria* larvae. Comparison of the morphology with the description and drawing by Miss M.V. Lebour in Brady (1910), who suggested that these worms were monorchic males with an indistinct *vas deferens* appear to indicate that these specimens were in fact immature females! Miss Lebour apparently did not find any diorchic male worms amongst her specimens to make a comparison.

Female worms with up to 179 free ovaries were recovered from some of the *Sialis lutaria* larvae. Merritt & Pratt (1964) describe this as being the mature form found developing in ostracods. Peura, Valtonen & Crompton (1986) described the development of free ovaries in the cystacanth stage of *Echinorhynchus gadi* as being a 'precocious' phenomenon. They envisaged that the degree of ovarian development

could be linked to the definitive host range, such that species with a wide host range such as *E.gadi* and *N.rutili* would benefit from accelerated ovarian development. This early reproductive maturity should ensure a short pre-patent period in the definitive host and perhaps an enhanced reproductive success.

7.4.2.10. *Size of* Neoechinorhynchus rutili *infecting* Sialis lutaria *larvae*

The worms recovered from the *S.lutaria* larvae were larger than those found in ostracods by Merritt & Pratt (1964) and those found in *S.lutaria* larvae by Villot (1885). It is difficult to explain this difference which might have resulted from different specimen preparation, examination and measurement techniques. However, it would be expected that larger individuals would be recovered from *S.lutaria* larvae, there being more space for the development available in the insect haemocoel. The pattern of mature female worms (f2) being longer than males is similar to the observation of Merritt & Pratt (1964) in ostracods. However, they described the juveniles of both sexes being simliar in length. The present data indicates that the f1 females and males are similar in size so the difference in size in more mature worms could be the result of differential growth rates of the sexes in the latter stages of development.

7.4.2.11. *Encapsulation*

Although encapsulation of *N.rutili* was not observed in *S.lutaria* larvae from Loch Monzievaird this phenomenon was seen in specimens collected from Bridge of Weir, Drumore Loch, Loch Maragan and Powder Works Dam Lochan. In some cases, the growth of *N.rutili* appears to have been restricted resulting in stunted forms. Dr A.M.Lackie examined the slides of sectioned encapsulated *N.rutili* and described the host response as only partial and probably only humoral in nature.

Dobson & Keymer (1985) described encapsulation as being an indication of incompatibility between host and parasite but the successful experimental infection and recorded size of worms indicate a limited effect of the immune system. Awachie (1966) described the occurrence of a brown extensible envelope of parasite origin covering the larva of *Echinorhynchus truttae* during the second phase of development in *Gammarus pulex* which he believed might offer some protection to the worm and this may also be the case for *N.rutili* . However, Gotz (1986) found evidence of cellular encapsulation in *Sialis lutaria* larvae both *in vitro* and *in vivo* and quotes a mean haemocyte number of 34,500 per mm^3 so the envelope could also be of host origin. Three processes could explain the observations. Firstly, that as worms grow they are able to break free from this encapsulating layer as seems to be the case in the Drumore Loch specimens in which only encapsulation of the smaller immature female worms was observed. Secondly, some worms may in some way be able to mask themselves so they are not recognised by the host as 'non-self' and hence are not encapsulated at any stage. Thirdly, initial infections with *N.rutili* are not recognised but subsequent individuals are recognised as non-self and are attacked by the host's immune systems. Another controlling factor may be temperature such that in winter, encapsulation would be less rapid as a consequence of a lower overall basic metabolic rate of the host. The male biased sex ratio observed in some samples may indicate sex differences in resistance to encapsulation. Obviously controlled experiments with individual insect larvae of differing instars challenged with *N.rutili* infections under different environmental conditions should resolve these problems and help to explain field observations.

7.4.2.12. *Orientation within the host*

Only a limited number of infected *S.lutaria* specimens were sectioned but it is interesting that in all cases the orientation was similar, with the longitudinal axes of host and worm parallel to one another. Muzzall & Rabalais (1975) reported a similar pattern for *Acanthocephalus jacksoni* cystacanth orientation in the isopod *Lirceus lineatus*. If this is a genuine phenomenon then it might be explained by the nature of the flow patterns in haemocoel circulation of the insect. Furthermore, on a purely speculative basis, since the worms appear to have fully formed reproductive organs this may indicate possible attempts at copulation. If successful insemination could occur in the insect host then this would result in the *S.lutaria* larval host definition being changed and the re-examination of *N.rutili* population dynamics. This proposal is not entirely speculative since Brattey (1980) observed active spermatozoa in male cystacanths of *Acanthocephalus lucii* in its intermediate host *Asellus aquaticus*.

7.4.2.13. *Effect of* Neoechinorhynchus rutili *on its host*

This aspect of the relationship has been discussed in Chapter 4 and in section 7.2.4.6.2 where the possibility of morphological, behavioural and physiological alterations of the host induced by *N.rutili* infection have been considered.

In terms of physiological effect, I was intrigued to know whether infected larvae would be able to pupate successfully and become fertile adults. Since the farmer at the Bridge of Weir site informed me that each May/June numerous adults were found at the waters' edge I decided to let the natural experiment continue and to examine the gonads of pupated adults from the site. The farmer was provided with a suitable fixative (10% formalin) and asked to collect at least 100

adults in the May/June of 1988. Unfortunately he only found 1 adult during this period and therefore this effect remained uninvestigated although I believe it merits further study since other acanthocephalan species are known to castrate their arthropod hosts (e.g. *Acanthocephalus dirus* cystacanths in *Asellus intermedius* reported by Seidenberg (1973)). Three adult *Sialis lutaria* from the shores of Loch Maragan were dissected in June 1987 and none was found to harbour developmental stages of *N.rutili*.

No evidence of any pigmentation differences between infected and uninfected individuals was observed. As indicated in Chapter 4, behavioural experiments similar to those conducted by Moore (1983) with infected isopods would be suitable in this case. Dubois & Geigy (1935) described *Sialis lutaria* larvae as nocturnal animals actively foraging during darkness. A suitable behavioural change mediated by the parasite would be to make the insects active by day and more accessible to potential definitive hosts.

7.4.3. Transmission experiments

7.4.3.1. Sialis lutaria *and ostracods*

Although the experimental attempt to feed *S.lutaria* larvae with ostracods of the species *Herpetocypris reptans* was unsuccessful it is known that larvae of instars 1 to 10 will feed on ostracods in natural conditions (Giani & Laville, 1973). Griffiths (1973) described *S.lutaria* larvae from a moorland pond in Wales as eating *Cypria ophthalmica*, a known intermediate host of *N.rutili* (Walkey, 1967). *Herpetocypris reptans* is one of the largest ostracod species which occurs in Britain (Hounsome p.c.) being 2 to 2.5mm long and 0.88mm high (Tressler, 1959) and this may account for the inability of the larvae to consume them. By comparison, the species of ostracods shown to be intermediate hosts of *N.rutili* , *Candona angulata*, *Cypria*

turneri, *Candona neglecta*, *C.candida* and *Cypria ophthalmica* (Brady, 1910; Merritt & Pratt, 1964; Valtonen, 1979; Walkey ,1967) which are much smaller than *H.reptans* with lengths of 1.4-1.6mm (Brady, 1910), 1.35-1.55mm (Brady, 1910), 0.55mm (Tressler, 1959), 0.56-0.61mm (Tressler, 1959) and 1.05-1.22mm (Tressler, 1959) respectively could possibly be more easily consumed by *S.lutaria* larvae and therefore transmission by this route is more likely. If *S.lutaria* larvae do acquire their *N.rutili* infections by feeding upon infected ostracods it would be interesting to know what size and stage of parasite is capable of being transmitted and by which mechanism. The fact that the worms recovered from *S.lutaria* larvae were often twice as long as those recovered from ostracods by Merritt & Pratt (1964) implies that some form of growth occurs in the insect larvae. However, it seems probable that the acanthocephalans must be at an early stage when it is transmitted from the ostracod or else there would be severe damage to the host during migration of parasite larvae through the gut wall into the haemocoel.

7.4.3.2. Sialis lutaria *and shelled acanthors*

Merritt & Pratt (1964) studied the development of *N.rutili* in experimentally infected ostracods at 15°C and observed complete development of an infective female juvenile within 48 to 57 days post infection. Since the *S.lutaria* larvae in the present study were kept at 12°C it seemed reasonable to end the experiment after this period. Walkey (1967) carried out his experimental infections at 18°C and introduced 1500 acanthors into the experimental vessels. He found no successful infections of *S.lutaria* larvae after 25 days. Even though the larvae were offered pieces of gravid *N.rutili* to consume, presumably easier to handle and containing many shelled acanthors, no successful infection was found. If *S.lutaria* larvae do not become

infected in the wild by feeding upon infected ostracods then alternative modes of transmission must operate. It is known for some species of acanthocephalan species that success of infection can be host size related. For example Oetinger & Nickol (1982) found that only small (40-50 mm long) *Asellus intermedius* could be infected routinely with *Acanthocephalus jacksoni* in the laboratory. Gleason (p.c.) suggested that in the case of *Pomphorhynchus bulbocolli* feeding behavioural differences in hosts (*Gammarus*) influenced infection and that these changed with the size of host. The study of Dubois & Geigy (1935) reported that first instars of larvae of *S.lutaria* feed upon microoganisms and detritus and also have a mud burrowing habit and are commonly found in the littoral regions of the habitat. Older instars are usually found in the sub-littoral and profundal regions where most shelled acanthors will be located due to the movements of the definitive hosts. It seems reasonable to suggest that the young benthic feeding larvae would become infected in natural conditions at this stage. The present study dealt only with instars 8, 9 and 10 which may partially account for the lack of infection success. Walkey (1967) did not record the instars of the 30 *S.lutaria* larvae which he used for his experiment. Elliot (1977a) observed that older larvae of instars 7 to 10 occasionally eat younger larvae and this may provide a route for eupostcyclic transmission. This experiment would need to be repeated with a full range of larval instars. As Devine (1988) stated, only when *S.lutaria* larvae can be directly infected with *N.rutili* acanthors, which subsequently develop into cystacanths that are infective to suitable fish species, can *S.lutaria* be cited as a true intermediate host of *N.rutili*.

7.4.3.3. *Infection of ostracods with* Neoechinorhynchus rutili

The experimental attempt to infect *Herpetocypris reptans* with

N.rutili was unsuccessful, in accord with the results of Walkey (1967). Dissections of this species of ostracod from sites of *N.rutili* infection have not shown any evidence of *N.rutili* infection themselves. This can be interpreted in a number of ways: firstly, the field evidence may not be conclusive. Walkey (1967) found the prevalence of *N.rutili* infection in County Durham to be fairly low, 3.75 % in *Cypria ophthalmica* (n = 400) and 3.85 % in *Candona candida* (n = 26). Walkey (1963) found seasonal differences in the prevalence of infection and it is possible that the ostracods in the present study were examined at a time when the prevalence was low in the loch. So the number of ostracods dissected may have been insufficient to detect an infection. Secondly, as Devine (1988) noted, the ostracods which he collected were from the edge of Drumore Loch while the trout tended to frequent the centre of the loch. The shelled acanthors would tend to be more concentrated in the central area and ostracods living there would most likely show a higher prevalence of *N.rutili* infection than their littoral counterparts. All of the ostracods dissected during the course of this study were collected from the shore areas of the loch and this may account for the observed lack of infection by *N.rutili*. Thirdly, the lack of success with the experimental infections may be due not only to the unsuitability of this species as an intermediate host but also to the non-viability of the shelled acanthors used. These acanthors were removed from the body of gravid female worms and, although they appeared to be fully developed, they may not have been infective. A more extensive comparison of the ostracods from various locations at a single site would possibly resolve some of these problems, but the question still remains as to whether ostracods are part of the life cycle of *N.rutili* in Scotland or not.

7.4.3.4. *Rainbow trout and infected* Sialis lutaria *larvae*

The 2 experimental attempts to infect commercially obtained rainbow trout with *N.rutili* established in *Sialis lutaria* larvae were successful. The results of the first experiment were somewhat ambiguous because a single *N.rutili* specimen was recovered from the control group of fish. However the difference between the size of worm recovered from the 2 groups of fish and the mean intensity of infection strongly suggested that the *N.rutili* observed in the experimental group had originated from the *S.lutaria* larvae. In terms of transmission, the route appeared to be fairly successful: of a possible expected 9 worms from the 120 *S.lutaria* larvae eaten by the 3 experimental trout, 3 (33%) were recovered.

Due to the ambiguity of the first experiment, a similar experiment was undertaken using larvae from a different source (Bridge of Weir). In this case, the aims of the experiment were 2 fold: firstly to substantiate the findings of the first experiment and secondly to investigate the effects of encapsulation of *N.rutili* in the *S.lutaria* larvae on its transmission capabilities to rainbow trout. Since the results of experiment 1 were limited to those from 3 experimental fish the protocol for the second experiment included 6 experimental fish. Unfortunately 3 of these died and again the experiment was restricted to 3 individuals. However, of an expected 21 worms in the 135 *S.lutaria* larvae presented to these 3 fish, only 4 (19%) were found to establish successfully in the trout. An exact probability test, as described by Siegel (1956), comparing numbers of unestablished and established worms in both experiments (the former values being derived from the control *S.lutaria* dissections) indicated that there was no significant difference between the 2 groups ($P = 0.247$) and it must be concluded that in this case no adverse effect of

encapsulation on transmission was recognised. The view that encapsulation in *S.lutaria* larvae renders the larvae unsuitable for transmission is questionable. In contrast, the envelope may afford protection to the *N.rutili* larva as it passes through the acidic gastric environment of its potential fish definitive host. It seems unlikely that any density-dependent effects similar to those described by Uznanski & Nickol (1982) in an experiment involving infection of Green sunfish (*Lepomis cyanellus*), with more than 40 *Leptorhynchoides thecatus* cystacanths.

Of the 7 worms found reestablished in the trout guts, 5 were male which may either reflect a biased sex ratio in the *S.lutaria* larvae or a greater establishment success of male worms. No information is available for the former although at other sites, there did appear to be a slight bias towards male worms in the *S.lutaria* larvae. However evidence from other acanthocephalan species (e.g. *Acanthocephalus parksidei* in *Caecidotea mililtaris* (Amin, Burns & Redlin, 1980): *Polymorphus minutus* in *Gammarus pulex* (Crompton & Whitfield, 1968)) indicated that the ratio of female to male cystacanths in the intermediate host is around 1:1 so there is no reason to suppose that *N.rutili* is dissimilar. The finding of a male biased sex ratio of *Acanthocephalus lucii* cystacanths in *Asellus aquaticus* by Brattey (1986) offered support to the opposing view.

The majority of established worms were found in the more anterior regions of the rainbow trout guts. This is similar to the observations of postcyclic transmission of mature specimens of *N.rutili* from three-spined sticklebacks to rainbow trout (Chapter 8) in which it was concluded that worms probably established themselves in the first region of the gut which is physiologically suitable, that is posterior to the pyloric sphincter of the stomach.

These experimental results with rainbow trout have some bearing

upon field observations. Although at most sites infected brown trout have been found feeding upon *S.lutaria* larvae, at Bridge of Weir (site 6) both rainbow trout and brown trout, both infected with *N.rutili*, were found feeding upon *S.lutaria* larvae and therefore the inference that transmission of *N.rutili* by this route occurs for both trout species in natural situations is justified.

7.5. SUMMARY (see Fig. 7.11)

7.5.1. The historical background to the discovery of *Sialis lutaria* larvae acting as intermediate hosts of *N.rutili* is described with a reconsideration of its importance as a host at several Scottish locations.

7.5.2. The methods for the collection and preparation of *N.rutili* specimens from *S.lutaria* larvae as whole mounts, wax sections and scanning electron micrographs are given. Identification was made on the basis of body characteristics and proboscis morphology.

7.5.3. The methods of collection and maintenance and examination of alder fly larvae from various Scottish sites are described.

7.5.4. The source and maintenance of rainbow trout for infection experiments are described.

7.5.5. Several experiments were performed to investigate transmission. These involved *S.lutaria* larvae and ostracods, *S.lutaria* larvae and *N.rutili* acanthors, ostracods and *N.rutili* acanthors, rainbow trout and infected *S.lutaria* larvae (2 experiments).

7.5.6. The acanthocephalan found in Scottish *S.lutaria* larvae was identified as *Neoechinorhynchus rutili* (Eoacanthocephala) on the basis of proboscis morphology and body measurements which were similar to values given by other authors for this species. The proboscis measurements appeared to be the most reliable for

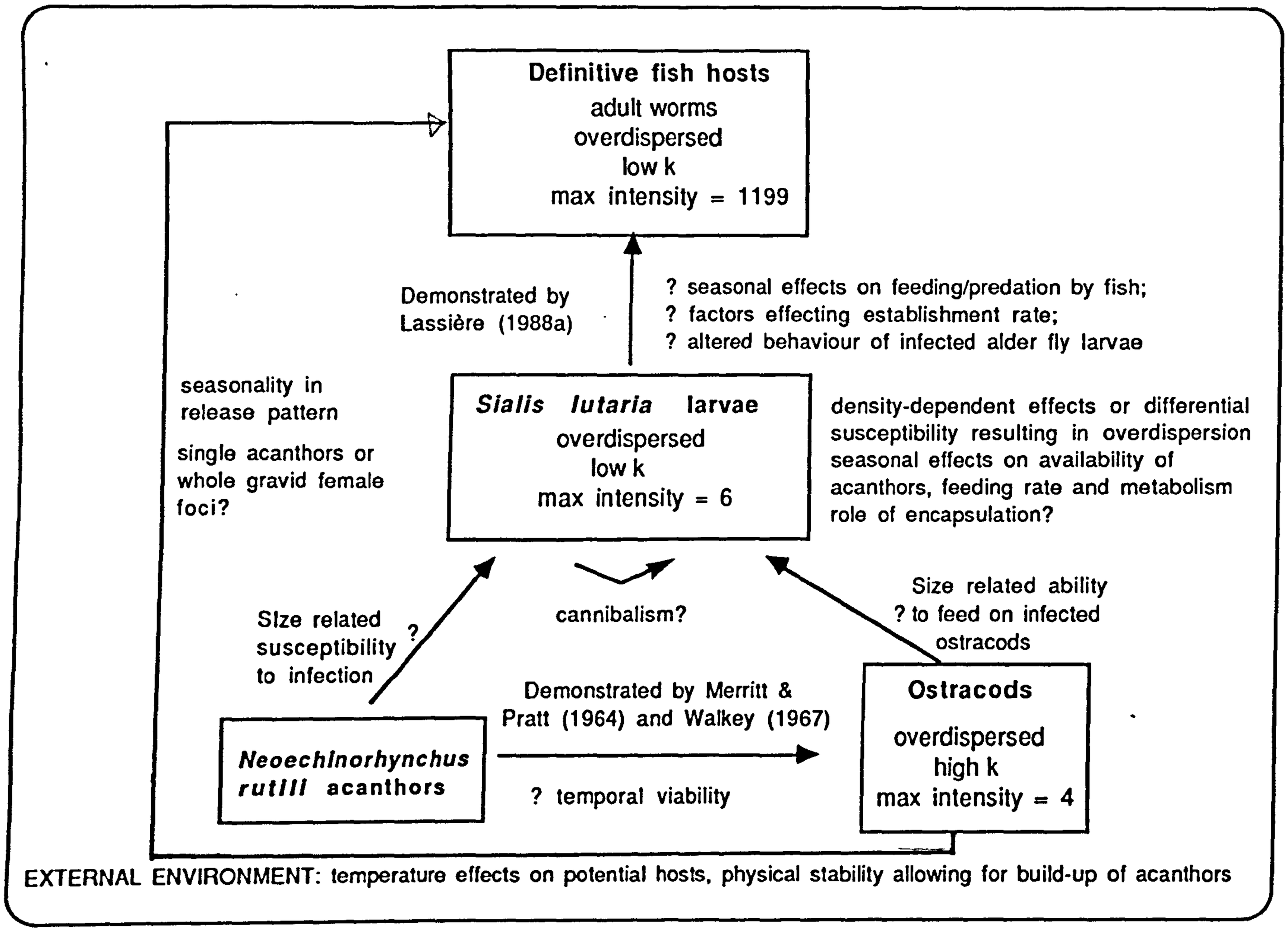


Fig. 7.11 The proposed role of *Sialis lutaria* in the life cycle of *Neoechinorhynchus rutili*

identification purposes.

7.5.7. The distribution of *N.rutili* infection in *S.lutaria* larvae was described in terms of the prevalence and intensity of infection in various larval instars. The overall distribution of numbers of *N.rutili* in the alder fly larvae was found to be overdispersed amongst samples from individual sites. Two patterns of infection with respect to larval instar were found.

7.5.8. Temporal changes the prevalence and intensity of infection were examined at two sites: Drumore Loch and Loch Monzievaird.

7.5.9. Immature and mature female (f1 & f2) and male worms were recovered from the *S.lutaria* larvae. Mature female worms were longer that the other worms and had up to 179 ovarian balls in their body cavity. Fresh worms were longer than those descrived by Merritt & Pratt (1964) from ostracods.

7.5.10. Worms in *S.lutaria* larvae tended to be oriented parallel to the longitudinal axis of the insect host and this may be as a result of the flow patterns in the haemocoel circulation of the insect.

7.5.11. Some form of encapsulation of *N.rutili* specimens was observed in *S.lutaria* larvae collected from 4 of the Scottish sampling sites. This encapsulation is likely to be of host origin, possibly with a parasite component and may serve to protect the worm as it enters the stomach of a potential definitive host.

7.5.12. Successful transmission of *N.rutili* was observed only in the experiments involving rainbow trout feeding on infected *S.lutaria* larvae. Reasons for the lack of success in the other experiments were proposed. The establishment rate in trout was up to 33% with worms tending to become attached in the more anterior regions of

the gut.

7.5.13. The overall prevalence and intensity of infection of *S.lutaria* larve were accounted for in terms of the physical characteristics of the hosts' habitats and the numbers of fish species harbouring mature *N.rutili* infections.

7.5.14. The observed seasonal changes in prevalence and intensity of infection were explained in terms of shelled acanthor input, *S.lutaria* migration and reproduction and the possible route for transmission of *N.rutili* to *S.lutaria* resulting in high late autumn, winter and early spring values and lower values in late spring and summer.

7.5.15. Two possible explanations for the observed distribution of infection with increasing larval instar are given which take into account susceptibility to infection and density-dependent effects of parasitism.

7.5.16. The possible mechanisms which result in an overdispersed distribution of *N.rutili* in *S.lutaria* larvae are discussed and the significance of this in terms of reproductive success is examined.

7.5.17. The possible effects of *N.rutili* on its *S.lutaria* host are discussed with reference to other acanthocephalan species.

CHAPTER EIGHT

POSTCYCLIC TRANSMISSION OF *NEOECHINORHYNCHUS RUTILI* TO TROUT

8.1. INTRODUCTION

8.1.1. Definition of postcyclic transmission

Postcyclic transmission was originally defined by Bozkov (1969) and has been observed to occur in a number of species of helminth including trematodes, cestodes and nematodes (Bozkov, 1976). In essence, postcyclic parasitism involves events by which mature helminths established in one host become re-established in new hosts as a result of feeding relationships. Bozkov (1976), who dealt specifically with vertebrate hosts of parasites, characterized postcyclic hosts as being either eupostcyclic or parapostcyclic hosts depending on whether the new host is conspecific with its prey, or not.

8.1.2. Reports of postcyclic transmission in the Acanthocephala

When Bozkov (1976) wrote his paper he reported that this phenomenon had not been established in the Acanthocephala but Nickol (1985b) has collated reports of at least 5 species of acanthocephalan, from all 3 classes, that were transferred to a new host by this route (Table 8.1). More recently Stoddart & Crompton (1988) have successfully transferred three-day-old *M.moniliformis* from one rat host to another by the oral route. Nickol (1985b) defined postcyclic transmission for the Acanthocephala as 'when ingested as adults within their definitive hosts, some acanthocephalans survive and parasitize the predator'. This concept implies that postcyclic transmission can only occur when an infected definitive host becomes food for another potential definitive host. Bozkov (1976) described that postcyclic transmission may be mediated not only via predation but also via cannabalism (i.e. predation of conspecifics), by consumption of fresh carcasses of hosts, of regurgitated food by animal parents to their young, or of mature helminths that have already vacated their host.

Obviously these different routes will not be relevant to all gut helminth species, for example, in fish, transmission of acanthocephalans via the consumption of fresh carcasses and vomited food seems unlikely.

Table 8.1: Reports of postcyclic parasitism in the phylum Acanthocephala

Class	Species	Host group	Reference
E	*Neoechinorhynchus cristatus*	Fish	Uglem & Beck (1972)
	Octospiniferoides chandleri	Fish	DeMont & Corkum (1982)
P	*Echinorhynchus salmonis*	Fish	Hnath (1969)
	Acanthocephalus ranae	Amphibia	Hammond (1968),
		''	Bozkov (1980)
A	*Moniliformis moniliformis*	Rat	Moore (1946),
		''	Stoddart & Crompton (1988)

E = Eoacanthocephala; P = Palaeacanthocephala; A = Archiacanthocephala

The previous studies on the postcyclic transmission of acanthocephalans can be subdivided according to the different routes as described by Bozkov (1976). It appears that only examples of cannabalism and feeding directly on mature worms outside the original host have been reported.

8.1.2.1. *Cannabalism*

An example of this route is provided by DeMont & Corkum (1982) who fed infected small mosquito fish (*Gambusia affinis*) to larger specimens. In their experiment 14 large uninfected fish were offered 39 very small fish as food. After 7 days, 3 of the large fish were found to contain a total of 7 *Octospiniferoides chandleri*.

8.1.2.2. *Feeding directly on mature worms outside the host*

Examples of this route of postcyclic transmission are the most common in the literature and often involve an experimental technique to introduce the worms into the new host. DeMont & Corkum (1982) offered 6 uninfected mosquito fish, living adult and juvenile *Octospiniferoides chandleri* in sections of alimentary tract from infected fish. Although the experimental fish were not observed to feed upon the gut sections, 1 of the 6 fish was found to contain a single female worm, 1 week later.

Hnath (1969) also gave sections of gut to his experimental fish but he introduced them to the new host fish via force feeding. Sections of the lower intestine of an adult coho salmon (*Oncorhynchus kisutch*), with 5 to 15 undamaged *Echinorhynchus salmonis* attached, were force fed to 8 uninfected brook trout (*Salvelinus fontinalis*). The trout were held in tanks at 10°C and were killed at intervals throughout a period of 12 weeks. Live worms were found attached to the fish intestines throughout this period.

Uglem & Beck (1972) force-fed adults and sub-adults (5 of each class) of *Neoechinorhynchus cristatus* and *N.crassus* collected from large-scale suckers (*Catostomus macrocheilus*) to speckled dace (*Rhinichthys osculus*), redside shiners (*Richardsonius balteatus*), squawfish (*Ptychocheilus oregonensis*) and rainbow trout (*Salmo gairdneri*), using a polyethylene tube and a syringe. Uninfected *C.macrocheilus* were used as controls. The fish were examined either 2 or 7 days after the experimental infections and only *N.cristatus* sub-adults were found to be attached to the posterior end of the intestine in all of the experimental species after 2 days. No infections were recorded after 7 days. Therefore, these species could not be considered to be genuine parapostcyclic hosts. In the control

C.macrocheilus infections, both sub-adult and adult *N.cristatus* were established by force feeding and therefore eupostcyclic parasitism was demonstrated.

Hammond (1968) transferred adult *Acanthocephalus ranae* between toads (*Bufo bufo*) by using a pipette inserted into the oesophagus. He gave his experimental toads 20 worms each and found the success rate of establishment in the new toad hosts to vary between 40 and 70%, between 1 and 3 days post infection. Bozkov (1980) experimentally transferred *Acanthocephalus ranae* from *Rana ridibunda* to *Bufo viridis*.

Moore (1946) attempted to transfer 41.5-hour-old *Moniliformis moniliformis* by an oral technique from one rat host to another but only 1 of the total of 26 worms introduced was found to be established in the intestine of the new rat which was autopsied 3 weeks post infection. He identified the effect of the gastric juices of the rat stomach as an explanation for the low success rate. When Stoddart & Crompton (1988) attempted to achieve experimental re-establishment of young adult *Moniliformis moniliformis* up to 7 days old via the oral route in rat hosts, the success rate was dramatically improved when the recipient rats were treated with Cimetidine 1 hour prior to transfer. This compound effectively reduced the acidity of the gastric environment. Worms transferred by this technique were found to be reproducing actively when they were 35 days old. This form of eupostcyclic transmission is unlikely to be important in naturally occurring *M.moniliformis* infrapopulations.

8.1.3. Postcyclic transmission as an explanation for field phenomena

As can be seen from these examples, the majority of reports of postcyclic transmission in the Acanthocephala have been of either of direct cannabalism or of feeding on mature worms artificially introduced into the new host. The value of experiments examining the

possiblity of postcyclic transmission via predation, which to date have not been reported, utilizing hosts of Acanthocephala known to coexist in the natural environment is therefore obvious. In the literature there are reports of definitive hosts which are infected with acanthocephalans but do not appear to feed extensively on the appropriate intermediate host. For example, Muzzall & Bullock (1978) found little evidence that fallfish (*Semoltilus corporalis*), fed on the ostracod intermediate host of *Neoechinorhynchus saginatus* but noted that several other fish species were susceptible to infection with subadults. Larger fallfish are known to be piscivorous in habit (Scott & Crossman, 1973) and this could explain why Muzzall & Bullock (1978) found such high intensities of infection with *N.saginatus* in larger fallfish. More generally, Bozkov (1976) concluded that this form of transmission may help to elucidate how certain species have become hosts for various helminths on an evolutionary time scale. It may also explain otherwise enigmatic observations of acanthocephalan and other helminth population dynamics.

8.1.4. *Neoechinorhynchus rutili* as a model species for the examination of postcyclic transmission

Neoechinorhynhcus rutili is an interesting species for whom the potential of postcyclic parasitism can be considered. Firstly, it has been reported from more than 110 species of fish (see Appendix II), from 22 familes, inhabiting brackish and freshwater environments throughout the northern holarctic zone (Van Cleave & Lynch, 1950). These fish species include small species like minnows (*Phoxinus phoxinus*), stoneloaches (*Noemacheilus barbulatus*) and three-spined sticklebacks (*Gasterosteus aculeatus*) and larger predominantly piscivorous species like pike (*Esox lucius*), coho salmon (*Oncorhynchus kisutch*), lake trout (*Salvelinus namaycush*), brown trout (*Salmo*

trutta) and perch (*Perca fluviatilis*). Postcyclic transmission may partly explain how this observed wide distribution and host range has come about. Van Cleave & Lynch (1950), in their classification of different types of definitive host of *N.rutili*, indicated that feeding on definitive hosts already harbouring a mature infection may bring about the observed extensive distribution of this species although they did not specifically define this phenomenon. They described such hosts as 'adventitious' or 'fortuitous' hosts and gave as examples the chub (*Leuciscus cephalus*), the rock bass (*Ambloplites rupestris*), the yellow perch (*Perca flavescens*) and the Catostomidae (see Chapter 3).

Secondly, *N.rutili* has been reported from both small and large fish species and also from both small and large individuals of the same species in the same water body so there is real potential for postcyclic transmission to occur via predation or cannabalism in the natural environment (see Appendix III). I have found at least 37 reports of *N.rutili* occurring in a number of fish species in one water body, 7 of which were in Scotland. Sixty-one species of fish are included in these reports.

Thirdly, taking one species as an example, larger specimens of brown trout *Salmo trutta* have been reported to harbour as many as 1500 and 812 individual worms (Pike p.c.; Devine, 1988) but they do not appear to feed extensively on the usual ostracod intermediate host (Merritt & Pratt, 1964; Walkey, 1967). Postcyclic transmission could be the process by which such high intensities of infection develop in the wild.

One conclusion from the available knowledge is that more studies are required to assess the significance of postcyclic transmission. DeMont & Corkum (1972) stated in their discussion 'the efficiency of this method of transfer cannot be determined because the numbers of worms in the infective material and the consumption of this material

were not known'. Hammond (1968) described the postcyclic transmission of *Acanthocephalus ranae* to toads, *Bufo bufo*, as 40-70% efficient but gave no further details. Postcyclic transmission has already been demonstrated for 2 neoechinorhynchid species, *Octospiniferoides chandleri* (DeMont & Corkum, 1982) and *Neoechinorhynchus cristatus* (Uglem & Beck, 1972) but no real quantitative analysis was made. Clearly the potential for the successful transfer of *N.rutili* by this route exists and the discovery of a field site during the course of my studies where *Gasterosteus aculeatus*, *Salmo trutta* and *S.gairdneri*, were infected with *N.rutili*, provided an ideal opportunity to investigate this phenomenon. This site was Drumore Loch, Perthshire, By means of examination of the *N.rutili* population in the sticklebacks, coupled with experimental postcyclic transmission to rainbow trout it was intended to provide the first quantitative measure of this type of transmission. This in turn would provide some quantitative measure of the potential for this route of transmission in the wild. The main results of this experiment have been published by Lassiere & Crompton (1988).

8.2. MATERIALS AND METHODS

8.2.1. Source and Maintenance of sticklebacks

Three-spined sticklebacks (*Gasterosteus aculeatus*) infected with *Neoechinorhynchus rutili* were collected on 4 occasions between October and December 1987 (Table 8.2) from Drumore Loch, Perthshire (Grid Ref. O.S. map Sheet 43, 1:50,000 NO 165608) with either a hand net or small drag net. The fish were kept in the laboratory in 5 l tanks in copper-free water in a constant temperature room set at 12°C.

8.2.1.1. *Distribution of the* Neoechinorhynchus rutili *infection in sticklebacks from Drumore Loch*

Of the total 236 sticklebacks, 144 were dissected and examined for parasites. Details of the prevalence and mean intensity of infection of these fish with *N.rutili* is shown in Table 8.2.

Table 8.2: Details of stickleback collections from Drumore Loch, Perthshire

Date	No. of fish		Infection with *Neoechinorhynchus rutili*					
	collected	dissected	No. inf.	% prevalence	Range	x	s^2:x	Total worms
14.10.87.	16	16	9	56.25	1-13	2.56	5.57	41
31.10.87.	150	68	25	36.76	1-63	2.39	26.43	163
a		30	13	43.33	1-13	1.97	4.89	59
b		15	5	33.33	1- 7	1.13	5.15	17
c		22	7	31.18	1-11	1.09	6.28	24
d		1	1	100.00	63	-	-	63
11.11.87.	30	30	18	60.00	1-71	5.67	34.18	170
10.12.87.	30	30	9	30.00	1-130	5.43	103.32	163
All dates	226	144	62	43.06	1-130	3.75	49.97	540

x = Mean number of worms per fish

s^2:x = variance to mean ratio

a-d = fish all collected on 31.10.87.

a = inital dissection group to establish intensity and prevalence of infection
b = control group in transmission experiment
c = unconsumed fish at end of transmission experiment
d = an individual fish also collected on this date

The overall distribution of numbers of *Neoechinorhynchus rutili* in the 144 sticklebacks is shown in Table 8.3 and Fig. 8.1. The distribution was compared with a negative binomial distribution, the

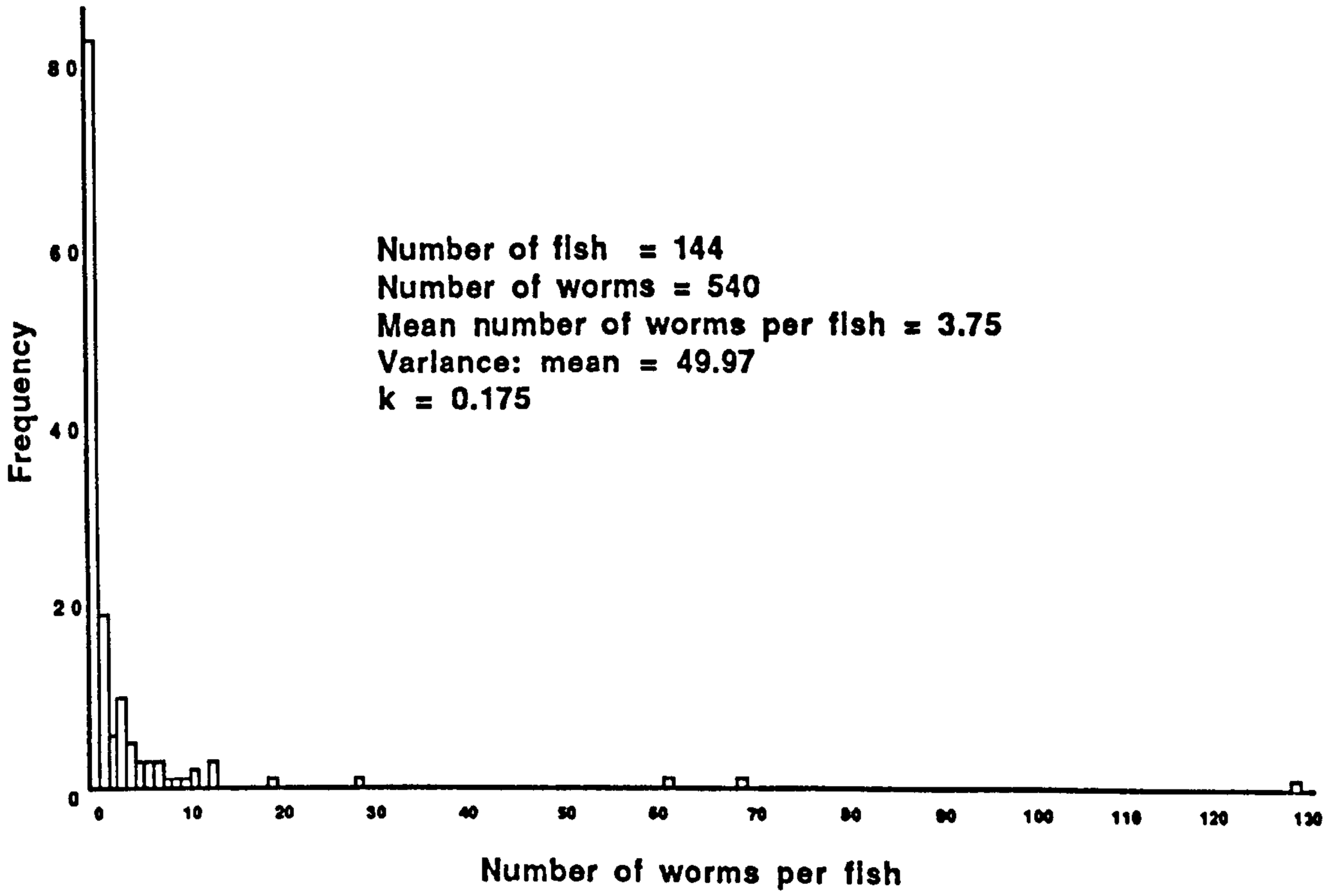


Fig. 8.1 Frequency distribution of numbers of *Neoechinorhynchus rutili* in three-spined sticklebacks from Drumore Loch, Perthshire

value for k being calculated as 0.175, using the maximum likelihood equation (Elliot, 1977b). For the experimental transmission, sticklebacks from the second October collection were used and 30 of these were dissected to measure the prevalence and intensity of infection with *N.rutili* (Table 8.4). The sample of sticklebacks was skewed towards the 21-30 mm length class which had a mean intensity of infection of 1.05 worms per fish.

Table 8.3: Frequency distribution of numbers of *Neoechinorhynchus rutili* in sticklebacks from Drumore Loch, Perthshire.

No. of worms per fish	Frequency	No. of worms per fish	Frequency
0	82	10	1
1	19	11	2
2	6	13	2
3	10	16	1
4	5	20	1
5	3	29	1
6	4	63	1
7	3	71	1
8	1	130	1
9	1		

Total number of fish = 144

Total number of worms = 540

Mean number of worms per fish = 3.75

Variance to mean ratio = 49.97

k = 0.175

Table 8.4: Distribution of *Neoechinorhynchus rutili* infection in 30 sticklebacks collected on 31.10.87.

Fish length class (mm)	No. of fish dissected	No. of fish with *N.rutili* infection	No. of worms recovered	% Prevalence	Mean intensity of infection (worms/fish)
11-20	1	0	0	0	0
21-30	21	7	22	33.3	1.05
31-40	5	3	11	60.0	2.20
41-50	2	2	17	100.0	8.50
51-60	1	1	9	100.0	9.00
All lengths	30	13	59	43.3	1.97

The sex, state of maturity and length of all worms found in the sticklebacks was determined by microscopic examination.

8.2.2. Source and maintenance of rainbow trout

Rainbow trout (*Salmo gairdneri*), were used for the experiments because they are much easier to maintain in the laboratory than brown trout. This approach was deemed acceptable since I had knowledge of infections of rainbow trout at Drumore Loch and also of both species being infected at another site in Scotland, Bridge of Weir (Site 6). Twenty trout, purchased on 29.10.87., were placed in 60 l tanks containing vigorously aerated copper free tap water, in a cold room set at 12°C with 4 fish per tank. These tanks were set up 6 days prior to the introduction of the fish. Fish for the experiment were chosen randomly, thus 7 fish were for the experiment, 12 for dissection to assess parasite status of the stock and 1 was a reserve. The experimental trout were placed in individual tanks and food was withheld for 7 days.

8.2.3. Transmission experiment

The rainbow trout were randomly sorted into a groups of 7 for experimental infection and a group of 13 for investigation of parasite status. No intestinal helminths were found in any of the group of 13 fish. One hundred and twenty sticklebacks were randomly arranged into 8 groups of 15 fish each. Each of the 7 experimental rainbow trout was exposed to at least 15 *N.rutili* established in the intestines of the groups of sticklebacks which were introduced into the same tanks as the trout (This estimate was based on the dissection of the 30 fish, see Table 8.4). This part of the experiment was conducted under Home Office approval. The eighth group was dissected as a control. The prevalence of infection was found to be 33.3% and the mean intensity of infection of was 1.13 (see Table 8.2). These results fitted well with the expected values on the basis of the initial examination (Table 8.4). The individual tanks containing the 7 trout were inspected daily and, 10 days after the introduction of the sticklebacks, the trout were killed, the number of sticklebacks eaten by each trout was recorded and surviving sticklebacks were dissected. The stomach contents of each experimental trout were examined and the number of undigested sticklebacks was recorded and deducted from the total number consumed by that fish. The gut was examined for the presence of *N.rutili* and their position was recorded in three regions of the gut, the duodenum, the ileum and the rectum, as described by Burnstock (1959).

8.3. RESULTS

8.3.1. *Neoechinorhynchus rutili* recovered from the experimental trout

Details of the numbers of sticklebacks consumed by each trout and numbers of *N.rutili* recovered are recorded in Table 8.5.

Neoechinorhynchus rutili were found attached in both the duodenal and ileal regions of the gut, but not in the rectal region. Three out of the 7 trout consumed all the sticklebacks in their tanks. On the basis of the numbers of worms recovered from the dissected control group, the survival and consequent establishment rate appears to be high for this type of transmission. Seventeen worms were recovered form the control group of sticklebacks. By comparison, trout numbers 2, 4 and 6 (Table 8.5) which had each caught and eaten 15 sticklebacks, contained 8, 13 and 14 worms respectively. One trout (No.5) which only caught 3 sticklebacks (it digested only 1) was not found to be harbouring any *N.rutili*. Another trout (No.7) had 24 *N.rutili* in its gut. This value exceeds that expected on the basis of the results from the control group. This observation can be accounted for by the known overdispersed distribution of *N.rutili* in the Drumore Loch sticklebacks (see Fig. 8.1). In the case of trout no.7 at least 1 heavily infected stickleback must have been included in the group of 15 fish which because of the known distribution, would be a rare selection event in a random sampling programme.

8.3.2. State of maturity and length of *Neoechinorhynchus rutili* in their hosts

Some of the group of sticklebacks caught on 31.10.87. contained reproducing females, with a copulatory cap in 1 case, and immature acanthors and mature shelled acanthors in the body cavity (in 2 cases and 1 case respectively). One mature female was found in the control group of sticklebacks and mature gravid female worms were found in the unconsumed sticklebacks (from the same source) at the end of the transmission experiment.

Table 8.5: Details of the numbers of sticklebacks consumed by each trout and numbers of *Neoechinorhynchus rutili* in transmission experiment

Trout number	Number of sticklebacks eaten	digested n/15	Numbers of *N.rutili* recovered Duodenum	Ileum	Total
1	0	0	0	0	0
2	15	15	7	1	8
3	13	13	12	2	14
4	15	13	12	1	13
5	3	1	0	0	0
6	15	15	8	6	14
7	14	14	17	7	24

Table 8.6: Sex and state of maturity of *Neoechinorhynchus rutili* found established in rainbow trout guts

Trout number	Number of worms: sex and state of maturity f1	f2	f3	fa	m	total	Ratio f:m
2	0	3	1	4	4	8	1.00
3	0	9	0	9	5	14	1.80
4	4	3	0	7	6	13	1.17
6	2	3	2	7	7	14	1.00
7	1	14	0	15	9	24	1.67
Total	7	32	3	42	31	73	1.35

f1 = immature female; f2 = female with free ovaries;
f3 = female with free ovaries and acanthors; m = male

Worms of a wide range of sizes were found re-established in the guts of the experimental trout (Table 8.7). Therefore it appears that both immature and mature individuals of both sexes can be successfully transferred in this way. The length data for worms recovered from the control group of 15 sticklebacks is shown in Table 8.7. In general, all the values for the worms from the sticklebacks were lower than those for the worms collected from the rainbow trout guts both in

terms of the upper limit of the length range and the mean. This may indicate that growth occurred in the new host or more simply that the physiological conditions of the trout gut is different to that of the stickleback such that the water balance alters the size of the worms.

Table 8.7: Size range of *Neoechinorhynchus rutili* in their stickleback and new rainbow trout hosts

Source	Stickleback control group				Rainbow trout			
State of maturity/sex	f1	f2	f3	m	f1	f2	f3	m
Length range um	-	1664-3200	3200	1056-2176	1280-3072	1344-4000	3200-4608	1440-3680
No. of worms	0	6	1	10	7	32	3	31
Mean length um	-	2752	3200	1693	1902	2973	3936	2511

f1 = immature female; f2 = female with free ovaries;
f3 = female with free ovaries and acanthors; m = male

The ratio of the number of female to male worms in the sticklebacks offered to the trout (control group + uneaten individuals) was 20:21 = 0.9524. In the trout, this ratio was 42:31 = 1.35 (see Table 8.6). Therefore, it appears that there might be a slightly higher success rate in re-establishment for females but the ratios for individual trout do not indicate this to be so. However, a X^2 test indicated that the difference in proportions was not significant (X^2 =0.8, P>0.05).

8.3.4. Re-establishment efficiency of *Neoechinorhynchus rutili* in experimental trout

In order to calculate the establishment efficiency the mean number of worms per stickleback was calculated for the control group +

the uneaten individuals (n=37) and that for the consumed sticklebacks, on the basis of the numbers of worms recovered from the trout divided by the total number of sticklebacks consumed. The mean values and variance to mean ratios of the two groups were 1.1081, 5.654 and 1.02816, 7.027 respectively. Utilizing the mean values the establishment rate equals 1.02816/1.1081= 92.79%. The variance to mean ratios for both groups are greater than unity, indicating an overdispersed distribution (Anderson & Gordon, 1982), and the fact that these values are of the same order of magnitude may add support to the suggestion that the re-establishment rate is high.

8.4. DISCUSSION

This experiment represents the first attempt to quantify the success rate of postcyclic transmission of *Neoechinorhynchus rutili* from three-spined sticklebacks to rainbow trout where there is some knowledge of the distribution of the parasites in the prey population. The estimated success rate of 92.8% indicates that this could form a very significant element of *N.rutili* population dynamics in the natural environment when several species of host, related via feeding relationships are infected. This factor may explain some of the enigmatic observations of acanthocephalan biology observed for other species in fish (see Muzzall & Bullock, 1978).

Worms of both sexes, various states of maturity and of a range of sizes were transferred in this way. This is of obvious importance if the parasite is to achieve any reproductive success after the transfer. This is contrary to the results of Uglem & Beck (1972) who found that only sub-adults of *N.cristatus* were re-established in the various new host species which they experimented with. Also contrary to their results, the present study found worms firmly attached to the gut mucosa 10 days after exposure to the infected prey species. It

therefore seems that the physiological stress suffered by the *N.rutili* in the trout stomach is not sufficient to adversely affect the worms, which consequently were able to reattach themselves to the gut mucosa of the new host. Further experiments could determine how long *N.rutili* is able to remain in the gut. The results of Hnath's (1969) study, in which he found experimentally transferred *Echinorhynchus salmonis* attached to the gut wall 12 weeks after their introduction into new hosts suggest that a similar pattern would arise for *N.rutili*. The fact that worms of both sexes re-established in the gut, including gravid females, indicates that successful reproduction and release of mature acanthors is likely to occur in the new host although this was not examined experimentally.

Moore (1946) accounted the low success rate (1/26 worms successfully re-established = 3.85%) in transmission of young *Moniliformis moniliformis* to a new rat host to the adverse effect of the gastric juices of the rat stomach. The study of Stoddart & Crompton (1988) in which this effect was controlled for (with Cimetidine, a gastric secretion inhibitor), of 390 worms (aged 3 and 7 days), 122 worms were found re-established in their new hosts which represents a success rate of 31.28%. In this case, it appears that the low p.H. of the stomach contents adversely affects the worms. How then, are the *N.rutili* able to resist the acid conditions in the trout stomach? Vonk (1939) collated reports of the p.H. values in the stomachs of various vertebrate species and found the rat stomach to have an average p.H. value of 4.6 (under normal feeding conditions) and pike (*Esox lucius*) to show values as low as 2.4, 52 hours after eating a prey item. If the values in the trout are comparable to that of the pike then the worms would be likely to experience high acid stress. My personal observations of only partially digested individual sticklebacks in regions posterior to the pyloric sphincter of the

stomach suggest (both in this experiment and in minnows eaten by brown trout at Loch Maragan, Scotland) that the partially digested host body tissues afford protection to the worms as they pass through the stomach.

In all the experimental rainbow trout the worms re-established in the more anterior duodenum and ileum and not in the rectum. This must infer that these regions offer suitable conditions for the establishment of the worms. In my own observations of young (small) *N.rutili* newly infecting the brown trout at Loch Maragan, they are most commonly found located more anteriorally in the gut. Awachie (1966) reported that older adult *Echinorhynchus truttae* in the guts of trout are found more posteriorly. In the present study there was no indication that the more mature worms were established further down the gut. This site extension effect may well be manifested when either the number of new worms introduced is greater or the spatial distribution is examined after a longer period. Such extensions of site range have been observed for a number of helminth species (see Crompton, 1973) and specifically the acanthocephalan *Acanthocephalus clavula* in their eel (*Anguilla anguilla*) hosts (Kennedy & Lord, 1982).

The proboscis of *Neoechinorhynchus rutili* bears only 18 hooks which is relatively few compared to other genera of fish Acananthocephala. For example, *Echinorhynchus truttae* has up to 352 hooks on its proboscis (Brown, Chubb & Veltkamp, 1986). I have observed the rapid eversion and inversion of the proboscis of *N.rutili* specimens freshly removed from the host intestine. When maintained in saline (0.9% NaCl solution) the same worms rapidly became reattached to sections of gut lying close to them. This may partly explain the very high observed rate of re-establishment in the host in the more anterior gut regions. Although *Echinorhynchus*

salmonis has a larger proboscis and many more hooks, Hnath (1969) reported that they can still successfully re-establish in parapostcyclic hosts. The lower establishment rate of *Moniliformis moniliformis* described by Stoddart & Crompton (1988) in rats may in part be explained by the proboscis morphology. In this context, an experiment involving sticklebacks concurrently infected with *N.rutili* and *E.truttae* would provide some answers to possible effect of proboscis morphology on the success of postcyclic transmission. In species where the proboscis becomes deeply embedded in the gut mucosa and a host tissue response results, for example in *Pomphorhynchus laevis*, it is unlikely that postcyclic transmission would be very successful.

In terms of the population dynamics of *Neoechinorhynchus rutili* this experiment has shown the important role that postcyclic transmission may play in natural situations, where several species of fish are host to *N.rutili* simultaneously. The proposed model for acanthocephalan population dynamics of Dobson & Keymer (1985) will have to be modified to incorporate this aspect of the life history. More specifically, the possibility of this type of transmission operating in trout in the natural environment is supported by 3 pieces of circumstantial evidence. Firstly, there are numerous reports of large trout feeding as predators on small fish (Swynnerton & Worthington, 1940; Ball, 1961; Hunt & Jones, 1972; Crozier, 1985). Secondly, *N.rutili* has been reported to occur concurrently in both small and large fish species from a number of locations (see Appendix III). Thirdly, brown trout from Drumore Loch have been found to harbour up to 1500 *N.rutili* per fish (Pike, p.c.). Perhaps this high intensity of infection in the predator reflects the high prevalence of the parasite in the prey. Since it is only larger trout that are piscivorous in habit, this form of transmission will only be important

in the larger size classes in the natural environment. Field studies of smaller host predation by larger classes of alternative host species would be necessary to assess the significance of such transmission in the overall population dynamics of the worm. Postcyclic transmission might also facilitate transfer to fish which might not commonly feed upon the invertebrate hosts of the parasite. *N.rutili* appears to have a low definitive host specificity so this route of infection would be an appropriate one for the species. This type of transmission is likely to promote the dispersal of *N.rutili* since some of the species of host are also migratory e.g. sea trout (*Salmo trutta*) and may therefore be a partial explanation for its wide distribution in the Northern holarctic zone as described by Van Cleave & Lynch (1950). In terms of a selective advantage, worms moving from small fish species to larger fish species by this route may experience more favourable gut conditions, for example reduced crowding effects and may consequently achieve more of their reproductive potential.

Current knowledge shows that *N.rutili* has either a simple direct life history in which transmission to the definitive host depends on their feeding on infected ostracods (Merritt & Pratt, 1964; Walkey, 1967; Valtonen, 1979) or a more complex pattern involving infected alder fly larvae (Lassiere, 1988a). In natural systems involving *N.rutili*, it appears that a more complex life cycle may exist in which both postcyclic transmission and additional arthropod hosts may play roles in the stability of the parasite suprapopulation. In general, this study has revealed something of the flexibility of acanthocephalan life histories in terms of routes of transmission.

8.5. SUMMARY

8.5.1. Postcyclic transmission is defined generally and specifically for the Acanthocephala.

8.5.2. Examples of postcyclic transmission in the Acanthocephala through cannabalism and experimental introduction of mature worms to a new host are reviewed.

8.5.3. Postcyclic transmission is described as a phenomenon which may account for enigmatic observations made on Acanthocephala and other helminth population dynamics in the natural environment.

8.5.4. *Neoechinorhynchus rutili* is considered as a model species for the study of postcyclic transmission for 3 reasons:

8.5.4.1. It has a wide definitive host range including small and large species which are associated via feeding relationships.

8.5.4.2. These fish species often occur in the same water body and provide the potential for postcyclic transmission via predation of one upon the other.

8.5.4.3. Observations of brown trout, which do not feed extensively on the intermediate host species, harbouring up to 1500 worms may be explained by postcyclic transmission.

8.5.5. Through a knowledge of the distribution of *N.rutili* in the prey population this study aimed to investigate the efficiency of re-establishment in rainbow trout.

8.5.6. Wild caught infected three-spined sticklebacks (*Gasterosteus aculeatus*) from Drumore Loch, Scotland, and uninfected rainbow trout (*Salmo gairdneri*) were used for the experimental transmission.

8.5.7. The distribution of the *N.rutili* in the three-spined sticklebacks was overdispersed.

8.5.8. Seven experimental trout were exposed to at least 15 *N.rutili* established in the guts of 15 randomly selected three-spined sticklebacks from Drumore Loch.

8.5.9. Ten days post-exposure to the three-spined sticklebacks, 5 trout were found to be harbouring between 8 and 24 *N.rutili* in the duodenal and ileal regions of the intestine. These areas are considered to be the first physiologically suitable regions of the gut for worm establishment.

8.5.10. Individuals of both sexes, various states of maturity and of a range of sizes were successfully transferred. There did not appear to be a bias in the sex ratio in terms of transmission.

8.5.11. On the basis of mean worm numbers per fish in the prey and the trout (the latter based on the number of three-spined sticklebacks consumed) the re-establishment efficiency was calculated to be 92.8%.

8.5.12. It is suggested that the partially digested three-spined stickleback body tissues afford protection to the worms as they pass through the acid conditions of the trout stomach.

8.5.13. The small, sparsely hooked, easily everted and inverted proboscis of *N.rutili* may facilitate successful re-establishment in postcyclic hosts.

8.5.14. The possiblity of postcyclic transmission of *N.rutili* occurring between brown trout and its prey species in the natural environment is supported by three pieces of circumstantial evidence:

8.5.14.1. The piscivorous habit of trout is well documented.

8.5.14.2. Both trout and their prey species have been reported as being infected with *N.rutili* in the same locality.

8.5.14.3. High intensities of infection in brown trout suggest a high prevalence of infection of *N.rutili* in their prey.

8.5.15. In general this study has shown something of the complexity of Acanthocephalan life-histories in terms of transmission. Specifically for *N.rutili* it has indicated that its life cycle is

more complex than originally envisaged and that this form of transmission may account for its observed wide distribution in the northern hemisphere.

CHAPTER NINE

GLADHOUSE RESERVOIR: BROWN TROUT HEALTH CHECK 1987

A trout health check was carried out during the 1987 fishing season at Gladhouse Reservoir, Lothian for the Lothian Regional Council. In October 1988 a 66 page report of the work was submitted to the council (specifically A.D. Jamieson) and a precis of this report follows. Where appropriate reference to the results of this work appear in the rest of this thesis.

9.1. SUMMARY

9.1.1. Complaints from anglers about the presence of "worms" in the brown trout caught at Gladhouse Reservoir resulted in Glasgow University Zoology Department being consulted to identify the parasites in May, 1986.

9.1.2. Trout were found harbouring 2 gut parasites, *Eubothrium crassum* and *Echinorhynchus truttae* and further samples of brown trout were heavily infected by these parasites. A thorough survey of the trout parasites was proposed for the 1987 fishing season.

9.1.3 The project had 2 objectives: firstly, to investigate the importance of the 500 stocked trout in the fishery and, secondly, to investigate the gut parasite fauna. The stocked trout were marked, prior to release at 3 sites at the reservoir on 11$^{th.}$ March with a blue dye spot over the heart.

9.1.4. Anglers were informed about the proposed project by means of a poster and were asked to collect gut samples and record details about their catch on Health Check Courtesy Cards during each month of the season.

9.1.5. Two hundred and sixty seven cards returned on 109 days of the fishing season (59.6%) with details of 884 trout captured during the 1987 fishing season were returned by

anglers.

9.1.6. Two hundred and sixty four *Gammarus pulex* were collected on 3 occasions. The prevalence of infection with *Echinorhynchus truttae* was found to be at a maximum in June, 1987 (2.88%).

9.1.7. Sixty three preserved gut samples, of which 42 were analysed were collected by fishermen during the 1987 fishing season. Samples were collected during every month of the season, except August.

9.1.8. The distribution of marked trout captures was found to be random with respect to depth throughout the season, although unmarked trout were caught more frequently in areas where the water was deeper.

9.1.9. One hundred and sixty eight of the 500 marked trout released into the reservoir during the 1987 fishing season were recorded as being captured. Captures of marked trout were well distributed throughout the reservoir, indicating good mixing with the indigenous population. The stocked trout are an important element of the Gladhouse Reservoir fishery.

9.1.10. The proportion of marked trout caught during each month decreased as the season progressed.

9.1.11. An assessment of the condition factor of the trout was limited because the majority of morphometric data provided by the fishermen were in Imperial Units. However, the values indicated that heavily parasitized trout were not in bad condition.

9.1.12. A total of 2337 individual dietary items from 26 dietary elemental classes were counted in the dietary analysis. The diet of Gladhouse Reservoir brown trout showed seasonal fluctuations in composition similar to those described at other European locations.

9.1.13. Four visceral and gut parasite species were indentified in the Gladhouse Reservoir gut samples: 2 nematodes, *Cystidicola farionis* and *Eustrongylides* sp., 1 cestode, *Eubothrium crassum* and 1 acanthocephalan, *Echinorhynchus truttae*.

9.1.14. The 2 nematode species occurred in only a few of the gut samples and, therefore, no assessment of their patterns of percentage prevalence and intensity of infection was feasible.

9.1.15. Over the fishing season, the percentage prevalence and mean intensity of infection of Gladhouse Reservoir trout with *Eubothrium crassum* showed similar patterns to those described by Rosen (1919) and Robertson (1953) at Dunalastair Reservoir, Scotland. The percentage prevalence was 61.9% and the mean intensity of infection 1.48 worms per fish for the whole season.

9.1.16. The distribution of the *Eubothrium crassum* infection of Gladhouse Reservoir brown trout was typically overdispersed with trout harbouring between 1 and 7 worms each.

9.1.17. *Eubothrium crassum* adult worms were found attached to the pyloric caeca in the duodenal region of the gut.

9.1.18. The intermediate host for *Eubothrium crassum* at Gladhouse Reservoir was not indentified.

9.1.19. The intermediate host for *Echinorhynchus truttae* at Gladhouse Reservoir was indentified as *Gammarus pulex* which formed and important part of the diet of Gladhouse Reservoir brown trout.

9.1.20. The distribution of the *Echinorhynchus truttae* infection of Gladhouse Reservoir brown trout was typically overdispersed,

individual trout harbouring between 2 and 1165 worms.

9.1.21. *Echinorhynchus truttae* adult worms were found in the stomach, duodenum, ileum and rectum of Gladhouse Reservoir brown trout throughout the 1987 fishing season. No established worms were found in the stomach.

9.1.22. Adult female *Echinorhynchus truttae* were longer than adult male *E.truttae*, although there was some overlap. A significant difference in lengths of worms recovered from different hosts, in different months, of different sex was found for female worms recovered from the duodenal and rectal regions of the gut, worms from the former region being significantly smaller.

9.1.23. The percentage prevalence of *Echinorhynchus truttae* infection for the whole season was 92.86%.

9.1.24. The mean intensity of *Echinorhynchus truttae* infection for the whole season was 156.4.

9.1.25. In general, male *Echinorhynchus truttae* were found to occupy more anterior regions in the gut than female worms.

9.1.26. As the female worms matured they were found more posteriorly in the gut.

9.1.27. The ratio of female to male worms in the monthly samples of *Echinorhynchus truttae* remained fairly constant throughout the season until September when female worms dominated the sample.

9.1.28. A dynamic situation existed in the *Echinorhynchus truttae* infrapopulations inhabiting the guts of Gladhouse Reservoir brown trout such that new infections occurred throughout the season and the output acanthors from mature female worms was limited to the warmer months of the season when young intermediate hosts were likely to be abundant.

CHAPTER TEN

CONCLUSION

The Acanthocephala is a phylum of parasitic species that exhibits great flexibility in its life history patterns. *Neoechinorhynchus rutili* is certainly no exception and this is confirmed by its apparent flexibility in Scottish populations. The main conclusions of this work are that:

1. *Neoechinorhynchus rutili* is a widely distributed parasite of a number of freshwater fish species in Scotland both in natural and man made sites.
2. The life cycle is rather complex involving the insect larva *Sialis lutaria* and possibly postcyclic transmission. To date the role of ostracods in the life cycle in Scottish systems has not been resolved.
3. The population dynamics of *N.rutili* in its brown trout hosts at Loch Maragan results from processes which are indirectly under the influence of temperature. A seasonal pattern of growth, maturation and occurrence was observed. Its distribution amongst both *Sialis lutaria* and the definitive hosts ensured that successful transmission, establishment and reproduction occurred during each year of the study. Estimates of the worm population size (based on a knowledge of the host size and population structure and the dispersal pattern of the worms) and fecundity indicated that *N.rutili* is a typical r selectionist. Observations from other sites suggested that there are local differences between species and locations in Scotland and that many populations exhibit apparently long term temporal stability.
4. *Neoechinorhynchus rutili*, when present in low numbers, apparently has no adverse effects upon its host, however the stocking of other water bodies with heavily infected fish from fish farms needs to be monitored as it may cause conservation problems.

5. Collaboration with members of the public in scientific research has great potential but needs to be closely monitored if the results are to be of sufficient quality.

A number of topics merit further investigation. These are set out below.

1. Are there any differences between the morphological features of *N.rutili* specimens from hosts of different sex, age and species from Scotland? The results shiould be compared with the previous work of Skryabina (1978) in U.S.S.R.
2. Can the morphology of the giant nuclei be used as a means of defining the reproductive status of natural populations?
3. How does the fine structure of the reproductive tissue of male and female *N.rutili* from *Sialis lutaria* larvae and from fish hosts compare with that of other acanthocephalan species? Are worms sexually mature in insect larvae? If so what is the significance of the orientation in the hosts with respect to mating possibilities?
4. Do all stages of *N.rutili* occur in *Sialis lutaria* larvae and is there gut damage as a result of acanthor migration? Does the insect gonad morphology indicate any possible effects of parasitization upon fertility?
5. What are the effects of environmental parameters upon the establishment, survival and fecundity of worm populations in controlled laboratory infections of definitive hosts using infected *Sialis lutaria* larvae? How do the results compare with field observations? What effect does host sex have upon infection dynamics? Are there any density-dependent effects of the intenstiy of *N.rutili* infection upon host fecundity?
6. What factors determine the dynamics of establishment in terms of

spatial arrangement of worms in the alimentary tract?

7. What are the effects of interspecific competition upon the establishment, spatial distribution, survival and fecundity of *Echinorhynchus truttae* and *N.rutili* simultaneously introduced into trout?
8. What is the most efficient method for retrieving shelled acanthors as they are released from the host to allow for an assessment of worm fecundity?
9. How do environmental parameters effect acanthor viability and how many losses are there in the passage to either ostracod or larval *Sialis lutaria* hosts?
10. What role, if any, do ostracods play in the life cycle of *N.rutili* in Scotland?
11. Are there any behavioural changes in *Sialis lutaria* larvae infected with *N.rutili* which make them more susceptible to predation by potential definitive hosts?
12. What roles do infected *Sialis lutaria* and postcyclic transmission play during winter in the population dynamics of *N.rutili*?
13. Are there any behavioural and physiological effects of *N.rutili* infections upon small definitive hosts which are likely to be predated upon larger piscivorous fish?
14. How great is the potential for postcyclic transmission of *N.rutili*, specifically to larger fish specimens, in natural populations? How is the survival and fecundity of worms transferred in this way effected in their new hosts?
15. If a fish consumes a smaller specimen that is concurrently infected with *Echinorhynchus truttae* and *N.rutili*, is the postcyclic transmission of these two species equally successful?
16. Does an *N.rutili* infection have an effect upon reproduction of *Sialis lutaria* larvae?

17. What is the significance of encapsulation of *N.rutili* in *Sialis lutaria* larvae, how does it come about and from where does it originate? How significant is this phenomenon in terms of the transmission success and population dynamics of *N.rutili*?
18. Does cannabalism between *Sialis lutaria* larvae play a role in the transmission of *N.rutili* between instars?
19. Do birds have a role to play in the transmission of *N.rutili* acanthors between sites?
20. To what extent is the observed *N.rutili* distribution in Scotland brought about by human activities?
21. What are the effects of acanthocephalan infections upon brown trout health in terms of their survival and fecundity?
22. Can collaboration between the universities and other establishments e.g. local government, fish farms and fishing clubs be extended to stimulate both interest in university research and public cooperation?

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APPENDIX I

RECORDS OF THE WORLDWIDE DISTRIBUTION OF *NEOECHINORHYNCHUS RUTILI* IN ITS DEFINITIVE HOST SPECIES

The following comprises an alphabetical list of fish species from freshwater, brackish and marine environments reported to be infected by *Neoechinorhynchus rutili* (including species in which *N.rutili* was not seen to mature). Species names are given as in the original papers and as far as is possible synonyms are as quoted by Margolis & Arthur (1979), Scott & Crossman (1973), Sterba (1973) and Wheeler (1969). Synonyms are marked with an * and the present names are also included in the list. The localities of the reports are given in as much detail as the original papers and references with no localities given are represented by NS (No site). For each species, the localities are given in alphabetical order of the references.

Fish species	Locality	Reference
Abramis ballerus	Rybinsk Reservoir,USSR	Izyumova (1960)
	Dabie Lake, Poland	Wierzbicka (1978)
Abramis bjorkna	NS	Van Cleave & Lynch (1950)
Abramis brama	NS	Golvan (1959)
	Druzno, Poland	Kozicka (1959)
	Rybinsk Reservoir,USSR	Izyumova (1958)
	Macha Lake fish pond System, Bohemia, Czech.	Moravec (1984a)
	NS	Van Cleave & Lynch (1950)
	Dabie Lake, Poland	Wierzbicka (1978)
Abramis sp.	NS	Yamaguti (1963)
*Acerina cernua**= (*Gymnocephalus cernua*)	NS	Golvan (1959)
Alburnus alburnus	NS	Golvan (1959)
	Dijon, France	Lespes (1884) in Walkey (1963)
	Lake Pestovo, USSR	Pozdnyakova (1963)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	NS	Van Cleave & Lynch (1950)

Fish species	Locality	Reference
Alburnus sp.	NS	Yamaguti (1963)
Ambloplites rupestris	NS	Golvan (1959)
	Madison, Wisconsin USA	Van Cleave & Lynch (1950)
Ambloplites sp.	NS	Yamaguti (1963)
*Amiurus nebulosus**= (*Ictalurus nebulosus*)	Lakes of Pinsk area, USSR	Chechina *et al* (1953)
Anguilla anguilla	Lake Lagoda, USSR	Barysheva & Bauer (1958)
	Karlskrona, Sweden	Lundstrom (1942)
	USSR	Markevich (1963)
	NS	Meyer (1932)
	Macha Lake fish pond System, Bohemia, Czech.	Moravec (1984a)
	Lake Vatia, Finland	Valtonen p.c.
	NS	Van Cleave & Lynch (1950)
Anguilla vulgaris	NS	Golvan (1959)
	Finland	Van Cleave & Lynch (1950)
Anguilla sp.	NS	Yamaguti (1963)
Barbus barbus	NS	Golvan (1959)
	NS	Van Cleave & Lynch (1950)
Barbus sp.	NS	Yamaguti (1963)
Blicca bjoerkna	NS	Golvan (1959)
	Dnepr Delta, USSR	Komarova (1964)
	Dabie Lake, Poland	Wierzbicka (1978)
Blicca sp.	NS	Yamaguti (1963)
Carassius carassius	NS	Van Cleave & Lynch (1950)
Carassius carassius auratus	NS	Golvan (1959)
Carassius sp.	NS	Yamaguti (1963)
Carpoides carpio	NS	Golvan (1959)
Catostomus ardens	NS	Golvan (1959)

Fish species	Locality	Reference
Catostomus catostomus	British Columbia, Canada	Bangham & Adams (1954)
	Alberta, Canada	Mudry & Anderson (1977)
Catostomus commersonni	NS	Golvan (1959)
Catostomus macrocheilus	British Columbia, Canada	Bangham & Adams (1954)
	NS	Golvan (1959)
	King Co. Washington Lake Washington, Seattle USA	Van Cleave & Lynch (1950)
Catostomus sp.	NS	Yamaguti (1963)
Chondrostoma nasus	NS	Golvan (1959)
	NS	Van Cleave & Lynch (1950)
Chondrostoma sp.	NS	Yamaguti (1963)
Cobitis latnia	NS	Golvan (1959)
	NS	Van Cleave & Lynch (1950)
Cobitis taenia	NS	Golvan (1959)
Cobitis sp.	NS	Yamaguti (1963)
Coregonus albula	NS	Golvan (1959)
Coregonus lavaretus	Lake Lagoda, USSR	Barysheva & Bauer (1958)
	Lake Pestovo, USSR	Pozdnyakova (1963)
Coregonus lavaretus maraenoides	Lake Pestovo, Novgorod area, USSR	Petrushevski (1954) Petrushevski & Bauer (1953)
Coregonus muksun	NS	Golvan (1959)
Coregonus nasus	Yenisei at Ust Port & Didinka, USSR	Bauer (1948a)
	North East Bay of Bothnia, Finland	Valtonen (1979), Valtonen & Crompton (1989)
Coregonus peled	Yenisei at Ust Port & Didinka, USSR	Bauer (1948a)
	NS	Golvan (1959)
	River Yenisei Kolyma River USSR	Skryabina (1978)

Fish species	Locality	Reference
Coregonus sardinella	NS	Golvan (1959)
	Alaska, USA	Schmidt p.c.
	Rivers Ob, Kolyma, Yenisei, USSR	Skryabina (1978)
Coregonus tugan	NS	Golvan (1959)
Coregonus sp.	NS	Yamaguti (1963)
Cottus asper	British Columbia, Canada	Bangham & Adams (1954)
Cottus bairdi	Lake Erie, Ontario Canada	Dechtiar (1972a)
	NS	Golvan (1959)
	Taylor Co. Wisconsin USA	Van Cleave & Lynch (1950)
Cottus cognatus	Yukon Territory, USA	Arthur *et al* (1976)
Cottus gobio	NS	Golvan (1959)
Cottus rhotheus	British Columbia, Canada	Bangham & Adams (1954)
*Cottus scorpius**= (*Myoxocephalus scorpius*)	Utvalnas, Sweden	Lindstrom (1942)
Cottus sp.	NS	Van Cleave & Lynch (1950)
	NS	Yamaguti (1963)
Couesius plumbeus	British Columbia, Canada	Bangham & Adams (1954)
Ctenopharyngodon sp.	NS	Yamaguti (1963)
Culaea inconstans	Wisconsin, USA	Amin (1986)
	Lake Huron, Ontario Canada	Bangham (1955)
	Lake of the Woods, Ontario, Canada	Dechtiar (1972b)
Cyprinus carpio	NS	Golvan (1959)
	Fish Ponds, USSR	Layman (1946)
	Macha Lake fish pond System, Bohemia, Czech.	Moravec (1984a & b)
	Germany	Luhe (1911)
	NS	Meyer (1932)
	Lake Alakul, USSR	Smirnova (1944)
	Fish Ponds, South Bohemia Czechoslovakia	Tesarcik (1970, 1972)
	NS	Van Cleave & Lynch (1950)

Fish species	Locality	Reference
Cyprinus sp.	USSR	Markevich (1963)
	NS	Yamaguti (1963)
Diplophysa dorsalis	NS	Golvan (1959)
Diplophysa strauchi	NS	Golvan (1959)
Diptychus dybowskii	NS	Golvan (1959)
Esox lucius	Yukon Territory, Canada	Arthur *et al* (1976)
	British Columbia, Canada	Bangham & Adams (1954)
	Loch Leven, Scotland	Campbell (1974)
	Llyn Tegid, Wales	Chubb (1967)
	River Lugg, Herefordshire England	Davies (1967)
	NS	Golvan (1959)
	Ribynsk Reservoir, USSR	Izyumova (1960)
	Moldavia, USSR	Iamandi & Iamandi (1936)
	River Blackwater tributaries at Kanturk, County Cork, Ireland	Kane (1966)
	Depnr Delta, USSR	Komarova (1964)
	Lake Konche, Karelia USSR	Malakhova (1961)
	USSR	Markevich (1963)
	Wisconsin, USA	Marshall & Gilbert (1905)
	Macha Lake fish pond system, Bohemia, Czech	Moravec (1979, 1984a)
	NS	Meyer (1932)
	New Vygozero Lake, Karelia USSR	Nagibina (1957)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	Lake Monona, Madison Wisconsin, USA	Van Cleave & Lynch (1950)
Esox sp.	NS	Yamaguti (1963)
*Eucalia inconstans**= (*Culaea inconstans*)	NS	Golvan (1959)
	Wisconsin USA	Van Cleave & Lynch (1950)
Fundulus heteroclitus	Newfoundland, Canada	Dickinson & Threlfall (1975)
Gadus morhua	NS	Petrochenko (1956)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)

Fish species	Locality	Reference
Gasterosteus aculeatus	British Columbia, Canada	Bangham & Adams (1954)
	Burn of Savoch, Scotland	Bwathondi (1976)
	Pond, Baildon Moor Yorkshire, England	Chappell (1969a,b,c)
	Hadleigh Marsh, England	Dartnall (1972)
	Drumore Loch, Scotland	Devine (1988)
	NS	Golvan (1959)
	Labrador, Canada	Hanek & Threlfall (1970)
	Bridge of Weir, West of Scotland Trout Farm, Scotland	Lassiere (Unpub.)
	Drumore Loch, Scotland	Lassiere & Crompton (1988)
	Loch Monzievaird, Scotland	Lassiere (Unpub.)
	Vancouver area, Canada	Lester (1975)
	Germany	Luhe (1911)
	NS	Meyer (1932)
	Pitlochry, Scotland	Pike & Edwards (1983)
	Beith, Scotland	Ritchie (1915)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	Cultus Lake, British Columbia, Canada	Van Cleave & Lynch (1950)
	Monkton pond, County Durham, England	Walkey (1967)
G.aculeatus microcephalus	NS	Golvan (1959)
	Kodiak Island, British Columbia, Canada	Van Cleave & Lynch (1950)
Gasterosteus gymmurus	NS	Golvan (1959)
*Gasterosteus pungitius**= (*Pungitius pungitius*)	Bernard Harbour, North West Territories, Canada	Jennes (1916) in Van Cleave & Lynch (1950)
Gasterosteus sp.	USSR	Markevich (1963)
	NS	Yamaguti (1963)
Gila atraria	NS	Hoffman (1967)
Gobio gobio	NS	Golvan (1959)
	Macha lake fish pond System, Bohemia, Czech.	Moravec (1984a)
	NS	Van Cleave & Lynch (1950)
Gobio sp.	NS	Yamaguti (1963)
*Gobius minutus**= (*Pomatoschistus minutus*)	NS	Golvan (1959)
	NS	Van Cleave & Lynch (1950)

Fish species	Locality	Reference
Gymnocephalus cernua	USSR	Markevich (1963)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
Hybognathus hankinsoni	Holt Creek, Nebraska, USA	Nickol & Samuel (1983)
	Missouri River at Brownville, Nebraska USA	Samuel *et al* (1976)
Hypophthalmichthys sp.	NS	Yamaguti (1963)
Ictalurus nebulosus	British Columbia, Canada	Bangham & Adams (1954)
*Idus idus**= (*Leuciscus idus*)	NS	Golvan (1959)
Lampetra fluviatilis	Bothnian Bay, Finland	Valtonen & Crompton (1989)
Leuciscus atrarius	NS	Golvan (1959)
Leuciscus cephalus	River Lugg, Herefordshire, England	Davies (1967)
	NS	Golvan (1959)
Leuciscus erythrophthalamus	NS	Van Cleave & Lynch (1950)
Leuciscus grislagine	NS	Golvan (1959)
Leuciscus idbarus	NS	Golvan (1959)
Leuciscus idus	New Vygozero Lake, Karelia USSR	Nagibina (1957)
	Lake Oyeren, Norway	Oien (1976, 1979)
	Kola Peninsula, USSR	Skryabina (1978)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	NS	Van Cleave & Lynch (1950)
Leuciscus idus orfus	Poprad & Strbske Lakes Czechoslovakia	Dyk (1957)
Leuciscus leuciscus	Lake Lagoda, USSR	Barysheva & Bauer (1958)
	River Lugg, Herefordshire, England	Davies (1967)
	NS	Golvan (1959)
	Britain	Kennedy (1974)
	NS	Van Cleave & Lynch (1950)

Fish species	Locality	Reference
Leuciscus phoxinus	NS	Meyer (1932)
Leuciscus rutilus	NS	Meyer (1932)
	NS	Van Cleave & Lynch (1950)
	NS	Yamaguti (1963)
*Leuciscus schmidti** (=*Squalius schmidti*)	NS	Golvan (1959)
Lota lota	Yukon Territory, Canada	Arthur *et al* (1976)
	Lake Huron, Ontario, Canada	Bangham (1955)
	British Columbia, Canada	Bangham & Adams (1954)
	River Biebrza, Poland	Ejsymont (1970)
	NS	Golvan (1959)
	Lake Konche, Karelia USSR	Malakhova (1961)
	USSR	Markevich (1963)
	Alaska, USA	G. Schmidt p.c.
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	NS	Van Cleave & Lynch (1950)
Lota sp.	NS	Yamaguti (1963)
Micropterus dolomieui	Lake Huron, Ontario,	Bangham (1955)
Morone chrysops	Lake Erie, USA	B.B. Nickol p.c.
Morone saxatilis	Kouchibouguac River, New Brunswick, Canada	Hogans (1984)
Mylocheilus caurinus	British Columbia, Canada	Bangham & Adams (1954)
	Lake Washington, Seattle Washington, USA	Van Cleave & Lynch (1950)
	Cultus Lake, British Columbia, Canada	
Myoxocephalus quadricornis	Bothnian Bay, Finland	Valtonen & Crompton (1989)
Myoxocephalus scorpius	Utvalnas, Sweden	Lindstrom (1942)

Fish species	Locality	Reference
Noemacheilus barbulatus	NS	Golvan (1959)
	Germany	Luhe (1911)
	Beith, Scotland	Ritchie (1915)
	Lake Pestovo, USSR	Pozdnyakova (1963)
	NS	Van Cleave & Lynch (1950)
Nemacheilus sp.	Indian Tibet & Kashmir	Datta (1936)
	NS	Yamaguti (1963)
Notropis dorsalis	NS	Golvan (1959)
	Holt Creek, Nebraska, USA	Nickol & Samuel (1983)
	Missouri River at Brownville, Nebraska, USA	Samuel *et al* (1976)
Notropis hudsonius	Lake Huron, Ontario, Canada	Bangham (1955)
	Lake Erie, Ontario, Canada	Dechtiar (1972a)
Oncorhynchus keta	British Columbia, Canada	Arai (1969)
Oncorhynchus kisutch	British Columbia, Canada	Arai(1969)
	British Columbia, Canada	Bangham & Adams (1954)
	NS	Golvan (1959)
	Cultus Lake, British Columbia, Canada	Van Cleave & Lynch (1950)
Oncorhynchus nerka	British Columbia, Canada	Arai (1969)
	British Columbia, Canada	Bangham & Adams (1954)
	Lake Huron, Ontario,Canada	Collins & Dechtiar (1974)
	NS	Golvan (1959)
	British Columbia, Canada	Margolis (1956, 1957, 1963)
	Cultus Lake, British Columbia, Canada	Van Cleave & Lynch (1950)
Onchorhynchus nerka nerka	Suttle Lake, Cascade Range Oregon, USA	Merritt & Pratt (1964)
Oncorhynchus tschawytscha	NS	Golvan (1959)
Onchorhynchus sp.	NS	Yamaguti (1963)
Osmerus mordax	Lake Huron, Ontario, Canada	Collins & Dechtiar (1974)
Parabramis sp.	NS	Yamaguti (1963)

Fish species	Locality	Reference
Perca flavescens	Lake Huron, Ontario, Canada	Bangham (1955)
	Lake Huron, Ontario, Canada	Collins & Dechtiar (1974)
	Lake Erie, Ontario, Canada	Dechtiar (1972a)
	NS	Golvan (1959)
	Lake Washington, Seattle Washington, USA	Lynch (1936)
	Wisconsin Lakes, USA	
	King Co. Washington USA	Van Cleave & Lynch (1950)
Perca fluviatilis	Loch Leven, Scotland	Campbell (1974)
	NS	Golvan (1959)
	Moja, Sweden	Lindstrom (1942)
	Lake Konche, Karelia USSR	Malakhova (1961)
	USSR	Markevich (1963)
	Macha lake fish pond System, Bohemia, Czech.	Moravec (1984a), Scholz (1987)
	New Vygozero Lake, USSR	Nagibina (1957)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	NS	Van Cleave & Lynch (1950)
Perca schrenki	NS	Golvan (1959)
Perca sp.	NS	Yamaguti (1963)
Phoxinus czekanowskii	River Kolyma, USSR	Skryabina (1978)
Phoxinus laevis	NS	Van Cleave & Lynch (1950)
Phoxinus percnurus	River Kolyma, USSR	Skryabina (1978)
Phoxinus phoxinus	Frongoch Lake, Wales	Bibby (1972)
	NS	Golvan (1959)
	Loch Maragan, Scotland	Lassiere (Unpub.)
	Dijon, France	Lespes (1884) in Walkey (1963)
	River Brerachan, Scotland	Pike & Edwards (1983)
	Dobsina Dam, Slovakia, Czechoslovakia	Zitnan (1973)
Phoxinus lagowskii oxycephalus	NS	Golvan (1959)
Phoxinus sp.	NS	Yamaguti (1963)
Pimephales promelas	Holt Creek, Nebraska, USA	Nickol & Samuel (1983)
	Missouri River at Brownville, Nebraska, USA	Samuel *et al* (1976)
Platichthys flesus	Bothnian Bay, Finland	Valtonen & Crompton (1989)

Fish species	Locality	Reference
Pleuronectes flesus	Aspo, Sweden	Lindstrom (1942)
Pomatoschistus minutus	NS	Golvan (1959)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	NS	Van Cleave & Lynch (1950)
Prospium transmontanus	Suttle Lake, Cascade Range, Oregon, USA	Merritt & Pratt (1964)
Prosopium williamsoni	British Columbia, Canada	Bangham & Adams (1954)
Ptychocheilus oregonensis	British Columbia, Canada	Bangham & Adams (1954)
	NS	Golvan (1959)
	NS	Petrochenko (1956)
	Cultus Lake, British Columbia, Canada	Van Cleave & Lynch (1950)
Pungitius pungitius	Lake Huron, Ontario, Canada	Bangham (1955)
	Newfoundland, Canada	Dickinson & Threlfall (1976)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	Bernard Harbour, North West territories, Canada	Van Cleave & Lynch (1950)
Pungitius sp.	NS	Yamaguti (1963)
*Pygosteus pungitius**= (*Pungitius pungitius*)	NS	Golvan (1959)
	N W Territories, Canada	Jennes (1916) in Walkey (1963)
Rhinichthys cataractae	Holt Creek, Nebraska, USA	Nickol & Samuel (1983)
	Missouri River at Brownville, Nebraska, USA	Samuel *et al* (1976)
Richardsonius balteatus	British Columbia, Canada	Bangham & Adams (1954)
Rutilus rutilus	River Yenisei	Bauer (1948a)
	River Lena	Bauer (1948b)
	Llyn Tegid, Wales	Chubb (1967)
	River Lugg, Herefordshire, England	Davies (1967)
	NS	Golvan (1959)
	River Glomma, Norway	Halvorsen (1972)
	Lough Mask, County Mayo, Ireland	Kane (1966)
	Lake Konche, Karelia USSR	Malakhova (1961)
	Macha lake fish pond System, Bohemia, Czech.	Moravec (1984a)
	Denmark	Muller (1776)

Fish species	Locality	Reference
Rutilus rutilus	Lake Lagoda, New Vygozero Lake,Karelia, USSR	Nagibina (1957)
	Lake Oyeren, Norway	Oien (1976, 1979)
	Konchozero, USSR	Petrushevski & Bykhovskaya (1935)
	Lakes Peurunkajarvi, Sarvesi & Vatia, Finland	Valtonen p.c.
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	River Ob, USSR	Volkova (1941)
	Lake Geneva, Switzerland	Zschokke (1884)
Rutilus rutilus heckeli	Dnepr Delta, USSR	Komarova (1964)
Salmo clarki	British Columbia, Canada	Bangham & Adams (1954)
	NS	Golvan (1959)
	Silver Lake, Washington State, USA	Mamer (1978)
	King Co. Washington, USA	Van Cleave & Lynch (1950)
	Vancouver Island, British Columbia, Canada	
Salmo gairdneri	British Columbia, Canada	Bangham & Adams (1954)
	Drumore Loch, Scotland	Devine (1988)
	NS	Golvan (1959)
	Bridge of Weir, West of Scotland Trout Farm, Scotland	Lassiere (Unpub.)
	River Doon trout Company Dalrymple, Scotland	Lassiere (Unpub.)
	Silver Lake, Washington State, USA	Mamer (1978)
	Suttle Lake, Cascade Range, Oregon, USA	Merritt & Pratt (1964)
	Trout farm, Germany	Steinstrasser (1936)
	NS	Van Cleave & Lynch (1950)
	Hanningfield Reservoir, Essex, England	Wootten (1973)
Salmo hucho	NS	Golvan (1959)
Salmo huso	NS	Van Cleave & Lynch (1950)
*Salmo irideus**= (*Salmo gairdneri*)	NS	Golvan (1959)
	Hungary	Jaczo (1943) in Walkey (1963)
Salmo ischchan gegarkuni	Lake Issyk-kul USSR	Iksanov (1954)

Fish species	Locality	Reference
Salmo salar	Llyn Padarn, Wales	Chubb (1967)
	White Sea, USSR	Dogiel & Petrushevski (1935)
	NS	Golvan (1959)
	New Brunswick, Canada	Hare & Frantsi (1974)
	Miramichi River system New Brunswick, Canada	Hare & Burt (1975a, 1975b, 1976)
	Switzerland	Heitz (1917, 1918)
	NS	Van Cleave & Lynch (1950)
Salmo trutta	Chirk Hatchery & Llyn Tegid, Wales	Aderounmu (1966)
	Hampshire, England	Baylis (1928)
	River Don & Burn of Savoch Scotland	Bwathondi (1976)
	Llyn Padarn, Llyn Tegid Wales	Chubb (1967)
	Loch Lomond, Scotland	Copland (1957)
	Drumore Loch	Devine (1988)
	Poprad & Strbske Lakes, Czechoslovakia	Dyk (1957)
	NS	Golvan (1959)
	Lough Mask, County Mayo & Celbridge, County Kildare	Kane (1966)
	Malham Tarn, England	Kennedy & Burrough (1978)
	Black Clauchrie Burn, Barrhill, Scotland	Lassiere (Unpub.)
	Bridge of Weir, West of Scotland Trout Farm, Scotland	Lassiere (Unpub.)
	Carbeth Loch, Scotland	Lassiere (Unpub.)
	Lochan Creag nan Caorann, Scotland	Lassiere (Unpub.)
	Lochan Duin, Scotland	Lassiere (Unpub.)
	Loch Essan, Scotland	Lassiere (Unpub.)
	Loch Maragan, Scotland	Lassiere (Unpub.)
	Loch Monzievaird, Scotland	Lassiere (1988a)
	Loch Oss, Scotland	Lassiere (Unpub.)
	Powder Works Dam Lochan, Tignabruaich, Scotland	Lassiere (Unpub.)
	Suttle Lake, Cascade Range, Oregon, USA	Merritt & Pratt (1964)
	NS	Meyer (1932)
	Pitcarmick Loch, Scotland	Pike & Edwards (1983)
	Lake Pestovo, USSR	Pozdnyakova (1963)
	River Severn, England	Rawson (1952)
	Beith, Scotland	Ritchie (1915)
	Dunalastair Reservoir Scotland	Robertson (1953)
	Trout farm, Germany	Steinstrasser (1936)
	River Teify, Wales	Thomas (1964a)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	NS	Van Cleave & Lynch (1950)

Fish species	Locality	Reference
Salmo trutta	Hannningfield Res, England	Wootten (1973)
Salmo sp.	NS	Yamaguti (1963)
Salvelinus alpinus	NS	Golvan (1959)
	Karluk Lake, Kodiak Island Alaska, USA	Morton (1942)
	Kodiak Island Alaska USA	Van Cleave & Lynch (1950)
'Salvelinus fontinalis	British Columbia, Canada	Bangham & Adams (1954)
	Poprad & Strbske Lakes, Czechoslovakia	Dyk (1957)
	NS	Golvan (1959)
	Vancouver Island, British Columbia, Canada	Van Cleave & Lynch (1950)
	Wisconsin, USA	
Salvelinus malma	British Columbia, Canada	Bangham & Adams (1954)
	NS	Golvan (1959)
	Karluk Lake , Kodiak Island, Alaska, USA	Morton (1942)
	Kodiak Island, Alaska, USA	Van Cleave & Lynch (1950)
Salvelinus namaycush	Yukon Territory, Canada	Arthur *et al* (1976)
Salvelinus sp.	NS	Yamaguti (1963)
Saurogobio sp.	NS	Yamaguti (1963)
Scardinus erythrophthalamus	NS	Golvan (1959) Moravec (1984a)
Scardinus sp.	NS	Yamaguti (1963)
Schizothorax planifrons	NS	Golvan (1959)
Schizothorax pseudasksaiensis issykkuli	NS	Golvan (1959)
	Issykkul Lake, Turkestan USSR	Meyer (1932)
Schizothorax sp.	NS	Yamaguti (1963)
Semotilus atromaculatus	NS	Golvan (1959)
	Holt Creek, Nebraska, USA	Nickol & Samuel (1983)
	Missouri River at Brownville, Nebraska, USA	Samuel *et al* (1976)

Fish species	Locality	Reference
Squalius schmidti	Issykul Lake, Turkestan, USSR	Meyer (1932)
Squalius sp.	NS	Yamaguti (1963)
Thymallus arcticus	Yukon Territory, Canada	Arthur *et al* (1976)
	Chauna River, USSR	Skryabina (1978)
Thymallus thymallus	Llyn Tegid, Wales	Chubb (1967)
	River Lugg, Herefordshire, England	Davies (1967)
	NS	Golvan (1959)
	USSR	Kazadaev (1954), Markevich (1963)
	Kola Peninsula, USSR	Skryabina (1978)
Tinca tinca	NS	Golvan (1959)
	Flatholmen, Sweden	Lundstrom (1942)
	Macha Lake fishpond System, Bohemia, Czech.	Moravec (1985)
	NS	Van Cleave & Lynch (1950)
Tinca sp.	NS	Yamaguti (1963)
Umbra limi	Lake Huron, Ontario, Canada	Bangham (1955)
Zoarces viviparus	NS	Golvan (1959)
	Bothnian Bay, Finland	Valtonen & Crompton (1989)
	NS	Van Cleave & Lynch (1950)

APPENDIX II

LIST OF DEFINITIVE HOST SPECIES FOR *NEOECHINORHYNCHUS RUTILI* IN THE NORTHERN HEMISPHERE

Classification follows that given by Jordan (1963), Wheeler (1969) and Margolis & Arthur (1979). Refer to Appendix I for localities and references. One hundred and sixteen species are included.

Class MARSIPOBRANCHI
Order PETROMYZONIFORMES

Family PETROMYZONIDAE
Lampetra fluviatilis

Class OSTEICHTHYES
Order ISOSPONDYLI

Family SALMONIDAE
Onchorhynchus keta
O.kisutch
O.nerka
O.nerka nerka
O.tschawyscha
Salmo clarki
S.gairdneri
S.hucho
S.huso
S.ischchan gegarkuni
S.salar
S.trutta
Salvelinus alpinus
S.fontinalis
S.malma
S.namaycush

Family COREGONIDAE
Coregonus albula
C.lavaretus
C.lavaretus maraenoides
C.muksun
C.nasus
C.peled
C.sardinella
C.tugan
Prosopium transmontanus
P.williamsoni

Family THYMALLIDAE
Thymallus arcticus
T.thymallus

Family OSMERIDAE
Osmerus mordax

Order HAPLOMI

Family UMBRIDAE
Umbra limi

Family ESOCIDAE
Esox lucius

Order OSTARIOPHYSI

Family CATOSTOMIDAE
Carpiodes carpio
Catostomus ardens
C.catostomus
C.commersonni
C.macrocheilus

Family CYPRINIDAE
Abramis ballerus
A.bjorkna
A.brama
Alburnus alburnus
Barbus barbus
Blicca bjoerkna
Carassius carassius
C.carassius auratus
Chondrostoma nasus
Couesius plumbeus
Ctenopharyngodon sp.
Cyprinus carpio
Diptychus dybowskii
Gila atraria
Gobio gobio
Hybognathus hankinsoni
Hypophthalmichthys sp.
Leuciscus atrarius
L.cephalus
L.erythrophthalmus
L.grislagine
L.idbarus
L.idus
L.idus orfus
L.leuciscus
L.phoxinus
L.rutilus
L.schmidti
Mylocheilus caurinus
Notropis dorsalis
N.hudsonius
Parabramis sp.
Phoxinus czekanowskii
P.laevis
P.percnurus
P.phoxinus
P.lagowskii oxycephalus
Pimephales promelas

Family CYPRINIDAE

Ptychocheilus oregonensis
Rhinichthys cataractae
Richardsonius balteatus
Rutilus rutilus
R.rutilus heckeli
Saurogobio sp.
Scardinius erythrophthalamus
Schizothorax planifrons
S.pseudasksaiensis issykkuli
Semoltilus atromaculatus
Tinca tinca

Family COBITIDAE
Cobitis latnia
C.taenia
Diplophysa dorsalis
D.strauchi
Noemacheilus barbulatus

Family ICTALURIDAE
Ictalurus nebulosus

Order APODES

Family ANGUILLIDAE
Anguilla anguilla
A.vulgaris

Order ANACANTHINI

Family GADIDAE
Gadus morhua
Lota lota

Order THORACOSTEI

Family GASTEROSTEIDAE
Culea inconstans
Gasterosteus aculeatus
G.aculeatus microcephalus
G.gymmurus
Pungitius pungitius

Order MICROCYPRINI

Family CYPRINODONTIDAE
Fundulus heteroclitus

Order PERCIFORMES

Family PERCIDAE
Gymnocephalus cernua
Perca flavescens
P.fluviatilis
P.schrenki

Family	PERCICHTHYIDAE
	Morone chrysops
	M.saxatilis
Family	CENTRARCHIDAE
	Ambloplites rupestris
	Micropterus dolomieui
Family	GOBIIDAE
	Pomatoschistus minutus
Family	ZOARCIDAE
	Zoarces viviparus
Order	SCLEROPAREI
Family	COTTIDAE
	Cottus asper
	C.bairdi
	C.gobio
	C.rhotheus
	Myoxocephalus quadricornis
	M.scorpius
Order	HETEROSOMATA
Family	PLEURONECTIDAE
	Platichthys flesus
	Pleuronectes flesus

APPENDIX III

LIST OF SITES WHERE SEVERAL FISH SPECIES ARE REPORTED TO BE INFECTED WITH NEOECHINORHYNCHUS RUTILI IN THE NORTHERN HEMISPHERE

Reports of a total of 31 sites in 11 countries in which more than one species of fish were infected with N.rutili worldwide are listed. The references are presented in alphabetical order of fish species for each locality in each country (also presented in alphabetical order). The Scottish sites are listed in Table 3.1.

Country	Locality	Fish species infected with Neoechinorhynchus rutili	Reference
Canada			
	Lake Erie	Cottus bairdi	Dechtiar (1972b)
		Morone chrysops	Nickol (p.c.)
		Notropis hudsonius	Dechtiar (1972b)
		Perca flavescens	
	Lake Huron	Culaea inconstans	Bangham (1955)
		Lota lota	
		Micropterus dolomieui	
		Notropis hudsonius	
		Osmerus mordax	Collins & Dechtiar (1944)
		Perca flavescens	Bangham (1955)
		Pungitius pungitius	
		Umbra limi	
	Lake of the Woods, Ontario	Culaea inconstans	Dechtiar (1972b)
	Cultus Lake	Gasterosteus aculeatus	Van Cleave & Lynch (1950)
		Mylocheilus caurinus	
		Onchorhynchus kisutch	
		O.nerka	
		Ptychocheilus oregonensis	

Country	Locality	Fish species infected with Neoechinorhynchus rutili	Reference
Czechoslovakia			
	Macha Lake Fish Pond System, Bohemia.	Abramis brama	Moravec (1984a)
		Cyprinus carpio	Tesarcik (1970, 1972), Moravec (1984a, b)
		Esox lucius	Moravec (1979)
		Gobio gobio	Moravec (1984a)
		Perca fluviatilis	
		Rutilus rutilus	
		Scardinius erythrophthalamus	
		Tinca tinca	Moravec (1985)
	Propad & Strbske Lakes	Leuciscus idus orfus	Dyk (1957)
		Salmo trutta	
		Salmo fontinalis	
England			
	River Lugg, Hereford-shire	Esox lucius	Davies (1967)
		Leuciscus cephalus	
		Leuciscus leuciscus	
		Rutilus rutilus	
		Thymallus thymallus	
	Hanning-field Reservoir, Essex	Salmo gairdneri	Wootten (1973)
		Salmo trutta	
Finland			
	Bothnian Bay	Alburnus alburnus	Valtonen & Crompton (1989)
		Coregonus nasus	
		Esox lucius	
		Gadus morhua	
		Gasterosteus aculeatus	
		Gymnocephalus cernua	
		Lampetra fluviatilis	
		Leuciscus idus	
		Lota lota	
		Myoxocephalus quadricornis	
		Perca fluviatilis	
		Platichthys flesus	
		Pomatoschistus minutus	
		Pungitius pungitius	
		Rutilus rutilus	
		Salmo trutta	
		Zoarces viviparus	

Country	Locality	Fish species infected with *Neoechinorhynchus rutili*	Reference
Finland			
	Lake Vatia	*Anguilla anguilla* *Rutilus rutilus*	Valtonen (p.c.)
Germany			
	Trout farm	*Salmo gairdneri* *Salmo trutta*	Steinstrasser (1936)
Ireland			
	Lough Mask County Mayo	*Rutilus rutilus* *Salmo trutta*	Kane (1966)
Norway			
	Lake Oyeren	*Leuciscus idus* *Rutilus rutilus*	Oien (1976, 1979)
Poland			
	Dabie Lake	*Abramis ballerus* *Abramis brama* *Blicca bjoerkna*	Wierzbicka (1978)
U.S.A.			
	Lake Washing-, ton, Seattle.	*Catostomus macrocheilus* *Mylocheilus caurinus*	Van Cleave & Lynch (1950)
	Holt Creek Nebraska	*Hybognathus hankinsoni* *Notropis dorsalis* *Pimephales promelas* *Rhinichthys cataractae* *Semotilus atromaculatus*	Nickol & Samuel (1983)
	Missouri River River, Brownville Nebraska	*Hybognathus hankinsoni* *Notropis dorsalis* *Pimephales promelas* *Rhinichthys cataractae* *Semotilus atromaculatus*	Nickol & Samuel (1983)
	Suttle Lake, Oregon	*Onchorhynchus nerka nerka* *Prosopium transmontanus* *Salmo gairdneri* *Salmo trutta*	Merritt & Pratt (1964)

Country	Locality	Fish species infected with *Neoechinorhynchus rutili*	Reference
U.S.A.			
	Silver Lake, Washington	*Salmo clarki* *Salmo gairdneri*	Mamer (1978)
	Karluk Lake, Kodiak Island, Alaska.	*Salvelinus alpinus* *Salvelinus malma*	Morton (1942)
U.S.S.R.			
	Ribinsk Reservoir	*Abramis ballerus*	Izyumova (1960)
		Abramis brama (1958)	Izyumova
		Esox lucius (1960)	Izyumova
	Lake Pestovo	*Alburnus alburnus* *Coregonus lavaretus* *C.lavaretus maraeoides* *Nemocheilus barbulatus* *Salmo trutta*	Pozdnyakova (1963)
	Lake Lagoda	*Anguilla anguilla* *Coregonus lavaretus* *Rutilus rutilus*	Barysheva & Bauer (1958) Nagibina (1957)
	River Yenisei Ust Port.	*Coregonus peled* *Rutilus rutilus* *Coregonus sardinella*	Bauer (1948a) Skryabina (1978)
	Kolyma River	*Coregonus peled* *Coregonus sardinella* *Phoxinus czekanowskii* *Phoxinus percnurus*	Skryabina (1978)
	River Ob	*Coregonus sardinella* *Rutilus rutilus*	Skryabina (1978)
	Lake Konche	*Esox lucius* *Perca fluviatilis* *Rutilus rutilus*	Malakova (1961)

Country	Locality	Fish species infected with *Neoechinorhynchus rutili*	Reference
U.S.S.R.			
	New Vygozero Lake	*Esox lucius* *Leuciscus idus* *Perca fluviatilis* *Rutilus rutilus*	Nagibina (1957)
	Lake Issykkl Turkestan	*Leuciscus schmidti* *Salmo ischchan gegarkuni* *Schizothorax pseudasksaiensis issykkuli*	Meyer (1932) Iskanov (1954) Meyer (1932)
Wales			
	Llyn Tegid	*Esox lucius* *Rutilus rutilus* *Salmo salar* *Thymallus thymallus*	Chubb (1967)
	Llyn Padarn	*Salmo salar* *Salmo trutta*	Chubb (1967)

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With 2 figures in the text

Host–parasite relationships between larval *Sialis lutaria* (Megaloptera) and *Neoechinorhynchus rutili* (Acanthocephala)

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SUMMARY

The role of the larva of the alder fly *Sialis lutaria* as a host for *Neoechinorhynchus rutili* (Acanthocephala) has been investigated. Brown trout (*Salmo trutta*) infected with *N. rutili* were found to be feeding on *S. lutaria* larvae which contained juvenile acanthocephalans in their haemocoels. These acanthocephalans have been identified as *N. rutili*. A previous report of this host–parasite relationship indicated some form of host response to the larval acanthocephalan. Stained wax sections of *S. lutaria* larvae infected with *N. rutili* did not show any evidence of either a host response or encapsulation. Samples of *S. lutaria* larvae revealed an interesting pattern of infection. Only larvae of a particular size range were infected. An experiment to infect commercially obtained rainbow trout (*Salmo gairdneri*) with *N. rutili* by feeding them with infected *S. lutaria* larvae was successful. Since *S. lutaria* larvae are known to be prey items for trout it appears that this route of infection could commonly occur in the natural environment. Larval *S. lutaria* is considered as an additional intermediate host to several ostracod species which have been shown to play a role in the transmission of *N. rutili*.

INTRODUCTION

Neoechinorhynchus rutili is generally assumed to exhibit a typical acanthocephalan life-cycle involving 3 infrapopulations: shelled embryos or acanthors in the hosts' aquatic environment, larvae developing in arthropod intermediate hosts and dioecious adult worms in the alimentary tract of the fish definitive host. At least 110 species of fish (Meyer, 1933: Golvan, 1959: Bykovskaya-Pavlovskaya *et al.* 1962: Kennedy, 1974) have been observed to be infected with *N. rutili*, and Van Cleave & Lynch (1950) described its distribution as circumpolar throughout the northern holarctic region in 10 families of fish from fresh water and brackish environments. Less is known about the intermediate host species of *N. rutili* in most of these locations. Merritt & Pratt (1964) identified *Cypria turneri* (Ostracoda) as an intermediate host for populations of *N. rutili* involving a number of fish species in Suttle lake, Oregon, USA. Brady had earlier (1910) cited the ostracod *Candona angulata* as an intermediate host in Northumberland and Walkey (1967) found that the ostracods, *Cypria ophthalmica* and *Candona candida* served as intermediate hosts in a life-cycle having three-spined sticklebacks, *Gasterosteus aculeatus*, as the definitive host. Walkey (1967) also demonstrated the experimental infection of both species of ostracod with *N. rutili* acanthors. Valtonen (1979) identified another ostracod species, *Candona neglecta*, as an intermediate host for *N. rutili* which later became established in a species of whitefish, *Coregonus nasus*, from the Bothnian Bay, Baltic Sea. These observations may have overshadowed the early report by Villot (1885) of having found larval *N. rutili* apparently encapsulated in the fat body of larval alder flies (*Sialis niger* = *S. lutaria*). Walkey (1967), however, was not able to infect *S. lutaria* experimentally and suggested that Villot's observations might

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have indicated that the alder fly larvae were paratenic hosts (see Nickol, 1985) for *N. rutili*. These insect larvae are extremely predacious (Kimmins, 1962) and might have become infected with *N. rutili* by feeding on infected ostracods.

The work described in this paper arose from the discovery of larval *S. lutaria*, infected with *N. rutili*, in the guts of brown trout. The primary aim was to investigate the role of this insect in the biology of *N. rutili* and in its transmission to trout.

MATERIALS AND METHODS

Light and electron microscopy

Live worms were photographed with a Leitz 22 EB compound microscope using ASA32 Panatomic black and white print film. Specimens for scanning electron microscopy were fixed in 5% formalin, dehydrated in an ethanol series, critical-point dried in CO_2 using a Polaron 3000, and sputter coated using a Polaron E5000. Specimens were viewed at 3 kV on a Philips 500 scanning electron microscope (Brown, Chubb & Veltkamp, 1986).

Specimens of *S. lutaria* infected with *N. rutili* were fixed in Carnoy's fluid for 3 h, dehydrated in absolute alcohol for 2 h and cleared in Histoclear overnight. Specimens were transferred to molten wax for two periods of 2 h each and then embedded in paraffin wax. Serial sections of 7 μm thickness were cut through the insect larvae in both transverse and longitudinal planes and were stained with Mallory's triple stain. Sections were photographed as for live *N. rutili*.

Source and maintenance of rainbow trout

Twenty-one rainbow trout (*Salmo gairdneri*), having an average wet weight of approximately 230 g, were obtained from the River Doon Trout Company, Cassilis Mill, Ayr, Scotland. They were kept in 60 litre tanks with secure lids in well-aerated copper-free tap water in a constant temperature room set at 12 °C with from 1 to 3 fish per tank. The trout were left to settle for 2 days before any experimental manipulation.

Source of alder fly larvae

The alder fly larvae were collected from the southern shore of Loch Monzievaird near Crieff, Perthshire (Grid Ref. O.S. map Sheet 58, 1:50000 NN842233) during April and May 1987. The life-cycle of *S. lutaria* lasts 2 years and the larvae undergo 10 instars prior to pupation in the soil (Fitter & Manuel, 1986). Since *S. lutaria* larvae exhibit an allometric growth pattern, their relative sizes were compared by measurement of the head width using an ocular micrometer.

OBSERVATIONS AND RESULTS

Brown trout (*Salmo trutta*) caught at Loch Monzievaird in April 1987 were found to be feeding on the larvae of the alder fly *S. lutaria*. Routine dissection of these insects revealed the presence of an acanthocephalan parasite lying free and unencapsulated in the haemocoel. The trout also harboured in their intestines an acanthocephalan identified as *N. rutili* according to Brown *et al.* (1986). Collection of 215 insect larvae from the southern shore of Loch Monzievaird revealed a prevalence of 7% of *N. rutili*

larvae. The head widths in the sample ranged from 1·08 to 2·91 mm and the infected larvae were all in the size class with heads between 1·92 and 2·67 mm wide.

Identity of the acanthocephalan parasite in Sialis lutaria

The acanthocephalans found in the larvae belonged to the class Eoacanthocephala (Bullock, 1969; Fig. 1 A, B). The parasites from the alder fly larvae possessed characters listed by Yamaguti (1963) of the genus *Neoechinorhynchus*, including a small cylindrical body with 4–5 giant hypodermic nuclei on the dorsal side and 1–2 on the ventral side, a short, globular proboscis bearing 18 hooks arranged in 6 spiral rows of 3 hooks each, anterior proboscis hooks of considerably larger size than the more posterior ones, long and digitiform lemnisci and testes positioned in the mid-region or posterior half of the body. Furthermore, the worms have a syncytial cement gland with a rounded cement reservoir. These acanthocephalans from the larval alder flies were identified as *N. rutili* as described by Van Cleave & Lynch (1950). The male and female *N. rutili* from alder fly larvae show close similarities to *N. rutili* taken from brown trout (Fig. 1 E, F, G). The similarity in proboscis morphology is revealed in the scanning electron micrographs of the proboscides of *N. rutili* from alder flies and roach (*Rutilus rutilus*) (Fig. 2 A and B). Photo-micrographs of sections of *N. rutili* in larval *S. lutaria* are shown in Fig. 2 C and D.

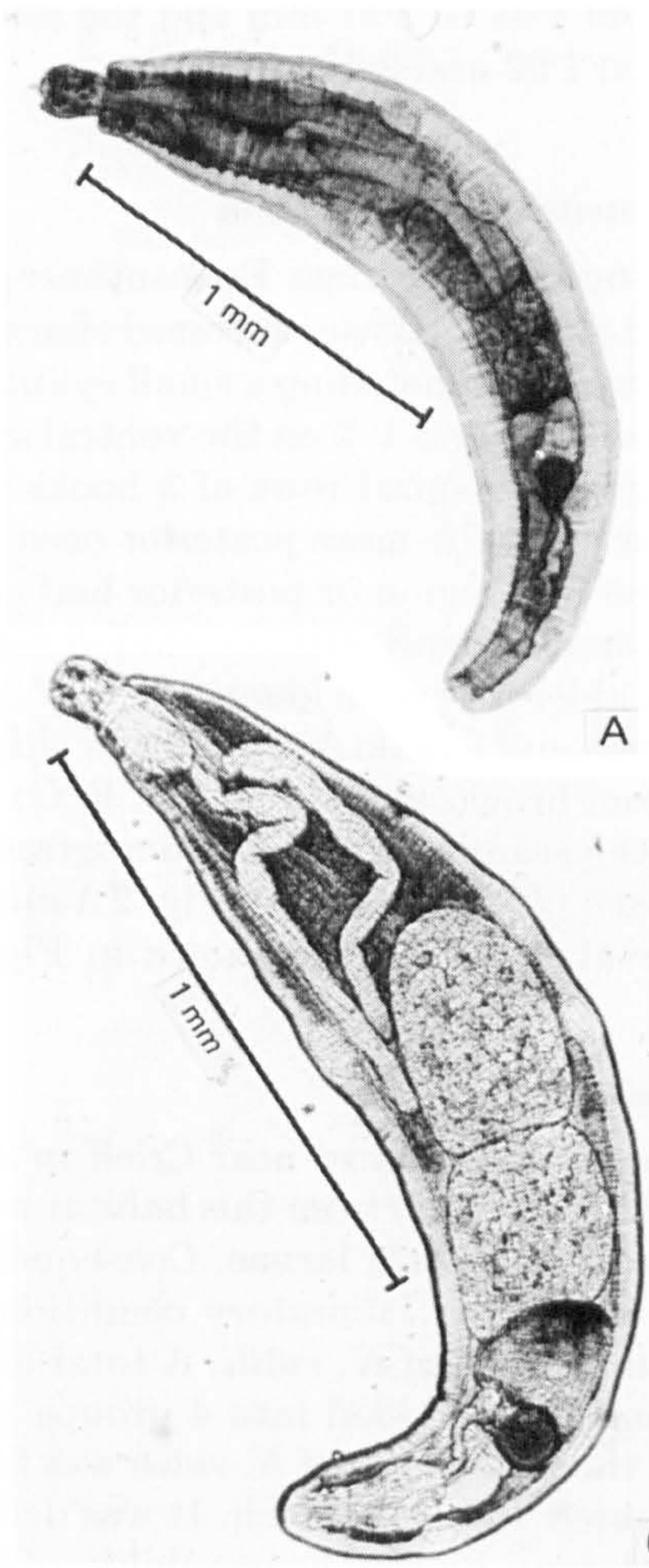
Transmission experiment

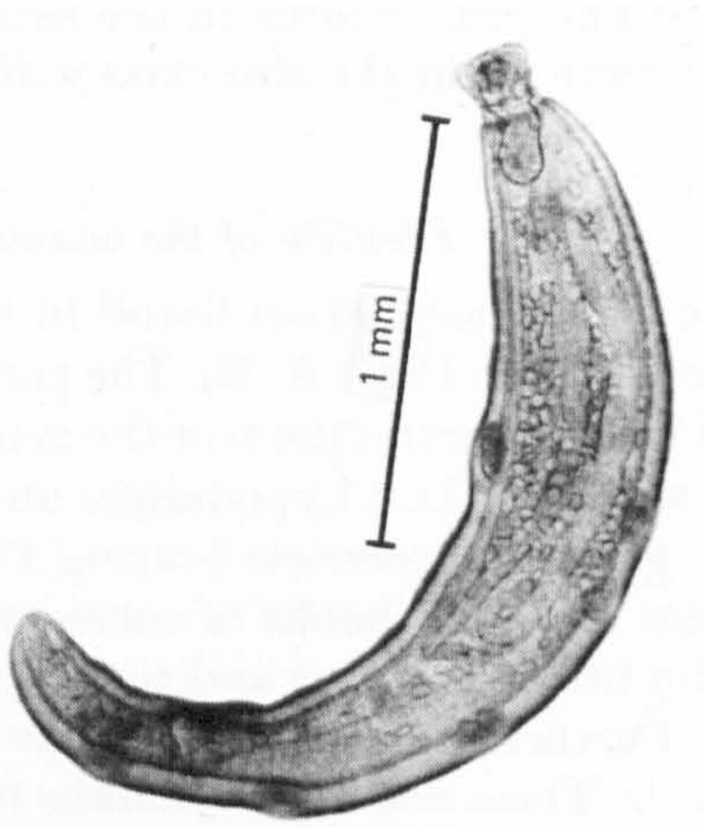
The observation that brown trout caught at Loch Monzievaird near Crieff in April 1987 were infected with *N. rutili* led to the proposition that fish from this habitat might have acquired their *N. rutili* infection by feeding on *S. lutaria* larvae. Consequently, rainbow trout (*Salmo gairdneri*) were allowed to feed under laboratory conditions on larval *S. lutaria* known to be harbouring natural infections of *N. rutili*. A total of 160 *S. lutaria* larvae from Loch Monzievaird were randomly divided into 4 groups of 40 larvae each. One of these groups was dissected and the prevalence of *N. rutili* was found to be 7·5% and each of the 3 infected larvae harboured 1 *N. rutili* each. It was decided to allow each of the 3 trout to feed on a group of 40 larvae, giving the possibility of their acquiring 3 *N. rutili* as a result. However, experience has shown that rainbow trout obtained from commercial supplies sometimes harbour adult *N. rutili* in their intestines. In order to be confident that the prevalence and intensity of *N. rutili* in the trout had been taken into account, 18 trout from a batch of 21 were dissected and 1 *N. rutili* was recovered. This was a female worm measuring 6·08 mm in length.

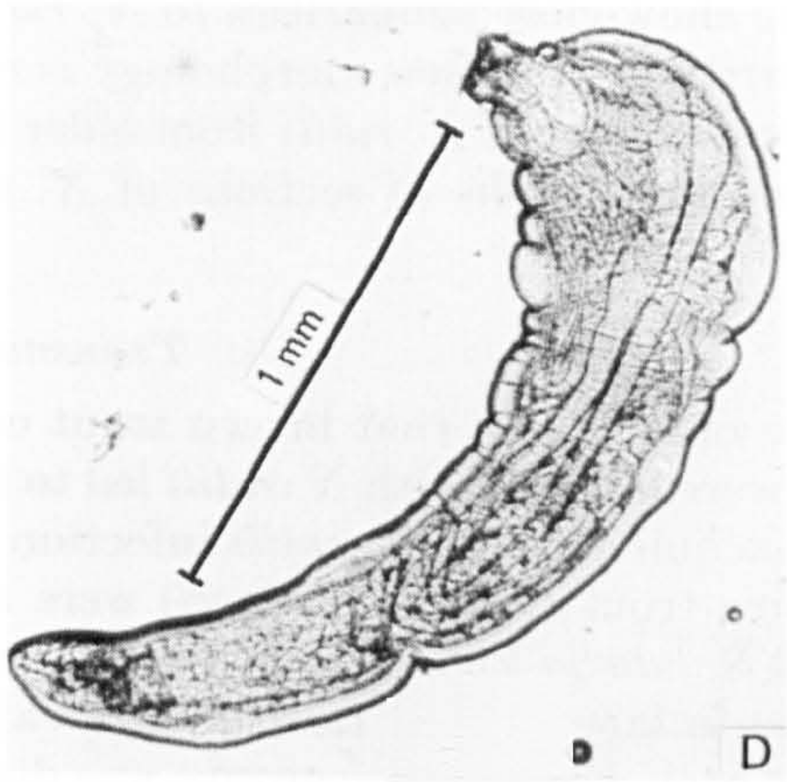
Seven days after the *S. lutaria* had been exposed to the 3 trout, the experiment was terminated and 2 of the fish were found to be infected with *N. rutili* attached to the intestinal mucosa. One fish contained an attached female worm (2·08 mm long) and a male (1·82 mm long) in the ileum, one contained one male worm (1·82 mm long) attached in the rectum (Fig. 1 C, D). It seems reasonable to assume that the *N. rutili* in the experimental trout were obtained when they fed on the groups of *S. lutaria* larvae. Also, the single *N. rutili* found in the trout dissected to monitor parasite status was a female three times the length of the female believed to have originated from the *S. lutaria*.

DISCUSSION

Alder fly (*S. lutaria*) larvae collected from Loch Monzievaird, Scotland have been found to harbour an acanthocephalan parasite identified as *N. rutili*. *N. rutili* from







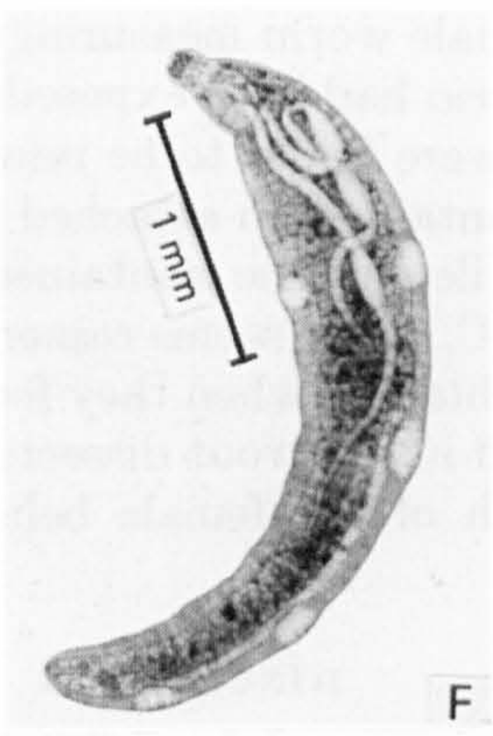


Fig. 1. For legend see opposite.

S. lutaria were considerably smaller in terms of body size than adult worms from a variety of fish hosts, but the proboscis dimensions fell within the ranges of values known for this parasite in its definitive host (Van Cleave & Lynch, 1950; Skryabina, 1978). The scanning electron micrograph of the proboscis of *N. rutili* from *S. lutaria* is similar to that of *N. rutili* described by Brown *et al.* (1986) and also to *N. rutili* collected from *Rutilus rutilus* from the Bothnian Bay (Fig. 2B).

Larval *N. rutili* were found in alder fly larvae by Robertson (1953) at Dunalastair Reservoir, Perthshire, and she also observed the presence of *N. rutili* in brown trout caught there. Observations of infected brown trout from Loch Monzievaird that were also feeding on infected alder fly larvae indicate that this insect may play a role in the transmission of *N. rutili* to trout. Further indirect evidence that *S. lutaria* may serve as an intermediate host for *N. rutili* comes from the fact that Pyefinch (1960) noted that benthic invertebrates, such as alder fly larvae, form the largest fraction of the food of trout in Scotland. Frost & Smyly (1952), who studied the seasonal diets of brown trout in Three Dubs Tarn, Lake District, noted that *S. lutaria* larvae were a common component of the diet.

The presence of *Echinorhynchus clavaeceps* (Zeder) = *Neoechinorhynchus rutili* (Muller) in alder fly larvae (*Sialis niger* = *Sialis lutaria*) was first reported by Villot (1885) in Grenoble, France. He observed this worm in *S. lutaria* larvae and noticed a number of differences from the adult form. The larval *N. rutili*, found in the fatty tissues of the insect, were described as being smaller than the adult, transparent and appeared not to be sexually mature although their reproductive apparatus was prominent. Most interestingly, Villot reported the larva as being wrapped in a sort of cyst which was thin and transparent and moulded exactly to the shape of the body and hence preventing any movement by the larva. The cyst was also covered with a thickish brown exudate. Although it is not clear on how many specimens Villot based his description, he measured the length of the worms as 480–640 μm and the greatest diameter as 160 μm. He also described the presence of 18 hooks on the proboscis with the same shape, size and arrangement as that of adult *N. rutili*. Although similar in various ways to the specimens described by Villot, the bodies of the Scottish *N. rutili* from the alder fly larvae were generally longer ($\times 2$) and wider ($\times 1{\cdot}5$). Specimens quite often fell out of the haemocoel of the insect larvae upon dissection and were observed to move about actively. Such larvae were not enveloped by any form of cyst or brown exudate. Some were observed still attached to the body wall of the insect larva. The sample of alder fly larvae from Loch Monzievaird revealed an interesting pattern of infection where only larvae in the middle of the size range were found to be infected. This may be an indication of reduced growth rate as a result of parasitic infection (Kennedy, 1985) or of differential infection rates according to host size. It is not known whether infected individuals of alder flies successfully pupate and produce fertile adult alder flies.

The view that *S. lutaria* can serve as an intermediate host for *N. rutili* must be

Fig. 1. Comparison between live unstained specimens of *Neoechinorhynchus rutili* collected from larval alder flies (*Sialis lutaria*) (A and B), experimentally infected rainbow trout (*Salmo gairdneri*) (C and D) and brown trout (*Salmo trutta*) (E, F and G). Specimens A, C and E are male; note the prominent testes and copulatory bursa (E). Specimens B, D, F and G are female; note the free ovaries and absence of testes. Specimen G has been inseminated and contains large numbers of acanthors. Note the size difference between specimens from different hosts and also the large subcuticular nuclei which are characteristic of the genus.

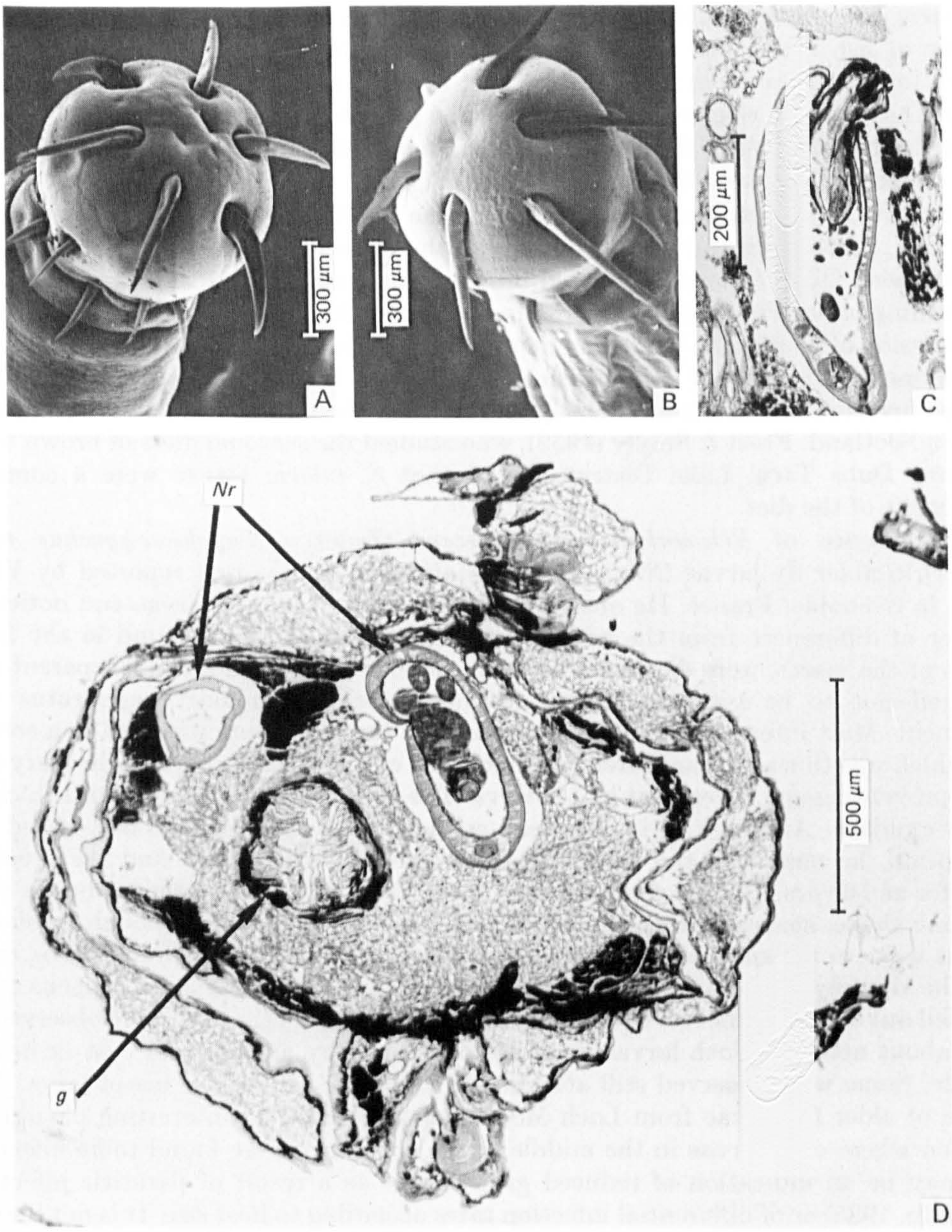


Fig. 2 (A and B). Scanning electron micrographs of the proboscides of *Neoechinorhynchus rutili* specimens collected from an alder fly larva (*Sialis lutaria*) and a roach (*Rutilus rutilus*) respectively. (Specimen from roach supplied by E. T. Valtonen.)
(C and D). Mallory's triple stained wax sections through the abdomen of *S. lutaria* larvae containing *N. rutili*.
(C). Longitudinal section showing a female *N. rutili*; note anterior hook on proboscis.
(D). Transverse section showing male *N. rutili* (*N.r*) lying close to the gut (*g*) of the insect.

considered in the light of knowledge of the relationship between *N. rutili* and ostracods as the intermediate hosts. Merritt & Pratt (1964) followed the development of *N. rutili* experimentally in the ostracod, *Cypria turneri*. The juvenile stage was attained 48–57

days post-infection and had a length of between 0·4 and 1·0 mm (proboscis inverted). There was little difference in the lengths of the male and female juveniles. The worms recovered from the alder fly larvae of Loch Monzievaird were considerably larger than those from Merritt & Pratt's ostracods, with male worms tending to be slightly smaller than females. Walkey (1967) observed that the larger sticklebacks fed on the larvae of *S. lutaria* during the months of February, March and April but his attempts to infect alder fly larvae with *N. rutili* were unsuccessful. This evidence, coupled with the stable high prevalence of young *N. rutili* in sticklebacks throughout the year, led Walkey to conclude that *S. lutaria* was not a likely intermediate host at this site. Since *S. lutaria* larvae are carnivorous in habit, Walkey suggested that *S. lutaria* was most likely to act as a paratenic host, acquiring its infection through feeding on infected ostracods. It is important to discover exactly how *N. rutili* becomes established in *S. lutaria*.

This work was undertaken while the author was the recipient of a studentship from the Natural Environment Research Council, (GT4/85/ALS/10). I wish to thank Mr Van Groot, the proprietor of Loch Monzievaird, for permission to sample insect larvae and catch trout, Alan MacGregor for catching the first trout, Dr E. T. Valtonen for supplying specimens of *N. rutili* from Finland, Dr R. Crowson for help with the identification of *Sialis lutaria* and Professor D. W. T. Crompton for his encouragement.

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Evidence for post-cyclic transmission in the life-history of *Neoechinorhynchus rutili* (Acanthocephala)

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SUMMARY

Field observations at one site on brown trout (*Salmo trutta*) and three-spined sticklebacks (*Gasterosteus aculeatus*) concurrently infected with mature *Neoechinorhynchus rutili*, together with the knowledge that large trout can be piscivorous in habit led to the proposition that the post-cyclic transmission of *N. rutili* may occur between these fish species. This route of transmission has been suggested for a number of acanthocephalan species. A laboratory experiement was conducted and it was demonstrated that uninfected rainbow trout (*Salmo gairdneri*) can acquire a *N. rutili* infection as a result of feeding on sticklebacks already carrying established worms in their intestines. This finding may help to explain how *N. rutili* is found in a wide range of fish definitive hosts throughout the northern holarctic region. More generally this example provides further evidence of the flexibility within acanthocephalan life-history patterns.

INTRODUCTION

Large brown trout (*Salmo trutta*) are known to be piscivorous in habit and minnows (*Phoxinus phoxinus*), perch (*Perca fluviatilis*), loach (*Noemacheilus barbulatus*), eel (*Anguilla anguilla*), three-spined sticklebacks (*Gasterosteus aculeatus*), bullhead (*Cottus gobio*), gudgeon (*Gobio gobio*), young salmon (*Salmo salar*), young trout (*Salmo trutta*) and roach fry (*Rutilus rutilus*) have been found in their stomachs (Frost & Brown, 1967; Crozier, 1985). Predatory trout tend to feed on fish which habitually form shoals, for example sticklebacks and minnows, in preference to species which do not (Frost & Brown, 1967). The piscivorous habit of large trout may provide a route for the transmission of endoparasitic helminths from an already appropriate definitive host to another. This postulated route of transmission, which is termed post-cyclic transmission (Nickol, 1985), has been proposed for a number of acanthocephalan species. Observations indicating the potential importance of post-cyclic transmission of fish acanthocephalans have been made by Hnath (1969), Uglem & Beck (1972) and DeMont & Corkum (1982).

Infections of *Neoechinorhynchus rutili* have been found in 10 families of fish from freshwater and brackish environments throughout the northern holarctic zone (Van Cleave & Lynch, 1950). One proposition from this observation is that larger fish species observed to be infected with *N. rutili*, while not often feeding on the usual ostracod intermediate host (Merritt & Pratt, 1964; Walkey, 1967), may have obtained their infection through post-cyclic transmission. Field work at Drumore Loch, Perthshire, Scotland revealed that both sticklebacks and brown trout were infected with sexually mature *N. rutili*. The purpose of the work described in this paper was to establish whether trout could become infected with *N. rutili* as a result of feeding on sticklebacks that were already harbouring the acanthocephalan in their alimentary tracts. The

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Table 1. *Prevalence and intensity of* Neoechinorhynchus rutili *infection in sticklebacks from Drumore Loch*

Fish length class (mm)	No. of fish dissected	No. of fish with *N. rutili* infection	No. of worms recovered	Prevalence (%)	Intensity of infection (no. of worms/fish)
11–20	1	0	0	0	0
21–30	21	7	22	33·3	1·05
31–40	5	3	11	60·0	2·20
41–50	2	2	17	100·0	8·50
51–60	1	1	9	100·0	9·00
All lengths	30	13	59	43·3	1·97

experiment consisted of exposing individual rainbow trout (*Salmo gairdneri*) to a number of sticklebacks carrying an estimated population of *N. rutili*.

MATERIALS AND METHODS

Source and maintenance of sticklebacks

Three-spined sticklebacks (*Gasterosteus aculeatus*) infected with *N. rutili* were collected in October 1987 from Drumore Loch, Perthshire (Grid ref. O.S. map Sheet 43, 1:50000 NO 165608) Scotland and were then kept in the laboratory in 5 litre tanks in copper-free water in a constant temperature room set at 12 °C. Thirty of the sticklebacks were dissected to measure the prevalence and intensity of infection with *N. rutili* (Table 1).

The prevalence was 43·3% and the mean intensity of infection was 1·97 for all lengths of fish. The sample of fish was skewed towards the 21–30 mm length class which had a mean intensity of infection of 1·05. A group of 15 sticklebacks of this size would therefore be expected to contain at least 15 worms. Accordingly the rainbow trout were exposed to an expected number of *N. rutili* which were already established in the guts of the sticklebacks of this size class.

Source and maintenance of rainbow trout

Twenty rainbow trout (*S. gairdneri*), having an average wet weight of approximately 254 g, were obtained from the River Doon Trout Company, Cassilis Mill, Ayr, Scotland. They were kept in 60 l tanks with secure lids in well aerated copper-free tap water in a constant temperature room set at 12 °C with 1 fish per tank. The trout were left to settle and food was withheld for 7 days prior to experimental manipulation. Rainbow trout were used because they are much easier to maintain in a laboratory aquarium than brown trout. Also we regularly observe *N. rutili* in the intestines of both brown and rainbow trout in Scotland (Lassière & Crompton, unpublished observations).

Transmission experiment

The rainbow trout were randomly sorted into a group of 7 for experimental infection and a group of 13 for investigation of parasite status. No intestinal helminths were found in any of the group of 13 fish. A total of 120 sticklebacks were randomly arranged

Table 2. *Details of numbers of sticklebacks consumed by each trout and numbers of* Neoechinorhynchus rutili *in transmission experiment*

	Number of sticklebacks		Numbers of *N. rutili* recovered		
Trout number	Eaten	Digested × /15	Duodenum	Ileum	Total
1	0	0	0	0	0
2	15	15	7	1	8
3	13	13	12	2	14
4	15	13	12	1	13
5	3	1	0	0	0
6	15	15	8	6	14
7	14	14	17	7	24

into 8 groups of 15 fish each. Each of the 7 experimental rainbow trout was exposed to at least 15 *N. rutili* established in the intestines of the groups of sticklebacks which were introduced into the same tanks as the trout. The eighth group was dissected as a control. The prevalence of the *N. rutili* infection was found to be 33·3% with a mean intensity of infection of 1·13. These results fitted well with the expected values on the basis of the initial examination (Table 1). The individual tanks containing the 7 trout were inspected daily and, 10 days after the introduction of the sticklebacks, the trout were killed, the number of sticklebacks eaten by each trout was recorded and surviving sticklebacks were dissected. The stomach contents of each experimental trout were examined and the number of undigested sticklebacks was recorded and deducted from the total number consumed by that fish. The gut was examined for the presence of *N. rutili* and their position was recorded in three regions of the gut, the duodenum, the ileum and the rectum, as described by Burnstock (1959).

RESULTS

Details of numbers of sticklebacks consumed by each trout and numbers of *N. rutili* recovered are recorded in Table 2. *Neoechinorhynchus rutili* were found attached in both the duodenal and ileal regions of the gut, but not in the rectal region, and 3 out of the 7 trout consumed all the sticklebacks in their tanks. On the basis of the numbers of worms recovered from the dissected control group, the survival and consequent establishment rate appears to be high for this type of transmission. A total of 17 worms were recovered from the control group of sticklebacks. By comparison, trout numbers 2, 4 and 6 (Table 2) which had each caught and eaten 15 sticklebacks, contained 8, 13 and 14 worms respectively. One trout (No. 5) which only consumed 3 sticklebacks was found to be harbouring no *N. rutili*. Another trout (No. 7) had 24 *N. rutili* in its gut. This value exceeds that expected on the basis of the results from the control group. This observation can be accounted for because numbers of *N. rutili* per stickleback at Drumore Loch are known to be over-dispersed and occasionally a fish with a high intensity of infection will appear in the sample. For example, from a sample of 144 fish the mean number of *N. rutili* per fish was observed to be 3·76 and the variance was 186·60 (Lassiere, unpublished observations). The variance to mean ratio for this distribution of *N. rutili* in sticklebacks is 49·63, confirming that the parasite was over-dispersed in this sample of hosts (Anderson & Gordon, 1982). Random sampling would not favour selecting one of these fish.

DISCUSSION

Experimental post-cyclic transmission of fish acanthocephalans has been observed before when DeMont & Corkum (1982) fed infected small mosquito fish (*Gambusia affinis*) to larger specimens. In their experiment 14 large uninfected fish were offered 39 very small fish as food. After 7 days, three of the large fish were found to contain a total of 7 *Octospiniferoides chandleri*. Hnath (1969) fed sections of gut with attached *Echinorhynchus salmonis* from a coho salmon (*Oncorhynchus kisutch*) to uninfected brook trout (*Salvelinus fontinalis*) and subsequently found live attached worms in the trout intestines over a period of 12 weeks. Hnath (1969) suggested that the observed high prevalence and intensity of infection of coho salmon and Lake trout (*Salvelinus namaycush*) in Lake Michigan was due to the piscivorous habit of these fish and consequent re-establishment of acanthocephalans from the guts of prey items. Uglem & Beck (1972) fed young and mature stages of *N. cristatus* and *N. crassus* to a number of fish species by means of a polythene tube and a syringe. Only the subadults of *N. cristatus* were found to establish in the guts of the fish.

The possibility of this type of transmission operating in trout in the natural environment is supported by three pieces of circumstantial evidence. Firstly, there are numerous reports of large trout feeding as predators on small fish (Ball, 1961: Crozier, 1985: Hunt & Jones, 1972: Swynnerton & Worthington, 1940). Secondly, *N. rutili* has been reported to occur concurrently in both small and large fish species from a number of locations. For example, Campbell (1974) found *N. rutili* in both pike and perch at Loch Leven. Thirdly, brown trout from Drumore Loch have been found to harbour up to 1500 *N. rutili* per fish (personal communication A. W. Pike). Perhaps this high intensity of infection in the predator reflects the high prevalence of the parasite in the prey. Post-cyclic transmission might facilitate transfer to fish which might not commonly feed upon the invertebrate hosts of the parasite. *Neoechinorhynchus rutili* appears to have a low definitive host specificity so this route of infection would be an appropriate one for the species. This type of transmission is likely to promote the dispersal of *N. rutili* since some of the species of host are also migratory, for example sea trout. Worms moving from small fish species to larger fish species by this route may experience more favourable gut conditions, for example, reducing crowding effects, and may consequently achieve more of their reproductive potential.

Current knowledge shows that *N. rutili* has either a simple direct life-history in which transmission to the definitive host depends on their feeding on infected ostracods (Merritt & Pratt, 1964; Walkey, 1967; Valtonen, 1979) or a more complex pattern involving infected alder fly larvae (Lassiere, 1988). In natural systems involving *N. rutili*, it appears that a more complex life-cycle may exist in which both post-cyclic transmission and additional arthropod hosts may play roles in the stability of the parasite suprapopulation. In general, this study has revealed something of the flexibility of acanthocephalan life-histories in terms of routes of transmission.

This work was undertaken while O.L.L. was the recipient of a studentship from the Natural Environment Research Council, (GT4/85/ALS/10). We would like to thank Dr A. W. Pike, Department of Zoology, University of Aberdeen, for introducing us to Drumore Loch and for providing information about the parasite status of brown trout from that site and Mr John Harvey for permission to study the fauna there.

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