Ecology of helminth infections in salmonid fish

by

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DECLARATION

I declare that the research described in this thesis has been carried out by myself unless otherwise cited or acknowledged. It has not, in whole or in part, been submitted for any other degree. During the course of this research, various aspects of the work have been reported elsewhere, either as published scientific papers, or in conference presentations. The presentations and the thesis chapters to which they relate are listed on pages V and VI.

DEDICATION

I dedicate this work to my mother, memory of my father and the rest of my family.

Published papers

Dorucu, M., Crompton, D.W.T., Huntingford, F.A. and Walters, D.E. (1995). The ecology of endoparasitic helminth infections of brown trout, *Salmo trutta*, and rainbow trout, *Oncorhynchus mykiss*, in Scotland. *Folia Parasitologica* **42**, 29-35. (Chapter 3).

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GENERAL SUMMARY

The ecology of helminth infections has been investigated in Scottish salmonid fish populations. Examination of 240 brown trout and 49 rainbow trout from 21 locations in Central Scotland indicated that *Crepidostomum farionis* (Digenea) was the most widely distributed helminth species, followed by *Eubothrium crassum* (Cestoda), *Diphyllobothrium dendriticum* and *Diphyllobothrium ditremum* (Cestoda), *Neoechinorhynchus rutili* (Acanthocephala), *Echinorhynchus truttae* (Acanthocephala), *Eustrongylides* sp. (Nematoda), *Capillaria salvelini* (Nematoda), *Cyathocephalus truncatus* (Cestoda), *Raphidascaris acus* (Nematoda) and *Cystidicola farionis* (Nematoda) in that order. The wide distribution of *Crepidostomum farionis* may be explained through the variable habits of the definitive host species and possibly interactions with human and avian factors. The frequency distribution of numbers of worms per fish was observed to be overdispersed. Autogenic species were generally the dominant form and responsible for most of the similarity in patterns of infection within and between localities. No evidence was found to indicate host morbidity due to the helminth infections. An analysis of pairs of associations between species of helminths revealed a significantly positive association between *N. rutili* and *C. farionis*. This may be because one species improves either the establishment or survival of the other. In contrast, there was no clear evidence of competition between helminth species. The results of this study were discussed in terms of observed patterns in the helminth communities in freshwater fish host.

The endoparasitic helminth fauna was examined from three sympatric morphs of Arctic charr, *Salvelinus alpinus*, (small-headed benthic, large-headed benthic and pelagic) from Loch Rannoch, Scotland. Six species of endoparasitic helminth were

recovered from the fish and the morphs had different patterns of infection. Overall, infections in pelagic charr were heavier than in the large-headed benthic morph, which in turn was heavier than in small-headed benthic even though the benthic morph lives longer than the pelagic. Pelagic fish had high prevalences and intensities of pseudophyllidean tapeworms, the first intermediate hosts of which are copepods. The prevalence and intensity of metacercariae of *Diplostomum* sp. (the first intermediate host of which are snails) were high in the benthic morphs. The results were discussed in terms of the effects of ecological factors on transmission of helminth parasites to their hosts and the evolution of host-parasite associations.

The natural levels of infection with procercoids of pseudophyllidean cestodes (assumed to belong to *Diphyllobothrium* spp.) in *Cyclops strenuus abyssorum* in Loch Lomond near Rowardennan, Scotland were investigated. Water temperature varied from 3.2°C to 16°C with the lowest value recorded in January and the highest in July. Infection of *C. str. abyssorum* with procercoids of *Diphyllobothrium* spp. appeared to occur throughout the year in Loch Lomond, but judged to be low in winter months compared with summer months. This may be due to negative effects of low water temperature on eggs hatching and unavailability of definitive hosts in winter.

Investigation of prevalence and intensity of *Diphyllobothrium* spp. infections in powan in Loch Lomond showed that powan were heavily infected, presumably because they feed mainly on zooplankton. No adverse effect upon the health of powan in Loch Lomond could be attributed to the presence of plerocercoids of *Diphyllobothrium* spp., nor did the sex of the fish have any influence on the infection parameters.

The epidemiology of the adult stage of *Eubothrium crassum* in its definitive host, *Salmo salar* from a farm was described. The overall prevalence and mean

Chapter 1 General introduction

1.1 WHY THE STUDY OF FISH PARASITOLOGY IS IMPORTANT

Parasites present a continual and unacceptable threat to the well-being and economy of millions of people and to domesticated, farmed and wild animals in all parts of the world. In this context, the importance of fish parasites is related directly to the economic importance of the fish species that they may infect. Fish is the primary source of quality protein for humans in many parts of the world, especially in developing countries (Dick and Choudhury, 1995). As the world becomes still more crowded with people, all foodstuffs, especially fish are becoming increasingly valuable. As a recreational asset, fish rank at or near the top, both for sport fishing and as one of the attractions of nature. Additionally observing live fish, both in nature and in the display aquarium, is an important hobby for many people.

If some unusual environmental event occurs of either natural or human origin, the equilibrium between host and parasite may be disturbed and an epizootic of one or more species of parasite may occur, leading to a serious loss of fishes (Hoffman, 1967). High mortalities due to parasitic infections take place when fish are held under relatively crowded and confined conditions. Thus, aquaculture suffers great loss each year due to parasitized fish which have to be condemned for human consumption and can be sold only for animal feed at reduced price (Hoffman, 1962).

Against this background of the economic and social importance of fish parasitology, this thesis describes a programme of research into helminth infections of salmonid fishes from various sites in Scotland.

1.2 TERMINOLOGY AND CHARACTERISATION OF MACROPARASITIC INFECTION LEVELS IN HOST POPULATIONS

A number of descriptive variables have been used for quantifying the levels of parasitic infection in a population of hosts. Most commonly used are the prevalence and intensity of infection and the frequency distribution of parasite numbers per host (with the associated pattern of dispersion).

1.2.1 Prevalence

Prevalence is a widely used epidemiological statistic indicating the proportion (usually expressed as percentage) of the host population infected with a parasite at the time samples were taken. Prevalence can be measured by direct observation of the parasites in the host, e.g. *post-mortem* examination for intestinal helminths or by indirect means, such as the examination of host faeces for parasite eggs or host body fluids for protozoan stages.

1.2.2 Intensity of infection

An index of the intensity of infection is ideally based on the total numbers of parasites per infected host. The intensity of helminth infection in the fish is usually defined as the number of parasite (either larvae or adult stage) per infected host and can only be determined at *post-mortem* examination for endoparasites.

1.2.3 Frequency distribution of parasite numbers per host

Prevalence and intensity combine to dictate the frequency of parasite numbers per host. There are three basic forms that the distribution of parasite numbers would be represented by most host having similar numbers of parasites, parasite randomly distributed and all hosts uninfected except one which harbours all the parasite population. The distribution of parasites can also be randomly distributed throughout the host population.

Macroparasites (particularly helminths) are often observed to be aggregated or over-dispersed in their hosts. These distributions are associated with specific relationships between the variance and the mean: under-dispersed (variance/means < 1); random (variance/mean = 1) and over-dispersed (aggregated, variance/mean > 1) (Table 1.1). These can be approximated by the three probability distributions; the positive binomial, the poisson and the negative binomial respectively (Anderson and Gordon, 1982).

Many studies have described the frequency distribution of a range of host-parasite relationship and some have also attempted to understand the factors that generate the various patterns of dispersion. It was recognised that the death of heavily infected hosts could have a potentially strong regulatory effect on an over-dispersed parasite population and its hosts (Crofton, 1971; Bradley, 1972). Anderson (1978) was able to propose regulatory and destabilising processes which could influence population growth by the use of theoretical models of the host-parasite interaction and reference to naturally occuring host-parasite systems. He predicted, however, that the impact of these processes would be dependent on the distribution pattern of parasites and host. Anderson and Gordon (1982) used Monte Carlo simulation experiments, based on

models of the growth and decay of parasite populations, to investigate the dynamic mechanisms that generate the various patterns of dispersion in the distributions of parasites and their hosts. These are summarised in Table 1.1:

Table 1.1. The factors that generate dispersion patterns (Reproduced with permission from Cambridge University Press, Anderson and Gordon, 1982).

$s^2/x \leftarrow 0$	$s^2/x = 1$	$s^2/x \rightarrow \infty$
All host harbour the same number of parasites	Parasites randomly distributed	All hosts uninfected except one which harbours all the parasite population
Under-dispersion $s^2/x < 1$	Random $s^2/x = 1$	Over-dispersion $s^2/x > 1$
←		→
Factors which generate under-dispersion (1) Parasite mortality (2) Density-dependent processes (3) Parasite-induced host mortality (host death rate positively correlated with parasite burden)		Factors which generate over-dispersion (1) Heterogeneity in host susceptibility to infection (2) Direct reproduction with the host (3) Heterogeneity in the ability of hosts to kill parasites whether by immunological or other types of response

Thus, the distribution of parasite numbers could result from opposing dynamic forces, some of which serve to increase the degree of dispersion, whilst others act to decrease dispersion (Anderson and May, 1982). Studies of dispersion in a host parasite system may indicate which are the important host and parasite processes and which hosts are likely to be important in regulating either the host or parasite populations. Tierney (1991) used controlled laboratory experiments to explore how dispersion patterns could arise in natural host-parasite associations in the *Schistocephalus solidus-Acanthocyclops viridis* system.

1.2.4 Terminology

During the course of this research, the following terms have been used as described and defined below.

Parasitology is the study of parasites and their interactions with their host (Cox, 1993).

Infection is the presence of the parasite within a host individual or population, whilst disease refers to a clinical condition that can be observed or measured.

Mean intensity: Total number of individuals of a particular parasite species in a sample of a host species ÷ number of infected individuals of the host species in the sample (Margolis *et al.*, 1982).

Abundance: Total number of individuals of a particular parasite species in a sample of host ÷ total number of individuals of the host species in the sample (Margolis *et al.*, 1982).

Parasitism is an ecological relationship between two organisms, one designated as the parasite and the other as host.

Crofton (1971) indicated that the essential features of the relationship are:

1. physiological dependence of the parasite on its host;

2. heavily infected host will be killed by their parasites;

3. the reproductive potential of parasites exceeds that of their host and

an overdispersed frequency distribution of parasites within the host population. That is in variance (s^2) of the parasite population is significantly greater than the mean ($\bar{x}$) of the parasite population.

A definitive host is the host in the life-cycle in which the parasite reaches sexual maturity.

An intermediate host is one that is required by the parasite to complete its life-cycle; and in which the parasite will undergo development.

Epidemiology is the study of the parasite population biology.

Epizootiology is the study of the population biology of parasites in other animals.

Epizootic: Massive infection rate among animals other than humans; identical to an epidemic in humans (Schmidt and Roberts, 1989).

Population is defined as a group of organisms of the same species occupying a given space in time and comprising a single gene pool.

Infrapopulation is defined as all of the parasites of a single species in one host.

Suprapopulation is defined as all the parasites of a given species in all stages of development within all hosts in an ecosystem.

Metapopulation is defined as all the infrapopulations of a species of parasite within all hosts of a given species in an ecosystem.

Autogenic species colonise new aquatic localities by the natural migration or human assisted movements of fish and/or invertebrate intermediate hosts harbouring intact parasites into the new localities.

Allogenic species may colonise this same way or in contrast by the liberation of helminth eggs from birds or mammals into new localities.

1.2.5 Berger-Parker dominance index (Southwood, 1978)

To describe helminth communities in each fish species at each locality, the B-P index was used, being number of species of helminths and the total number of helminth individuals. To focus attention on the dominant helminth species in each host at each

locality, the non-parametric Berger-Parker dominance index was used (Esch *et al.*, 1988).

$d = N_{max} / N_T$

where N_T is the total number of helminths in the community and N_{max} is the total number of helminths belonging to the dominant species (Southwood, 1978; Esch *et al*, 1988), which measures the proportion of the total catch that is due to the dominant species.

1.2.6 Percentage similarity index

Parasite communities within individual fish were compared within and between localities using a quantitative percentage similarity index:

$C_{\chi\gamma} = \Sigma_i \min (p_{xi}, p_{yi})$, where $p_{xi} = x_i/X$, the proportion of species *i* in the community X and $p_{yi} = Y_i/Y$, the proportion of species *i* in community Y (Hulbert, 1978; Esch *et al.* 1988), which measures the minimum proportion of overlap in the numbers of individuals of the same species between two communities. For each species of fish in each locality, the percentage similarity between each pair of fish in a sample was determined separately and the mean of all possible pair combinations was obtained. This procedure was carried out for autogenic and allogenic species separately. Comparison of community similarity between localities was carried out in a similar manner, resulting in a mean value for each pair of localities. This procedure was also followed separately for autogenetic and allogenetic species. Since the intent was to focus on similarity, to be meaningful that similarity must be calculated between each pair of fish; the derived data (mean percentage similarity) which from the basis of our study are not independent. This has the advantage that of using the best measure of

similarity available but it has the disadvantage of being unable to test for significant differences in variance, since the most basic assumption of a statistical test is that samples be independent (Esch *et al.*, 1988).

1.3 BIOLOGY OF SALMONID FISH

The Salmonidae is one of the world's best known families of fish. Although originating in the northern temperate zone of Europe, Asia and North America, members of this family have been introduced into the upland areas of tropical countries, where the rivers are sufficiently cool, and into the temperate lands in the southern hemisphere (Maitland and Campbell, 1992).

All the Salmonidae are predatory, feeding on invertebrates and fish; there is usually a change of emphasis from one type of food to another as the individual grows. Several members of the family are long-lived and there are records of wild brown trout, *Salmo trutta*, and Arctic charr, *Salvelinus alpinus*, and brook trout, *S. fontinalis*, living for more than 20 years. Salmonidae spawn in freshwater, so no member of the family is entirely marine, although a number of species spend most of their lives at sea. Species of salmonidae spawn during the coolest period of the year, usually in running water but sometimes in relatively still water in a lake. In either case they require a clean silt-free bottom for the survival of their eggs and larval stages (Maitland and Campbell,1992).

Trout are an important tourist and angler attraction in Scotland and it is of interest to understand the potential and actual effects of parasites on their fish hosts (Campbell, 1971; Lassiere, 1989). From the economic viewpoint many salmonid fish in fish farms might also be infected with helminths which are subsequently used to stock

natural water bodies. Salmonid fish have widespread economic and environmental importance. Thus, correct identification and understanding of their diseases are therefore vital if valuable stocks are to be maintained.

1.4 STUDIES OF PARASITE LIFE-CYCLES

Parasites show a great diversity in their life-cycles, often involving one or more intermediate hosts in the cycle. Parasites may use fish as definitive hosts or as intermediate hosts. Amongst the latter set are two types: those that use fish as both intermediate and definitive hosts (autogenic species), (e.g. *Eubothrium* spp., see Chapter 7) and those that use fish as intermediate host only as (allogenic species) (Lincoln *et al.*, 1982), (e.g. *Diphyllobothrim* spp., see Chapter 5). These latter parasite species mature in vertebrates other than fishes, generally in birds or mammals. The transmission of helminth infections from host to host is usually achieved by eggs or larvae. Eggs containing infective stages are usually directly ingested by a host. Larvae may be similarly ingested, or consumed while attached to a plant or eaten while located in an intermediate host which acts as a prey item for the next host in the life cycle (Cox, 1993).

1.5 THE EFFECTS OF HOST FEEDING HABITS ON PARASITE COMMUNITIES

The structure of parasite communities has been related to the habitat choice and food specialisation of hosts (Dogiel, 1961; Malmquist *et al.*, 1986; Walker *et al.*, 1988; Frandsen *et al.*, 1989; Dorucu *et al.*, 1995b). The nature of predator-prey relationships should therefore serve as a potential biological index for predicting the structure of the

parasite fauna in any given aquatic ecosystem. Esch (1971) suggested that the most significant selection force influencing the parasite fauna in each lake operates via characteristically structured predator-prey interactions. Analysis of host-parasite associations showed that animals with similar food preference tend to have similar kinds of parasites (Cameron, 1964). Often fish from different sites in the same area feed on different prey species. Because many of the fish parasites are transmitted through the food web by infected invertebrates or forage fish, certain of the observed differences between parasites communities probably reflect local variations in habitat condition, prey availability and fish feeding behaviour. A clear example is given by Due and Curtis (1995) that the local differences in prevalence and abundance of freshwater Arctic charr parasites were related to the feeding patterns of the fish. Due and Curtis (1995) found that Arctic charr feeding on chronomids were free of metazoan parasites, but those eating copepods were infected with cestodes. Walkey (1967) observed an evident correlation between ostracods in the diet and infection of *Gasterosteus aculeatus* with *Neoechinorhynchus rutili.*

1.6 THE ECOLOGY OF HELMINTH INFECTION IN NATURAL POPULATIONS OF SALMONID FISH

Species of salmonid fish are of major economic and recreational importance in the British Isles, so an understanding of their parasitic infections is potentially of practical importance. In the past, the study of parasites of brown trout, *Salmo trutta*, in the British Isles and other temperate countries has focused on morphological and taxonomic aspects, especially of those helminth parasites that cause damage to fish (Duguid and Sheppard, 1944; Hickey and Harris, 1947); Wootten, 1973; Batterton,

1974). The relationship between the parasite fauna of fish living in lakes and prevailing limnological conditions has been examined on a number of occasions. Some studies have suggested that there may be general relationships between the parasite fauna and the history, size and the geographical location of the lake (Dogiel, 1961; Kennedy, 1978b). Kennedy (1978b) has noted significant correlation between lake area, and number of parasite species harboured by trout. For instance, the number of parasite species increased with increasing lake area, but was unrelated to lake age, limnological structure and geographical location. This relationship between fish parasite fauna and the size of water body has also been studied by Dogiel (1961). His findings corresponded with those of Kennedy, who concluded that this may be due to larger water bodies having greater habitat diversity within them, so that a greater diversity and abundance of free-living animals that can serve as hosts to helminths.

In other studies, a relationship has been detected between the parasite fauna of the fish and the tropic status of lake (Wisniewski, 1958; Chubb, 1963, 1964, 1970; Esch, 1971; Wootten, 1973). Wisniewski (1958) and Chubb (1963, 1964, 1970) concluded that the tropic conditions of lakes ultimately determine the assemblage of fish, which in turn determines the parasite species present. Salmonids were characteristic of oligotrophic systems, whereas cyprinids and other fishes were more typical of eutrophic waters (Chubb, 1963, 1964). A further contribution to this subject was proposed by Wisniewski (1958) and Esch (1971), who made a distinction between the types of parasites found in oligotrophic and eutrophic systems. Both suggested that fish from oligotrophic lakes, owing to lack of interaction with organisms outside the system (e.g., birds and mammals), possess more adult parasites and fewer larval phases than fish from eutrophic lakes. Wisniewski (1958) found that most of the fish parasites

in the eutrophic Lake Druzno, Poland were larval forms, and Esch (1971) noted that larval phases predominated in centrarchids from two eutrophic lakes compared with similar fish from an oligotrophic system in Michigan. Halvorsen (1971) advanced the idea that relationships between hosts and parasites were constant, despite limnological or geographical differences. Wootten's (1973) data support Halvorsen's (1971) hypothesis, in that the same fish species in limnologically different localities had similar parasite assemblages. Marcogliese *et al.* (1991) studied metazoan parasites of *Salmo salar* and *Salvelinus fontinalis* at eight sites in Newfoundland and related the results to the lake characteristics, stressing their importance in structuring parasite communities of salmonids. They proposed that nine of 14 parasite species were salmonid specialists and because of the widespread distribution of intermediate hosts generally had a wider geographic distribution than the remaining generalist parasites. One exemption is provided by *Echinorhynchus lateralis*, which is probably the best colonist, owing to its ease of transmission. The intermediate host of *Echinorhynchus lateralis* is the amphipod *Hyalella azteca* which is common throughout Newfoundland. Marcogliese *et al.* (1991) also found that small ponds had low parasite richness and large lakes had high parasite richness, perhaps due to the availability of hosts. Their study was unusual in that it looked at the parasite fauna of 2 species, rather than considering the total fauna of fish parasites. Similarly, Kennedy (1978b) investigated the composition of the parasite fauna of *Salmo trutta* in nine British lakes from varying geographical locations and analysed the findings with regard to several physico-chemical parameters.

The number of parasite species that a fish species harboured was found to vary widely from one host to another and from one locality to another (Kennedy, 1978b; Kuris, *et al.* 1980; Leong and Holmes, 1981; Price and Clancy, 1983; Kennedy *et al.*,

Kuris, *et al.* 1980; Leong and Holmes, 1981; Price and Clancy, 1983; Kennedy *et al.*, 1986). Kennedy *et al.* (1986) examined the parasite fauna of 12 species of freshwater fish from 17 localities on Jersey in the Channel Islands and compared the composition, number and diversity of the parasite fauna of each species of fish in each locality. They recorded 13 species of parasites from 12 species of fish and found that the distribution, prevalence and abundance of each species varied, reflecting in large measure the distribution and abundance of their host species and the conditions prevailing in each location.

1.7 PARASITE FAUNA OF SALMONID FISH IN THE BRITISH ISLES

In the reference list of parasites recorded in freshwater fish from Great Britain and Ireland, Chappell and Owen (1969) list some 90 species while Kennedy (1974) made a more comprehensive check-list that dealt with 168 parasitic species and incorporated all published and some unpublished information to that date. Later, Chubb (1979, 1980) reviewed the seasonal occurrence of helminths in freshwater fishes and described the parasites of various species of fish in different climatic zones in the world. Few surveys have been carried out on the regional distribution of helminth parasites of fish in Central Scotland, which is an area with abundant and varied bodies of freshwater richly stocked with fish including the brown trout and rainbow trout *Salmo trutta* and *Oncorhynchus mykiss* respectively. Investigations of trout parasites in the British Isles have been mainly undertaken in England, Wales and West Scotland (Bwathondi, 1984). One such survey of helminth species infecting freshwater fishes in Scotland was begun in June 1953, by Copland (1957). Its aim was to obtain information about the prevalence, nature and distribution of these parasites; and in particular to

identify those that might have an adverse effect on the condition of the fish. This investigator studied the parasites of Loch Lomond fishes and recovered a wide variety of helminth species. In addition, he noted that little was known about the life histories and habits of these parasites or their effects on the health of their fish host. Bwathondi (1984) investigated the mode of infection and the life cycles of trout parasites in Strathbeg, Scotland. He examined 402 brown trout and recorded a total of 13 species of helminths and one species of parasitic bivalve. However, many of Scotland's numerous population of salmonid fish have never been subjected to a parasitological survey. One aim of the project reported in this thesis was to expand our information of helminth parasites in Scottish salmonid fish populations by expanding the number of locations from which data are available (Chapter 3, 4, 6).

1.8 PARASITE INTERACTIONS

Competition between parasite species occurs whenever two or more species use the same resources and when those resources are in short supply (Pianka, 1983; Esch *et al.*, 1988). Competition can appear either as exploitation or interference. According to Halvorsen (1976) exploitation is uncommon among helminth parasites, occuring only when intestinal parasites compete for limited food resources. Interference occurs when two individuals or species are vying for the same resources and there is some form of direct confrontation or interaction that reduces access to the resource for one or both individuals or species. For example, Stock and Holmes (1987) reported that for the smaller cestode itself or a pathological changes in the intestine induced by the cestode in the intestine were responsible for affecting both species richness and linear distributions, especially species which absorb nutrients directly through their surfaces.

Patterns of association of larval trematodes examined by Esch *et al.* (1988) suggested that negative and random associations are most common although some positive association has been detected. Where negative interactions between larval trematode species were found in snails (Lie, 1966, 1973; Basch and Lie, 1968; Lie *et al.*, 1968; Lim and Heyneman, 1972; Heyneman *et al.*, 1972), it seems the rediae of many echinostome species feed on and kill rediae and sporocysts of subordinate species. This direct type of interference competition resulted in the elimination of prior infection of subordinate species from the host in which subsequent infections of subordinate species failed to establish. Basch *et al.* (1969) also reported interference competition between species with only a sporocyst stage, resulting in the elimination of a subordinate species by a dominant species. Lie (1969) showed that if an infection of the echinostome *Echinostoma audyi* is present in a snail then the establishment of *Hypoderaeum dingeri* is prevented.

In certain cases, prior infection with one species may predispose that host to infection by a second species, resulting in a positive association at least temporarily. For example, *Echinostoma liei* preferentially infects snails previously infected by *Schistosoma mansoni* (Heyneman *et al.*, 1972). Lie *et al.* (1976) proposed that suppression of host cellular defence mechanisms by *S. mansoni* accounts for the synergistic increased rate of establishment of echinostomes in previously infected snails. In this instance, *E. liei* is strongly dominant and *S. mansoni* is subsequently eliminated in a few weeks (Heyneman *et al.*, 1972).

Some helminth species are obligate secondary invaders. For example, *Austrobilharzia terrigallensis* can only infect snails harbouring a previous infection of another trematode species. Such species are likely to be found in double

infections and would therefore be most prevalent where parasitism by other trematodes is common. It can be speculated that perhaps one species improves either the establishment or survival of the other species, but more information on this subject is needed. One aim of the study described in this thesis is to examine patterns of associations among helminth species in Scottish salmonid fishes (Chapter 3).

1.9 HOST-PARASITE INTERACTIONS: EFFECTS OF PARASITES ON THEIR HOSTS

The parasitic helminths (nematodes, cestodes, trematodes and acanthocephalans) are known as macroparasites. When compared with microparasites (the protozoans, viruses and bacteria), macroparasites are relatively large, have long generation times and are immunologically characterised by a greater diversity of antigens.

As discussed by Gulland (1995), although parasitism may be associated with morbidity and mortality of the host, the general belief has been that well adapted parasites do little harm to their hosts, so as to prevent their own elimination. This view has been reconsidered because theoretical studies have indicated that many co-evolutionary pathways may be followed depending upon the relationships between parasite pathogenicity and transmission efficiency (Anderson and May, 1982; May and Anderson, 1990; Dobson and Merenlender, 1991; Toft and Aeschlimann, 1991). Thus, although the literature reports individual mortality in wild animals caused by infections, these have been interpreted as resulting from an imbalance in the natural host-parasite interaction. Such imbalances may result from the introduction of an infectious agent into a wildlife population by humans.

Many parasites damage their hosts at least for some of the time during an infection, and much attention has been given to the physical, biochemical and cellular mechanisms by which organisms damage their hosts (Mackenzie *et al.*, 1987). A parasite inhabiting host tissue may exert pathological effects, for example by interfering with the normal function of the tissue. Infection of dace, *Leuciscus leuciscus,* with metacercariae of the eye fluke, *Diplostomum spathaceum,* results in impaired visual acuity and the extent of impairment is correlated with the intensity of infection (Crowden and Broom 1980). *Diplostomum* metacercariae located in the lens of rainbow trout have been shown to induce cataracts, to affect growth of the host and even cause mortality of smaller fish (Sharriff *et al.*, 1980). Parasites commonly reduce the nutritional levels of their host. For example, the intestinal tapeworm *Diphyllobothrium latum* can selectively absorb vitamin B_{12} and so induce a condition in humans similar to pernicious anaemia (van Bonsdorff, 1977). A nutritional deficit can be imposed by parasites found in many organs, but noticeable effects are exerted primarily by parasites in the body cavity (when the parasites are large in comparison with the size of the host), in the digestive tract (the nutrient acquiring system) or in the pulmonary or circulatory system (the oxygen acquiring and distributing system). In parasitism, there must be a phase in the life history when all the nutrients are obtained either directly from the host's food and digestion products or more commonly from assimilated food substances obtained from the host's tissues and metabolites (Crompton, 1991). A parasitized host may induce a nutrient deficit by one or more of the following four mechanisms.

1. Parasites may cause a direct or indirect nutritional drain. Parasites may compete with the host for energy or nutrients, may damage host tissues thereby stimulating costly

repair responses or may otherwise stimulate energy and nutrient-requiring host defensive responses.

2. Parasites may effect the assimilation efficiency of the affected host by altering gastrointestinal functions such as absorption or gut motility.

3. Infected hosts may eat less food.

4. The acquisition and delivery of oxygen to the tissues can be impaired by parasites that are found in pulmonary tissue or in the circulatory system or that feed extensively on blood cells.

These mechanisms are not independent. The interactions among gastrointestinal functions, food intake, nutrient or energy reserves and immunological responses are complex and have recently attracted considerable attention (Castro, 1988; Symons, 1989). They involve both local events (in the gastrointestinal tract) and system events (humoral or central nervous system) (Mettrick and Podesta,1974; Blalock, 1989). Pathological changes in the host caused by parasites may be accompanied by altered host behaviour. For instance, infection of dace with *D. spathaceum* results in reduced visual ability and, consequently, reduced feeding efficiency, and also an increase in the use of surface water (Crowden and Broom, 1980). The fish may become more susceptible to the avian definitive host of the parasite and thus, increase the probability of parasite transmission. Acute pathogenicity from *Eubothrium* infection may occur simply from physical damage imposed on a host fish harbouring a particularly large parasite burden. In such cases the intestinal tract may become blocked, resulting in death of the host (Mitchell, 1993). For many host-parasite associations, the presence and nature of any effects of infection and host condition have not been studied in

details. One aim of this project is therefore to examine the effects of helminth infections on various aspects of body condition in their fish host (Chapters 3, 4, 6, 7).

1.10 PARASITISM AND AQUACULTURE

Because of deleterious effects such as those described above, parasites often cause serious outbreaks of disease in cultured fish populations. Although compared with the viral and bacterial diseases of fish, tapeworm-induced mortality is relatively of minor importance, there are some cestode species that can seriously affect cultured and wild fish populations (Hoole, 1994). The presence of dense populations of fish kept in particular environmental conditions may favour certain parasite species, with the result that the parasite population increases to a very high level. The numbers of parasites necessary to cause harm to a fish varies considerably with the species and size of the host and its health status. Individual parasite species may have widely differing effects on different host species. Parasites have also a different role in sparsely populated ponds with large surfaces and in intensive cage and enclosure rearing. Parasites may be important in reducing fish numbers by mortality, by reducing fecundity or by reducing the weight of individual fish (Molnar, 1987). A subsidiary aim of this study, arising opportunistically from a report of helminth infection in a population of farmed fish, was to investigate the effects of *Eubothrium crassum* on its salmonid host (Chapter 7).

1.11 CULTIVATION OF PSEUDOPHYLLIDEAN CESTODE SPECIES *IN VITRO*

To understand fully the causes of any association found between, for example, body condition and natural helminth infection, experimental studies are needed in

which infection of known intensity are induced into fish of known nutritional status and supply of eggs from the helminth concerned is obviously a pre-requisite for such experiments. This requires systems for either *in vivo* or eggs production.

Since *in vivo* experiments, although often successful, are not altogether practical in a laboratory situation and also raise ethical problems, effective *in vitro* systems have been aimed for over 60 years. Several species of pseudophyllidean cestodes have been investigated *in vitro*, including *Schistocephalus solidus*, *Ligula intestinalis* and *Diphyllobothrium dendriticum* (Smyth, 1958, 1959; Mason, 1965; Sinha, 1967; Taylor and Baker, 1968). All three species have fish-eating birds or mammals as their definitive hosts, with freshwater fish and copepodes acting as intermediate hosts. Smyth (1946), a leading figure in the development of *in vitro* systems for cestode cultivation, demonstrated that the plerocercoids of *S. solidus* could be maintained under aseptic conditions at room temperature for over 300 days. This remarkable feat illustrates an unswerving devotion to the maintenance of culture conditions facilitating the survival of the plerocercoids. It does not however reflect conditions that would be encountered naturally within the definitive host and no sexual maturation was achieved. Through many experiments aimed at growing cestodes *in vitro*, a picture was built up of the various stages passed through in the development from plerocercoid to fully mature and reproductive adult worm in a number of cestode species (Bell and Smyth, 1958; Smyth, 1946; Smyth, 1959). Although time scales between the different species, a seven-staged maturation process has been suggested (see Chapter 8; Bell and Smyth, 1958; Smyth, 1959). Despite this common pattern of development in pseudophyllidean cestodes, it should be noted that some species such as *S. solidus* and *L. intestinalis* have progenetic plerocercoids, that is, advanced development of the genitalia whilst still within the fish

host (Smyth, 1959, 1994). Therefore *S. solidus* proves a useful *in vitro* parasite, with eggs produced in just 48 hours (Sinha, 1967). Although maturation of both *S. solidus* and *L. intestinalis in vitro* has been successfully achieved by a number of people (Taylor and Baker, 1968; Sinha, 1967), such success has yet to be paralleled with *Diphyllobothrium dendriticum*. It is important to pursue this aim not only to increase the repertoire of species available to *in vitro* cultivation (thus removing live hosts from the laboratory), but also because of the implications this has for the study of *Diphyllobothrium latum*, a related species and parasite of humans. An aim of this thesis was to develop techniques for *in vitro* culture of *Diphyllobothrim dendriticum* to maturation.

1.12 SUMMARY OF AIMS

To reiterate, the aims of this study were therefore:

(1) To investigate the distribution, prevalence and intensity of helminth parasite species in Scottish salmonid fish populations (Chapters 3, 4, 6, 7).

(2) To study interactions within helminth communities and to assess the importance of trout in the persistence of the helminths associated with them (Chapter 3).

(3) To examine how the feeding specialisation of host fish species might influence the structure of helminth communities (Chapter 4).

(4) To investigate effects of helminth infections on various aspects of the body condition of the fish host (Chapters 3, 4, 6, 7).

(5) To examine the epidemiology of *Diphyllobothrium* spp. infection in a first intermediate host (Chapter 5) and a second intermediate host (Chapter 6) population in

Loch Lomond; with regard to describing the transmission ecology of the parasite in this system.

(6) To revise the methods for maintaining the life-cycle of *Diphyllobothrium* spp., which is one of the most common species with a complicated life cycle, in the laboratory condition and to examine growth and survival of plerocercoids *in vitro* (Chapter 8).

Chapter 2 General materials and methods

The studies reported in the following chapter involve some specialised techniques that are described in the relevant section of text. Some histological procedures, however, were used in several studies, and to avoid replication these are reported in this brief chapter.

2.1 METHODS FOR PARASITOLOGICAL STUDY

In a parasitological study the parasite or parasites must first be identified, their role as a cause of disease should then be considered and the life cycle must be elucidated. These steps enable control or eradication to be implemented (Cox, 1993). In general epidemiological studies are carried out in the following order.

1- Sampling for epidemiological survey.

2- Removing the parasite from fish during *post-mortem* examination.

3- Identifying the parasite.

4- Obtaining a thorough knowledge of the parasite's life history.

5- Studying the ecological requirements of the parasite, including its host specificity, optimum temperature, pH, nutrition and metabolic requirements.

6- Mapping the geographic range of the parasite.

7- Determining the effect of the immunologic mechanisms of the host to the parasite.

8- Studying the control and treatment methods.

2.2 SOME FIXATION AND STAINING TECHNIQUES IN PARASITOLOGY

In order to store the helminths and also for further investigation specimens were fixed in AFA (alcohol-formalin-acetic acid). Malzacher's staining technique was used when difficulty with identifying worms was encountered (Pritchard and Kruse, 1982). AFA solution is excellent for general work since it acts as a good fixing agent and has the additional advantage that the specimens may be allowed to remain in it for some time without damage or hardening.

Formalin	6 parts
EtOH	50 parts
Glacial acetic acid	4 parts
Distilled water	40 parts

2.2.1 Malzacher's staining

Whole mounts of cestodes and trematodes stain particularly well with this method. It is used Borax-carmine stain and Astra blue stain to differentiate the various organs sharply.

Astra blue stain	
Astra blue	1g
Tartaric acid	3g
Distilled water	100ml
Borax-carmine stain	
Carmine	3g
Borax	4g

mix ingredients and boil for about 30 minutes or until carmine is dissolved. Cool and add

EtOH (100) ml allow to stand 1-2 days and filter.

2.2.1.1 Staining procedure

Transfer specimens from fixative into borax-carmine for 15 minutes. Transfer to distilled water until the parenchyma looks pale. Transfer to astra blue stain for a few (3-5) minutes. Wash in several changes of distilled water until the rinse is clear. Dehydrate (starting with 70 % EtOH), clear and mount.

2.2.2 Staining of histological slides using Haematoxylin and Eosin

Slides were processed using the following protocol.

Histoclear no. 1 for 5 minutes.

Histoclear no. 2 for 5 minutes.

Absolute alcohol for 3 minutes, twice.

Rehydrate through graded alcohols, i.e. 90 %, 70 %, 50 % and 30 % each for 3 minutes.

Wash in running tap water.

Haematoxylin for 1.5 to 2 minutes.

Wash in running tap water.

Acid alcohol for 10 seconds.

Wash in running tap water.

Scott's tap water for 10 seconds.

Wash in running water.

Eosin for 1.5 to 2 minutes.

Wash in running tap water.

Dehydrate through graded alcohols (30 % through to absolute) for 1 minute in each.

Histoclear no. 2 for 3 minutes.

Histoclear no. 3 for 3 minutes.

Specimens were then mounted using Histomount. Structures differentially stained were as follows. Nuclear chromatin-blue to purple; cytoplasm, collagen, keratin and erythrocytes-pink.

Chapter 3 The ecology of endoparasitic helminth infections of brown trout, *Salmo trutta*, and rainbow trout, *Oncorhynchus mykiss*, in Scotland

3.1 INTRODUCTION

Earlier studies of parasitic infections of trout in the British Isles have focused on morphological and taxonomic aspects of the different types of parasite, with particular attention being paid to those species associated with disease in their fish host (Duguid and Shepard, 1944; Hickey and Harris, 1947; Wootten, 1973; Betterton, 1974). More recently, emphasis has been placed on the circumstances which may have influenced the origins and evolution of trout-parasite relationships. For example, Kennedy (1978) noted that the diversity of parasite species harboured by the trout living in various lakes increased in relation to increasing surface area. He concluded that large lakes contained greater habitat diversity than small lakes and so harboured a greater variety of invertebrate hosts of parasites dependent on indirect life-history patterns. These ideas were developed further by Kennedy *et al.* (1991) and Hartvigsen and Kennedy (1993) in a consideration of the role of habitat in determining the diversity of trout parasites. The trophic status of lakes has also been related to the complexity of fish parasite-parasite faunas (Wisniewski, 1958; Esch, 1971; Chubb, 1979, 1980, 1982).

Few surveys have been carried out on the regional distribution of helminth parasites of fish in Central Scotland, an area with abundant and varied bodies of freshwater richly stocked with brown trout, *Salmo trutta,* and rainbow trout, *Onchorhynchus mykiss*. The oldest of these water bodies dates from about 15000 years BP when Scotland was being released from the grip of the Ice Age (Ratcliffe, 1977). One such survey was carried out by Copland (1957), who investigated the parasite fauna of fish, including trout, in Loch Lomond and another by Bwathondi (1984) who

identified thirteen species of helminths from a sample of 402 brown trout caught in Strangbeg.

3.2 AIMS

The aims of the investigation described in this chapter were to study the distribution and abundance of species of endoparasitic helminths in Scottish brown and rainbow trout, with emphasis on (1) the intensity of the infections, (2) interspecific associations between helminth species in the communities and (3) the status of the trout in the helminths' life cycle. The reasons for conducting the investigation were threefold. First, recent theory has indicated that for macroparasites intensity is the variable that exerts most influence over the regulation of host-macroparasite interactions (Anderson and Gordon, 1982), including parasite-induced morbidity and mortality. Secondly, much effort is being made to identify the processes that generate and stabilize communities of helminth species in fish (Esch *et al.*, 1990). Thirdly, it is of interest to determine the importance of trout in the survival of the species of helminth found in association with them.

3.3 MATERIALS AND METHODS

A total of 289 trout (240 brown trout, *Salmo trutta* L., 1758, and 49 rainbow trout, *Oncorhynchus mykiss*, (Walbaum, 1792)) were obtained from 21 locations in Central Scotland (Table 3.1) between October 1990 and August 1993. Some were caught with gill nets or hand nets and some were acquired from sport fishermen. Lengths and weights of fish were recorded and sex was determined. Many of the fish were examined for the presence of endoparasitic helminths immediately after capture,

but some were frozen at -20 °C and then thawed before examination. Fish eyes, gills, stomach, intestine, pyloric caeca, liver, pancreas, swim bladder, spleen and kidneys were examined. All helminths recovered were identified by reference initially to publications by Chubb *et al.* (1987), Kennedy (1974) and Brown *et al.* (1986) and the number of helminths per fish was counted. If helminths could not be identified quickly, they were fixed in AFA (Pritchard and Kruse, 1982), stained by using Mazacher's staining technique (see Chapter 2) and mounted before taxonomic keys were consulted. Routine statistical procedures were used for investigation of the data collected during the survey, provided that samples of at least 18 trout had been studied from each location. The customary method of investigating associations, by means of the log-odds ratios for 2x2 contingency tables, was hampered somewhat by very low prevalences of some of the parasites. In order to accommodate the systematic effect of location, a GLIM analysis was carried out which included "Location" in the model. Thus the results would summarise the association by an average value (over locations) of the log-odds ratio. This metric takes a value zero for complete independence with positive and negative values respectively denoting positive and negative associations.

A total of 6192 helminths representing 10 species from 10 genera (Table 3.1) were obtained from the 289 fish examined during the survey. The specific identity of the nematode placed in the genus *Eustrongylides* (Table 3.1) remains problematic (Kennedy and Lie,1976).

Table 3.1 The species of endoparasitic helminths found in *Salmo trutta* (brown trout) and *Oncorhynchus mykiss* (rainbow trout) from freshwater bodies in Central Scotland.

	Species status	Host Status	Microhabitat
Phylum PLATYHELMINTHES			
Class Trematoda			
Order Digenea			
Crepidostomum farionis (Müller, 1784) Nicoll, 1909	AU	DH	Posterior intestine and pyloric caeca
Class Cestoda			
Order Spathebothriidea			
*Cyathocephalus truncatus** (Pallas, 1781) Kessler, 1868	AU	DH	Pyloric caeca
Order Pseudophyllidea			
Diphyllobothrium dendriticum (Nitzsch, 1824)	AL	IH_2	Body cavity
Diphyllobothrium ditremum (Creplin, 1825) Lühe, 1910	AL	IH_2	Body cavity
Eubothrium crassum (Bloch, 1779) Nybelin, 1922	AU	DH	Pyloric caeca and anterior intestine
Phylum ACANTHOCEPHALA			
Class Eoacanthocephala			
Neoechinorhynchus rutili (Müller, 1780) Stiles and Hassal, 1905	AU	DH	Intestine
Class Palaeacanthocephala			
Echinorhynchus truttae (Schrank, 1788) Petrochenco, 1956	AU	DH	Intestine
Phylum NEMATODA			
Order Dioctophymidea			
Eustrongylides sp.	AL	IH	Encysted in musculature or body cavity
Order Trichuridea			
*Capillaria salvelini** (Polyanskii, 1952)	AU	DH	Pancreas and posterior intestine
Order Ascarididea			
*Raphidascaris acus** (Bloch, 1779) Dyk and Lucky, 1954	AU	DH	Encysted in liver
Order Spiruridea			
*Cystidicola farionis** (Müller, 1784) Baylis, 1931	AU	DH	Swim bladder

Abbreviations: (*) not observed in rainbow trout during the course of this survey, (AU) autogenic species, (AL) allogenic species [see Esch *et al.* 1988], see Kennedy (1974) and Chubb (1979, 1980, 1982) for checklists of helminth infections in freshwater fishes, (DH) definitive host, (IH) intermediate host, (IH_2) second intermediate host.

3.4 RESULTS

3.4.1 Prevalence of helminth infections

Information about the number of trout examined and the prevalences of the 10 species of helminth are given in Table 3.2. Overall, *Crepidostomum farionis* was found to be the most widely distributed species of helminth; not every individual fluke was examined but all those inspected in detail were judged to belong to this species. *Diphyllobothrium* spp., *Echinorhynchus truttae, Eubothrium crassum*, and *Neoechinorhynchus rutili* were the most widely distributed, in that order, after *C. farionis* (Table 3.2).

3.4.2 Intensity of helminth infections

Intensities of infections (the number of worms per infected fish; Margolis *et al.*, 1982) are given in Table 3.3. Some heavy infections were recorded, for example, 339 plerocercoids of *Diphyllobothrium* spp. and 118 adult *N. rutili*, 105 adult *E. truttae* and 100 *C. farionis* in individual brown trout. It was noticeable that, where sufficient numbers of infected fish had been obtained (samples of 18 or more from the different locations; Table 3.2), the frequency distribution of numbers of worms per fish was overdispersed with variance: mean ratio $(s^2/\bar{x})>1$ (Anderson and Gordon, 1982). Although intensity of infection would be expected to influence host health and condition, direct observations made at the *post-mortem* examination of whole fish did not indicate that even individual fish with the highest worm burdens were experiencing any overt morbidity. Furthermore, there was no evidence of any statistical correlation between host weight and worm burden.

3.4.3 Association between helminth species

The number of helminth species found in individual brown trout from the seven of the locations from which 18 or more fish were caught are shown in Table 3.4. The largest number of species found per fish was four. In cases where certain species were often found together or where species appeared not to coexist in the same host, the question arises as to whether these distributions are generated randomly or depend on either synergistic or antagonistic associations. The results of the log-odds ratio analysis to investigate estimate of the significance of association between pairs of helminth species in the brown trout from the seven locations (Table 3.4) is given in tables 3.5 and 3.6. Clear evidence was obtained for a positive association between *N. rutili* and *C. farionis* ($P< 0.01$) in brown trout, regardless of their locations, and some evidence for a possible positive association between *Diphyllobothrium* spp. and *E. crassum* was also found (Table 3.5). It needs to be emphasised, that the sensitivity of investigations of this sort are influenced by the level of infection. However, it should be noted that the associated pairs of species of helminths depend for their transmission on benthic and pelagic intermediate hosts.

Table 3. 2 The prevalence (%) of endoparasitic helminth infections in *Salmo trutta* (brown trout) and *Oncorhynchus mykiss* (rainbow trout) in Central Scotland.

Location	n	A	B	C	D	E	F	G	H	J	K
Loch Lomond (NS3597)	8	-	-	12.5	25	37.5	-	-	12.5	-	-
Loch Maragan (NN4027)	35	74.2	-	68.5	45.7	2.8	-	-	14.2	2.8	-
River Fillan (NN3726)	18	33.3	5.5	22.2	-	5.5	-	-	-	-	-
Aurs Burn	10	10	90	40	-	10	-	-	-	-	20
Carbeth Reservoir (NS5379)	20	15	15	10	10	10		-	-	-	-
Loch Awe (NN0722)	20	-	-	10	100	65	-	-	5	-	-
	4*	-	-	-	75	25	-	-	-	-	-
Dunalastair Reservoir (NN7158)	15	-	26.6	33.3	60	66.6	6.6	-	-	-	-
Jaw Loch (NS4975)	10	50	-	50		10	30	-	-	10	-
Cochno Loch (NS4976)	3	100	-	33.3	-	33.3	-	-	-	-	-
Loch Leven (NN0960)	3	-	-	-	33.3	33.3	-	-	-	-	-
	2*	-	-	-	-	-	-	-	-	-	-
Secret Loch	1	+	-	+	-	-	+	-	-	+	-
	1*	+	-	-	-	-	-	-	-	-	-
Hill Loch (NS5647)	7	-	-	-	57	-	-	-	-	-	-
	1*	-	-	-	-	-	-	+	-	-	-
Loch Rannoch (NN5957)	19	-	57.8	15.7	57.8	63.2	-	5.2	-	-	-
	7*	14.2	71.4	14.2	42.8	100	-	-	-	-	-
Burncrooks (NS4879)	4	25	-	25	25	25	-	-	-	-	-
Talla Reservoir (NT1121)	15	-	93.3	40	26.6	33.3	33.3	20	6.6	-	
Loch Venachar (NN5705)	2	-	-	50	50	100	-	-	-	-	-
Loch Rusky (NN6103)	13*	30.7	-	15.3	-	15.3	-	7.6	-	-	-
Carron Valley Reservoir (NS6983)	22	-	18.2	13.6	13.6	-	-	4.5			
Whiteadder Reservoir (NT6563)	19	10.5	78.9	36.8	15.7	5.2	-	5.2	-	-	36.8
Fruid Reservoir (NT0919)	9	-	88.8	55.5	22.2	11.1	-	33.3	-	-	
Lake of Menteith (NN5700)	21*	-	-	-	33.3	-	-	-	-	-	-

Abbreviations: n= Number of fish examined. Percentage of infected fish with (A) *Neoechinorhynchus rutili*, (B) *Echinorhynchus truttae*, (C) *Crepidostomum farionis*, (D) *Diphyllobothrium* spp., (E) *Eubothrium crassum*, (F) *Cyathocephalus truncatus*, (G) *Eustrongylides* sp., (H) *Capillaria salvelini*, (J) *Raphidascaris acus*, (K) *Cystidicola farionis*. (*) rainbow trout, (+) infected, (-) no worms found. Grid references taken from the *Ordnance Survey Gazetteer of Great Britain*, third edition (1992).

Table 3.3 Intensity of endoparasitic helminth infections in *Salmo trutta* (brown trout) and *Oncorhynchus mykiss* (rainbow trout) in Central Scotland.

Location	n	A		B		C		D		E		F		G		H		J		K	
		$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$	$\bar{x}$ ±SD	$s^2/\bar{x}$
Loch Lomond	8	-	-	-	-	1	-	2.0±1.4	0.9	1.6±1.1	0.7	-	-	-	-	1	-	-	-	-	-
Loch Maragan	35	21.3±40.4	77.0	-	-	45.8±30.5	20.3	27.1±34.6	44.1	2	-	-	-	-	-	1.6±0.8	0.4	1	-	-	-
River Fillan	18	3.6±2.8	2.1	30	-	10.5±11.2	11.9	-	-	13	-	-	-	-	-	-	-	-	-	-	-
Aurs Burn	10	1	-	2.3±1.3	0.7	4.2±3.3	2.5	-	-	1	-	-	-	-	-	-	-	-	-	1	-
Carbeth Reservoir	20	2.6±1.5	0.8	7.6±5.5	3.9	4.0±1.4	0.5	1.5±0.7	0.3	8.0±9.9	12.2	-	-	-	-	-	-	-	-	-	-
Loch Awe	20	-	-	-	-	5.0±4.2	3.5	23.8±12.5	6.5	3.3±2.9	2.5	-	-	-	-	1	-	-	-	-	-
	4*	-	-	-	-	-	-	5.0±3.6	2.5	1	-	-	-	-	-	-	-	-	-	-	-
Dunalastair Reservoir	15	-	-	6.0±8.0	10.6	26.2±27.4	28.6	18.7±14.4	11.0	2.9±3.3	3.7	1	-	-	-	-	-	-	-	-	-
Jaw Loch	10	28.4±50.4	89	-	-	10.3±13.9	18.7	-	-	1	-	2.0±1.0	0.5	-	-	-	-	3	-	-	-
Cochno Loch	3	12.0±9.4	7.3	-	-	3	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Loch Leven	3	-	-	-	-	-	-	4	-	1	-	-	-	-	-	-	-	-	-	-	-
	2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Secret Loch	1	336	-	-	-	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1*	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-
Hill Loch	7	-	-	-	-	-	-	68.2±90.1	119	-	-	-	-	-	-	-	-	-	-	-	-
	1*	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Loch Rannoch	19	-	-	5.0±7.2	10.3	2.3±1.1	0.5	8.0±13.2	21.7	3.5±2.6	1.9	-	-	1	-	-	-	-	-	-	-
	7*	-	-	11.6±9.9	8.4	4	-	20.6±12.6	7.7	3.8±2.7	1.9	-	-	-	-	-	-	-	-	-	-
Burncrooks	4	-	-	-	-	2	-	1	-	5	-	-	-	-	-	-	-	-	-	-	-
Talla Reservoir	15	-	-	31.0±34.0	37.2	1.8±1.3	0.9	4.2±2.9	2.0	1.2±0.4	0.1	2.4±1.6	1.0	3.0±2.6	2.2	6	-	-	-	-	-
Loch Venachar	2	-	-	-	-	2	-	2	-	4.0±0.0	-	-	-	-	-	-	-	-	-	-	-
Loch Rusky	13*	2.7±2.8	2.9	-	-	3.0±2.8	2.6	-	-	1.5±0.7	0.3	-	-	3	-	-	-	-	-	-	-
Carron Valley Res.	22	-	-	1.2±0.5	0.2	9.6±10.0	10.4	2.3±2.3	2.3	-	-	-	-	1	-	-	-	-	-	-	-
Whiteadder Reservoir	19	1	-	11.3±13.1	15.2	11.5±9.9	8.5	11.3±17.0	25.4	5	-	-	-	1	-	-	-	-	-	5.4±3.1	1.7
Fruid Reservoir	9	-	-	12.2±10.9	9.7	6.6±5.9	5.2	1.0±0.0	-	2	-	-	-	2.6±2.8	3.0	-	-	-	-	-	-

Abbreviations: n= Number of fish examined. Infection with (A) *Neoechinorhynchus rutili*, (B) *Echinorhynchus truttae*, (C) *Crepidostomum farionis*, (D) *Diphyllobothrium* spp., (E) *Eubothrium crassum*, (F) *Cyatocephalus truncatus*, (G) *Eustrongylides* sp, (H) *Capillaria salvelini*, (J) *Raphidascaris acus* and (K) *Cystidicola farionis*. (*) rainbow trout, (-) no worms found.

Table 3.4 The number of species of helminths found in individual *Salmo trutta* (brown trout) from seven locations in Central Scotland.

Location	n	0	1	2	3	4
Loch Maragan	35	1	7	16	10	1
River Fillan	18	10	4	4	-	-
Carbeth Reservoir	20	11	6	3	-	-
Loch Awe	20	-	7	10	3	
Loch Rannoch	19	2	5	5	5	2
Carron Valley Reservoir	22	12	9	1	-	-
Whiteadder Reservoir	19	3	4	5	6	1

(n) Number of fish examined, (0) number of fish with no infection, (1) number of fish with one infection and so on.

Table 3.5 The results of an investigation of associations between pairs of helminth species in *Salmo trutta* (brown trout) from seven locations in Central Scotland.

Species pairs	Log odds ratio (±SE)	Odds ratio	Result
A/B	0.75+/-1.08	2.12	
A/C	1.16+/-0.58	4.99	$P < 0.01$
A/D	-1.30+/-0.78	0.27	
A/E	-0.74+/-1.31	0.47	
B/C	1.24+/-0.70	3.46	
B/D	0.27+/-0.69	0.76	
B/E	-0.75+/-0.85	0.47	
C/D	0.16+/-0.53	1.18	
C/E	0.78+/-0.79	2.19	
D/E	1.32+/-0.77	3.75	$P < 0.10$

(A) *Neoechinorhynchus rutili*, (B) *Echinorhynchus truttae*, (C) *Crepidostomum farionis*, (D) *Diphyllobothrium* spp, and (E) *Eubothrium crassum*.
Note: positive association between pairs of species is denoted by a positive value for the log odds ratio and a negative association by a negative value.

Table 3.6 Characteristics of helminth communities in *Salmo trutta* (brown trout) from seven locations in Central Scotland.

	Locations						
Characteristics	LM	RF	CR	LA	LR	CVR	WR
Number of fish examined	35	18	20	20	19	22	19
Total no. of helminth species	6	4	5	4	5	4	7
No. of autogenic species	5	4	4	3	3	2	5
No. of allogenic species	1	0	1	1	2	2	2
Total No. of helminth individuals	2076	107	58	502	193	42	331
Proportion of autogenic individuals	0.80	1.0	0.95	0.11	0.54	0.81	0.89
Proportion of allogenic individuals	0.20	0.0	0.05	0.89	0.46	0.19	0.11
Berger-Parker dominance index	0.52	0.39	0.39	0.89	0.45	0.69	0.51
Character of dominant species	AU	AU	AU	AL	AL	AU	AU
Identity of dominant species	Cre	Cre	Ech	Dip	Dip	Cre	Ech
Mean % similarity between indiv. fish (AU spp.)	39.5	7.5	3.3	25.7	18.1	3.9	33.6
Mean % similarity between indiv. fish (AL spp.)	20.2	0.0	0.5	100	13.8	1.3	1.8

Abbreviations: AU, autogenic species; AL, allogenic species; Cre *Crepidostomum farionis*; Ech, *Echinorhynchus truttae*; Dip, *Diphyllobothrium* spp.; LM, Loch Maragan; RF, River Fillan; CR, Carbeth Reservoir; LA, Loch Awe; LR, Loch Rannoch; CVR, Carron Valley Reservoir; WR, Whiteadder Reservoir.

intensity of the infection within the farmed salmon population was 37.2 % and 1.34 respectively, to be weakly overdispersed ($s^2/\bar{x}$ = 1.36). It was though that salmon parr may acquire infection when they were stocked in freshwater. Although negative effects of infection on host condition and gonadosomatic index were observed, there was no evidence to suggest influence of infection on hepatosomatic index and percentage body fat of host fish.

A series of experiments was conducted to obtain *Diphyllobothrium dendriticum* eggs for experimental infections in the laboratory conditions. Since infection of *Diphyllobothrium dendriticum* could not be established in either chickens or domestic ducks by feeding them with plerocercoids, an attempt was made to cultivate pseudophyllidean cestodes *in vitro*. Plerocercoids of *Diphyllobothrium dendriticum*, (10-30 mm long) were recovered from the previsceral cavity of powan and kept *in vitro* in an artificial medium. In a number of trials, worms survived for a number of days, and showed significant growth. However, there was no evidence of maturation. Additions of amino acids and vitamins to the medium had no effect on enhancing maturation. Plerocercoids of *Schistocephalus solidus* were also cultured *in vitro* in medium containing homogenised duck embryos and Tyrode's Solution plus supplements (glucose and vitamin B_{12}). These worms showed full development into sexually mature adults and eggs were obtained. Further work will be required to complete the maturation of *Diphyllobothrium dendriticum in vitro*, but this achievement would greatly facilitate further research.

These various results are discussed in the general context of the ecology of helminth infections in fish.

3.5 DISCUSSION

The results from the current survey have added significantly to knowledge of the distribution and abundance of endoparasitic helminths infections in trout in Scotland (see Copland, 1957; Bwathondi, 1984). None of the species of helminths observed in the trout is a new host record, but it is only recently that *C. farionis* has been found in rainbow trout (Kennedy *et al.*, 1991). Nor was evidence obtained to suggest that any of the infections was associated with significant morbidity or poor condition of their fish hosts. The most interesting aspect of the study is the interpretation of the results in terms of helminth communities in freshwater fish in Great Britain (Esch *et al.*, 1988). The results undoubtedly show considerable variation in the helminth communities from the trout sampled in reasonable numbers from seven of the 21 locations studied (Table 3.4). Kennedy (1990) pointed out that such variation remains largely unexplained, although after an analysis of several data sets, Esch *et al.* (1988) were able to suggest a range of ecological processes that could account for the observed diversity. One of the data sets investigated by Esch *et al.* (1988) dealt with brown trout taken from 9 locations in the British Isles. Two of these locations were from Scotland; three species of helminth were found in 20 trout from Loch Long and nine species were recovered from as few as 13 trout from Loch Dunalastair. In Table 1 of their paper Esch *et al.* (1988) describe the characteristics of the helminth communities in the populations of trout they studied. A similar description is presented in Table 3.6 for the trout sampled from seven locations during our survey. Overall Scottish trout were infected dominantly by autogenic helminth species (Table 3.6). Eight out of 10 species recovered were autogenic. A similar observation was also made by Esch *et al.* (1988). *Crepidostomum farionis* was the dominant species in the helminth communities in brown trout in 3 of

the seven locations and *E. truttae* and *Diphyllobothrium* spp. in two each. There was little similarity between these results and those obtained by Esch *et al.* (1988), indicating the importance of the trout habitats in determining the structure of helminth communities (Kennedy, 1978b; Chubb, 1980, 1982). The concept of the compatibility and encounter filters proposed by Combes (1991) also helps to explain how helminth communities might vary between populations of the same host species living in a relatively small geographical area. The physiological and immunological properties of the trout will determine whether helminths will survive in them and little variation is to be expected in this aspect of the trout-helminth relationships. The infective stages of compatible helminth species that trout actually encounter, however, will depend particularly on the role of the water body in the ecology of intermediate hosts and vectors.

Within helminth communities evidence of only one interaction was found between helminth species, namely the positive association between *C. farionis* and *N. rutili*. Perhaps one species improves either the establishment or survival of the other. The fact that both species are restricted to the gut throughout their time in trout indicates that some effect of the host's immune response may not be involved. This association and other aspects of *C. farionis* in trout (Table 3.6) merit further investigation.

3.6 SUMMARY

1. A total of 6192 helminths representing 10 species belonging to 10 genera were recovered from the 289 trout (240 brown trout and 49 rainbow trout) obtained from 21 locations in Scotland.

2. *Crepidostomum farionis* (Digenea) was the most widely distributed helminth species in trout from Central Scotland, followed by *Eubothrium crassum* (Cestoda), *Diphyllobothrium dendriticum* and *D. ditremum* (Cestoda), *Neoechinorhynchus rutili* and *Echinorhynchus truttae* (Acanthocephala), *Eustrongylides* sp., *Capillaria salvelini* (Nematoda), *Cyathocephalus truncatus* (Cestoda), *Raphidascarus acus* and *Cystidicola farionis* (Nematoda), in that order.

3. The frequency distribution of numbers of worms per fish was overdispersed.

4. The largest number of helminth species found per fish was four (recorded in Loch Rannoch, Loch Maragan and Whiteadder Reservoir).

5. No evidence was found to indicate that even fish with the highest worm burdens (e.g. 339 plerocercoids of *Diphyllobothrium* spp.) were experiencing any obvious morbidity.

6. An analysis of pairs of associations between species of helminths revealed a significantly positive association between *N. rutili* and *C. farionis*.

7. There was no clear evidence to report competition between helminth species.

8. Autogenic species were generally the dominant element and responsible for most of the similarity within and between localities.

Chapter 4 Endoparasitic helminth infections of sympatric Arctic charr, *Salvelinus alpinus*, populations from Loch Rannoch: their use as biological tags to distinguish the morphs.

4.1 INTRODUCTION

Arctic charr, *Salvelinus alpinus* are disparate in many aspects of their biology including patterns of parasitic infection which is influenced by their ecological diversity. In a very recent study, Due and Curtis (1995) examined parasites of freshwater resident and anadromous Arctic charr, *Salvelinus alpinus* in Greenland and found 21 metazoan parasite species. In general, the quantitative aspects of the parasite communities varied according to sampling sites which can be related to ecological differences, but those parasite species associated with freshwater hosts (*Diphyllobothrium ditremum*, *Eubothrium salvelini*, *Proteocephalus longicollis* (Cestoda), *Crepidostomum farionis* (Digenea)) were usually numerically dominant even in anadromous fish. They also noted that fish from various sites in the same area were feeding on different prey species. It was concluded that the absence of some intermediate hosts like Mysidacea, Amphipoda, Ephemeroptera and Odonata from Greenland excluded some parasites including the cestode *Cyathocephalus truncatus*, nematodes of the genus *Cystidicola* and freshwater acanthocephalans.

4.1.1 Parasites as an indicator of host biology

Where they are host-specific, parasites can be used as biological indicators or natural markers to provide information on various aspects of host biology (MacKenzie, 1987). It is possible to use parasites as biological tags to identify separate stocks of fish

(Sinderman, 1961; Margolis, 1963; Pippy, 1969; Hare and Burt, 1976; Plat, 1976; Beverley-Burton and Pippy, 1978; Kennedy, 1978a; Dick and Belosevic, 1981; Boillon and Dempson, 1988). The purpose of stock separation studies is to identify subgroups within a host population. Such stocks may differ in various aspects of their life history such as having different spawning grounds or feeding areas and fisheries biologists need to know about this for management purposes. There are other well known examples of the use of parasites to separate stocks of Pacific salmon, *Oncorhynchus* spp., (Moser, 1991). Dick and Belosevic (1981) studied parasites of Arctic charr and used the results to separate anadromous and non-migrant charr. They recovered 9 species of parasites from non-migrating charr and 15 from sea-run charr. *Diphyllobothrium* spp., *Eubothrium salvelini* and *Proteocephalus longicollis* were chosen as good indicators of non-migrating charr by Dick and Belosevic (1981) as prevalence and intensity were high and dominance values constituted the majority of parasites in the system. *Brachyphallus crenatus*, *Brachyphallus sturionis* and *Prosorhynchus squamatus* were chosen as good indicators of anadromous charr because prevalence and intensity were high, their combined dominance values constituted the majority of parasites in those charr and they are marine and estuarian parasites.

Parasites have also been used to determine migration routes of juvenile fish and seasonal migration patterns of adult fish (Black, 1981; MacKenzie, 1987; Bouillon and Dempson, 1988). Black (1981) investigated metazoan parasites as indicators of seaward movements of anadromous brook charr and found that the marine trematode, *Brachyphallus crenatus* remained in brook charr, *Salvelinus fontinalis* during their movements into freshwater. Differences in the prevalence of this parasite between sampling localities in late July indicated that some charr, which undergo smoltification,

do not in fact go to sea in contrast to others. On this basis, infection with *Brachyphallus crenatus* enabled Black to make an estimate of the percentage of charr that had been to sea entering a tributary in late August.

A few studies have concentrated on the use of parasites in separating sympatric species of fish. Davis and Huffman (1977) studied the ecological differences between *Gambusia affinis* and *Gambusia geigeri* and Cloutman (1976) studied *Campostoma anomallum pullum* and *Campostoma oligolepis*. *Crasiphiala bulboglossa* (Digenea) was found to be significantly more abundant in *C. a. pullum* than in *C. oligolepis* suggesting that the two stoneroller forms were distinct species.

4.1.2 Determinants of parasite transmission

For a parasitic infection to occur both parasite and host must have been present at the same place and at the same time. Analysis of host-parasite associations accordingly shows that animals with comparable food habitats tend to have similar kinds of parasites and that related hosts tend to have related parasites (Cameron, 1964). Parasites are adapted to the ecology of their hosts and if this remains stable the population gaining successful admission will also remains stable. If the host's ecology is altered markedly, the parasite population may become abnormally large and disease, due to excessive numbers, may result. Since many of the fish parasites are transmitted through the food web by infected invertebrates or forage fish, certain of the observed differences probably reflect local variations in habitat condition, prey availability and fish feeding behaviour. Often fish from different sites in the same area feed on different prey species. Many invertebrate food items serve as intermediate host for parasites (Due and Curtis, 1995). Due and Curtis (1995) noted that the local differences in prevalence

and abundance of freshwater Arctic charr parasites were sometimes clearly related to the feeding patterns of the fish. Arctic charr feeding on chronomids were considered to be free of metazoan parasites, but those eating copepods were infected with cestodes. In a very recent study, analyses of statistical associations between the stomach contents and endoparasites of Arctic charr, from a small lake in northern Quebec showed that food items found in fish stomachs at the time of capture frequently consisted of intermediate hosts for the parasites infecting the fish (Curtis *et al.*, 1995). In the same study, the stomach contents of Arctic charr infected by *Diphyllobothrium ditremum, D. dendriticum* and *Eubothrium salvelini* tended to include copepods, while fish infected by the digenean *Crepidostomum farionis* more frequently contained insect larvae (ephemeropterans) and fish infected by the acanthocephalan *Echinorhynchus lateralis* most often had amphipods in their stomach.

In fish-helminth parasite interactions, the principal determinants of parasite transmission efficiency are mostly considered to be feeding and habitat biology of the fish (Kennedy, 1975; Frandsen *et al.*, 1989). Price and Clancy (1983) used the key and checklist by Maitland (1972) and Kennedy (1974) respectively to test predictions on host feeding preferences influencing the accumulation of parasites. For example, the top predator *Esox lucius* had a richer parasite fauna than *Phoxinus phoxinus* which feed on algae and invertebrates and in turns is likely to be fed upon by pike. Valtonen (1979) confirmed, from observations on *Neoechinorhynchus rutili* in *Coregonus nasus* in the Bay of Bothnia that the pattern of infection could be explained by fish feeding on ostracods. An increase in the number of *Echinorhynchus salmonis* in *Coregonus lavaretus* over three years could be correlated with feeding habits (Valtonen, 1983). Aspects of the ecology of *Neoechinorhynchus rutili* were discussed by Walkey (1967)

and Valtonen (1979). Walkey (1967) observed a distinct correlation between ostracods in the diet and infection of *Gasterosteus aculeatus* with *Neoechinorhynchus rutili*. Most parasites species occur in a limited number of invertebrate hosts (Kennedy, 1975). Most fish specialise on limited number of invertebrate prey, so the range of parasites to which fish are exposed is inevitably limited. Sympatric Arctic charr morphs tend to segregate in choice of food and habitat use (Nilsson and Filipsson, 1971; Klementsen and Grotnes, 1980; Hindar and Jonsson, 1982; Frandsen *et al.*, 1989). Thus, the helminth parasitism in Arctic charr from Loch Rannoch may be expected to differ between morphs.

4.1.3 General biology of Arctic charr

The Arctic charr has a northern circumpolar distribution and anadromous populations are found in the northernmost part of the geographical range of the species e.g. Canada, Greenland, Iceland and northern Norway. Non-migratory and land-locked populations are found both in these northern areas and in parts of North America and Europe further to the south. Charr exist quite widely in Ireland and northern Scotland and very locally in south-west Scotland, north-west England and north Wales. Charr occur in quite large numbers in some lakes, probably outnumbering the trout population where they co-exist. Some water bodies have been found to contain charr of several different distinct ecotypes or morphs (Johnson, 1981; Maitland, 1992; Sandlund *et al.*, 1992).

4.1.4 Classification of Arctic charr

Phylum	**Chordata**
Subphylum	**Vertebrata**
Class	**Pisces**
Subclass	**Crossopterygii**
Superorder	**Telostei**
Order	**Isospondyli**
Suborder	**Salmonidei**
Family	**Salmonidae**
Genus	***Salvelinus***
Species	***alpinus*** (Linnaeus, 1758)

4.1.5 Feeding polymorphism in Arctic charr

The nature of most parasite fauna is directly related to the type of food ingested by the host (Dogiel, 1962). In an aquatic ecosystem undergoing change, parallel changes should be expected in those parasite fauna related to potential intermediate and definitive hosts. The nature of predator-prey relationships should serve therefore, as a potential biological index for predicting the structure of the parasite fauna in any given aquatic ecosystem. The most significant selection force influencing the parasite fauna in each lake operates via a characteristically structured predator-prey interaction (Esch, 1971).

The feeding habits and habitat use of Arctic charr are variable (Hegge *et al.*, 1989; Hindar and Jonsson, 1992; Malmquist *et al.*, 1992; Sandlund *et al.*, 1992; Henning *et al.*, 1993). However, the general trend seems to be that they exploit littoral areas and feed on littoral zoobenthos and crustacean zooplankton (Giovinazzo, 1989; Langeland *et al.*, 1991) in situations of allopatry, whereas they are more confined to deep epibenthic and pelagic areas (feeding mainly on profundal zoobenthos and crustacean zooplankton) when they are in sympatry with other species particularly trout

(Hindar and Jonsson, 1982; Henning *et al.*, 1993). The sympatric morphs of Arctic charr in Loch Rannoch also use different habitats and show variation in their feeding habits. Pelagic charr feed in the surface water mainly on zooplankton while benthics live in the deeper water and feed on a wide range of benthic invertebrates (see Table 4.1 and Figure 4.2) (Walker *et al.*, 1988; Adams, pers. comn.).

Arctic charr, *Salvelinus alpinus*, in Scandinavia, Iceland and British Isles are polymorphic and occur as several types, each differing from the other with regard to genetics, spawning habitat, morphology and feeding behaviour (Walker *et al.*, 1988; Frandsen *et al.*, 1989; Curtis *et al.*, 1995).

Charr populations isolated from each other tend to develop distinguishing features (biological and morphological) such as to indicate some degree of genetic divergence. This suggests that, there is a little or no interbreeding between distinct types, but if the physical barriers to interbreeding were to be removed it would be expected to take place. The commonest types of barrier are geographical and ecological ones. The different forms of charr found in the lakes of Switzerland, Scandinavia and the British Isles are probably to be looked upon as subspecies of the widely distributed Arctic charr, *Salvelinus alpinus*, which is a migratory fish in the Arctic Ocean (Norman, 1975).

Within lakes, Arctic charr, *Salvelinus alpinus*, exhibit up to four variants or morphs. Different morphs may be distinguished by a number of traits, such as choice of food and habitat, individual growth rate, age and size at sexual maturity, time and place of spawning, body proportions, colouration and parasites (Johnson and Burns, 1984). Arctic charr like some other fish species, develop morphs through trophic differentiation or ecological polymorphism within breeding populations (Skreslet, 1973;

Campbell, 1979; Turner and Grosse, 1980; Hindar and Jonsson 1982; Kornfield *et al.* 1982; Grudzien and Turner 1984; Jonsson *et al.*, 1988).

Under such conditions a single population may split into several genetically different local stocks (Kirkpatrick and Selenders, 1979; Ryman *et al.*, 1979; Ferguson and Mason, 1981; Ryman, 1981; Hindar *et al.*, 1986) which at least in salmonids may be based on their well-documented reproductive homing behaviour (Stuart, 1953, 1957). From the perspective of evolutionary genetics, the discontinuous trophic variation found in Arctic charr may well be the type of niche-specific polymorphism. Maynard (1966) and Turner and Grosse (1980) have regarded such trophic variation as a fundamental prerequisite for sympatric speciation. It has been indicated that there were four phenotypical and ecological variants of Arctic charr in Thingvallavatn, Iceland (Snorrason, 1982, Snorrason *et al.*, 1989; Skulason *et al.*, 1989; Malmquist *et al.*, 1985, 1986).

Malmquist *et. al.*, (1986) studied the biology of charr and plerocercoid of the genus *Diphyllobohrium* spp. in four sympatric morphs of charr. They distinguished charr morphs on the basis of external morphology, colour pattern and the diet. Charr morphs, which they called dwarfs and snail charr, feed on zoobenthos in the littoral zone, pelagic charr feed mostly on zooplankton and piscivorous charr feed almost solely on three-spined stickleback. They found clear differences in frequency of infections of *Diphyllobothrium* spp. between the charr morphs. According to their observations, only 0.3 % of the dwarfs and 6.2 % of the snail charr were infected and on the other hand 76.9 % pelagic and 90.1 % piscivorous charr were infected. These morphs are small and large benthivorous charr and planktivorous and piscivorous charr.

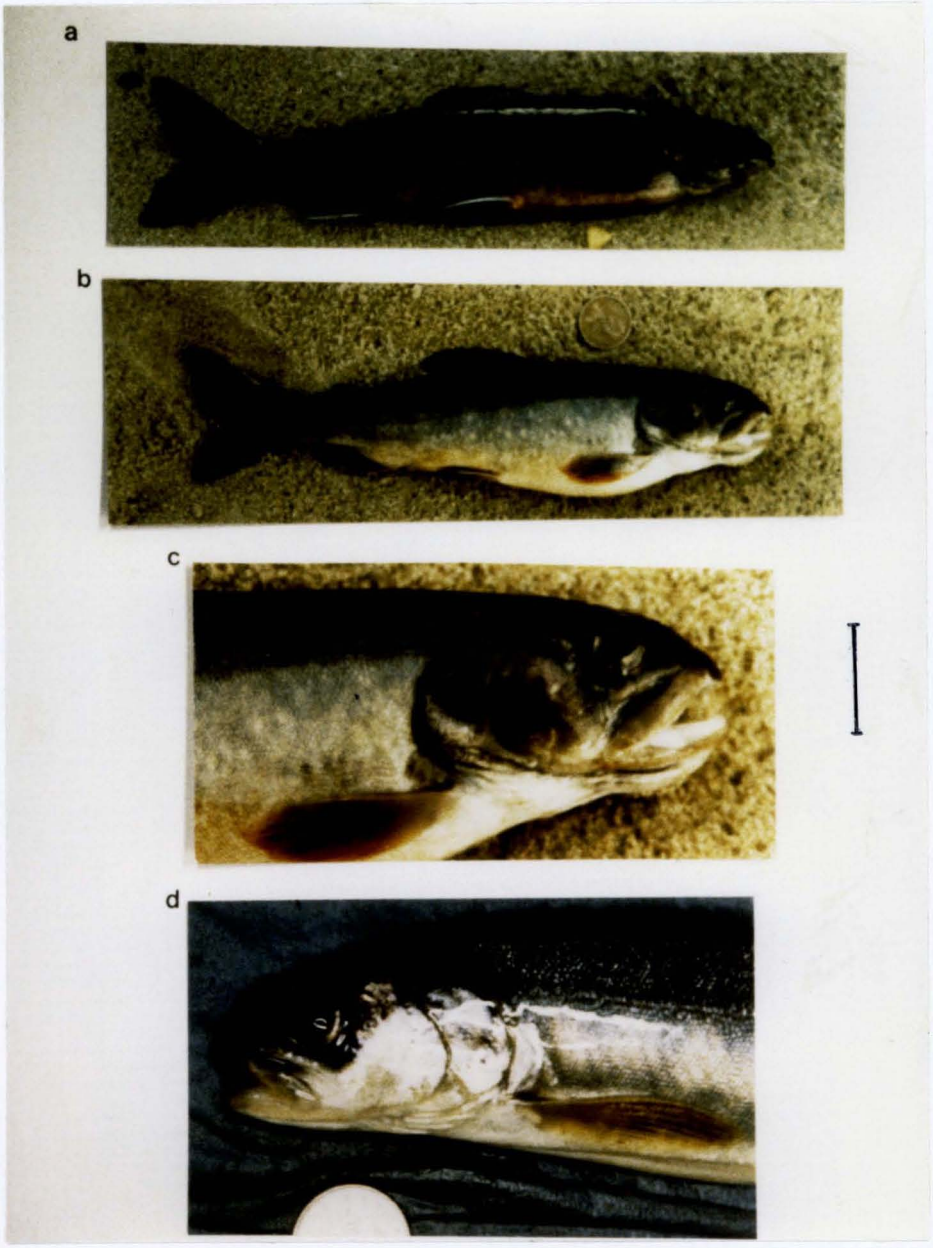


Figure 4.1 The Arctic charr morphs from Loch Rannoch. Whole bodies of (a) the pelagic and (b) the large-headed benthic form. Head region of (c) the large-headed and (d) the small-headed benthic form. Bar represents 4 cm for (a,b) and 2 cm for (c,d).

Table 4.1 Ecological and morphological differences in Arctic charr from Loch Rannoch.

	Pelagic	Small-headed	Large-headed
Habitat	pelagic	shallow water	deeper water
Food	mainly cladocera + copepoda	tricoptera, other insect larvae and some adult insects	chironomids and adult insects
Colouration | dorsal	black	dark brown	dark brown
Colouration | ventral	claret	pale	pale
Colouration | fin	dark red to black	pale brown	pale orange
Head shape	small	between pelagic and large-headed	large and robust
Spawning habitat	throughout littoral	2 tributaries in west basin	unknown
Sexual dimorphism	yes	no	no
Morphometry	highly streamlined	deep bodied	deep bodied
Spotting	well defined	very faint	very faint
White leading edges to fins	yes	no	no

Figure 4.2. The proportion of food items of pelagic and benthic charr from Loch Rannoch

Differences in life-history characteristics, including age at maturity, growth rate, feeding ecology, migrations and reproductive tactics can occur among populations of Arctic charr (Black, 1981; Dick and Belosevic, 1981; Nordeng, 1983; MacKenzie, 1987; Bouillion and Dempson, 1988). The only previous study on parasites of charr in Loch Rannoch carried out by Walker *et al.* (1988) is a comparison of the helminths of two morphs. In that study, they worked on two ecologically distinct (pelagic and benthic) forms of Arctic charr. A recent study showed that there are three sympatric morphs of Arctic charr in Loch Rannoch (Adams, unpublished data). These are namely large-headed benthic, small-headed benthic and pelagic charr (Figure 4.1). The morphs show differences not only in colour, head morphology and jaw structure, but also in their distribution and choice of feeding habitat within the Loch (Figure 4.1 and Table 4.2; Walker *et al.*, 1988; Hartley, 1992; Adams, unpublished data). The differences in trophic morphology and life history may correlate strongly with diet and habitat choice. The morphs have probably evolved through competitive niche segregation and trophic adaptation.

4.2 AIMS

The aims of the study described in this chapter were: (1) to study and compare the endoparasitic helminth fauna of three sympatric morphs of Arctic charr from Loch Rannoch and (2) to characterise those parasite species that may serve as indicators for these three ecological groups of charr. Thus, the effects of ecological factors on pattern and transmission of helminths had also to be investigated. (3) Another aim was to estimate the prevalence and intensity of infections and the impact of helminths on body

condition of fish. In addition, the relationships between sex and size of charr and prevalence and intensity of helminth infections were studied.

4.3 MATERIALS AND METHODS

4.3.1 Study Area

Loch Rannoch (National Grid Ref. NN 600580) is a narrow oligotrophic loch with a maximum depth of 134m. It is a mineral-poor loch (pH 6.0-6.5; alkalinity <6 ppm as calcium carbonate), with a stony shoreline. The length of the Loch is 16.7 km and it lies at an altitude of 204 m. Only about 25 % of its area of 1902 ha covers water less than 15 m deep (Figure 4.3). The surrounding land consists mostly of mixed relict deciduous and coniferous woodland with areas of rough grazing and marginal cultivation, lying on bedrock of the Moine series (Murray and Pullar, 1910; Walker *et al.*, 1988). The loch contains at least eight species of fish; pike, *Esox lucius*, perch, *Perca fluviatilis*, eels, *Anguilla anguilla*, three-spined stiklebacks, *Gasterosteus aculeatus*, minnows, *Phoxinus phoxinus*, salmon, *Salmo salar*, brown trout, *Salmo trutta*, (Walker *et al.*, 1988).

4.3.2 Collection and preservation of fish samples

The gill nets for pelagic and benthic charr were laid at a depth of between 10-35 m from the surface and left overnight. A sample of 253 Arctic charr were caught during October 1992 and July 1993. Thirty were of the small-headed benthic form, 50 were large-headed benthics and 173 were pelagic. Some of these were examined immediately after capture; the rest of them were frozen (-20 °C) until they could be dissected. Frozen fish were thawed before *post-mortem* examination.

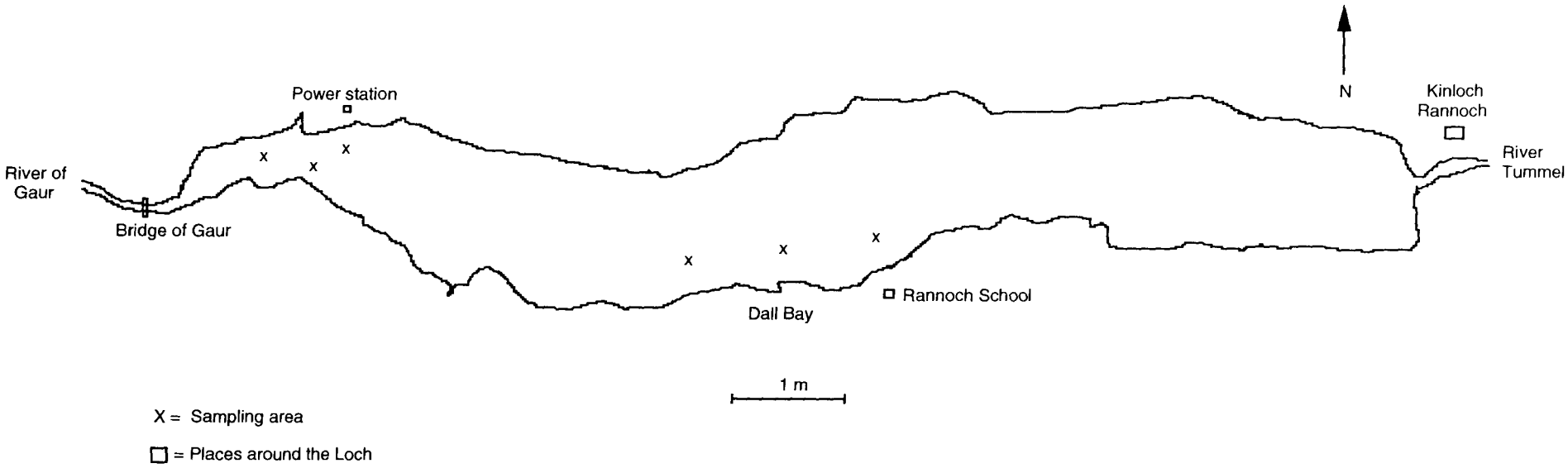


Figure 4.3 Map of Loch Rannoch showing the locations of sampling sites

4.3.3 Collection and preservation of helminths

The fish were examined for helminth infections using basic parasitological techniques (see Chapter 2), with eyes, gills, stomach, pyloric caeca, small intestine, liver, kidney, swim bladder, brain and skeletal muscle being examined separately.

All helminths recovered were identified by reference initially to publications by Kennedy (1974) and Brown *et al.* (1986), Chubb *et al.* (1987) and the number of parasites per fish was counted and recorded. If helminths could not be identified quickly, they were fixed in AFA (Pritchard and Kruse, 1982), stained by using Malzacher's stain technique and mounted before taxonomic keys were consulted.

4.3.4 Morphological measurement

The various length measurements in use have been described and discussed by Lagler (1956). Fork length which we applied is measured from the anteriormost extremity of the fish to the tip of the median rays of the tail. Fork length is regarded by some as the most convenient length and is particularly useful if specimens tend to abrade or otherwise lose a projecting extremity of the tail fin (Bagenal, 1978). For the study, fork length of charr examined were measured by means of a ruler before *post-mortem* examination.

Fish specimens to be weighed might be alive, anaesthetised, freshly dead, variously embalmed or frozen. Weights of fresh and preserved fish specimens are not comparable. Even for a single method of handling, a high level of precision is not possible, sometimes no better than within several per cent because of variations in stomach contents or in amount of water engulfed at capture. The metric system is

preferable for weight measurement (Bagenal, 1978). Weights of charr obtained were measured using a Sartorius handy balance before *post-mortem* examination.

The body condition factor is a standard variable which fisheries biologists measure to assess individual robustness within a given population (Esch and Fernandez, 1993). This index is used to compare the 'fatness' or 'well being' of fish and is based on the hypothesis that the heavier fish of a given length are in better condition than lighter fish (Bagenal, 1978). Lemly and Esch (1984) observed a significant negative relation between body condition of bluegill sunfishes, *Lepomis macrochirus,* infected with the trematode, *Uvulifer amboloplitis,* and parasite intensity. As parasite intensity increased, body condition declined and they concluded that the decline in body condition contributed to a fish's inability to survive the winter months. Many authors (e.g. Frost and Brown, 1967; Bagenal, 1978; Weatherley, 1987) have found it convenient to use a simple formula for determination of condition factor as follows:

$$K = \frac{W}{L^3} \times 10^2$$

Where:

K= Condition factor

W= Weight of fish in grams and

L= Fork length of fish in centimetres

This simple formula happens to fit the body form of salmonids better than it does many other groups. For instance, the condition factors for eels thus calculated are much less than unity, whereas for species of less elongated form the reverse may be true (Weatherley and Gill, 1987).

4.3.5 Sexing Fish

Inspection of the gonad is the most reliable means to determine the gender of a fish. In adult females, eggs are readily discernible in the ovaries. While in adult males the testes are typically smooth, whitish and non-granular in appearance. In immature specimens the shape of the gonad may be a guide to the gender (for example, testes have finger-like processes in many catfishes), but it is often necessary to use a dissecting microscope to determine the sex of small immature fish (Bagenal, 1978).

Sex of charr were determined by means of an internal examination when the *post-mortem* examination for endoparasites was carried out, the sex organs being found under the gut.

4.3.6 Statistical analysis

The data were checked for normality and transformed where necessary. For normally distributed variables parametric statistics were used and for non-normally distributed variables non-parametrics statistics were used. Differences in sex ratio between 3 morphs of Arctic charr was tested by χ^2 test. Differences in prevalence of infections between 3 morphs according to sex was tested by χ^2 test and intensity of infection was examined by Kruskal-Wallis test. Relationship between length and weight

for 3 morphs was investigated by Regression Analysis. Differences in size of fish (in terms of length and weight) between 3 morphs was inspected by One-way ANOVA. Differences in condition factor of fish between 3 morphs was also investigated by One-Way ANOVA. Relationship between intensity of infection and condition factor of fish in each morphs was tested by Spearman Correlation Coefficient. χ^2 test was employed to test differences in prevalence of infection between pelagic and benthic morphs. Differences in prevalence of *Diplostomum* sp.; *Diphyllobothrium* spp.; and *Eubothrium salvelini* between pelagic and benthic morphs.

4.4 RESULTS

4.4.1 Sex ratio

The sex ratio of the fish examined was 34 male and 16 female, 4 male and 26 female and 35 male and 138 female for large-headed, small-headed benthic and pelagic charr respectively. Thus, the female to male ratio was 0.5, 6.5 and 3.9 for large-headed, small-headed and pelagic respectively (Figure 4.4). Interestingly, 68 % of large-headed benthic were male compared to 13 % for the small-headed morph and 20 % for the pelagic (χ^2=47.11, df=2, p<0.000). No effect of host sex was observed on the prevalence or intensity of infection in 3 morphs (χ2 = 2.338, df = 1, p <0.1), so no difference in the susceptibility of the sexes to infection status was identified.

4.4.2 Length and weight relationships

Relationships between log-length and log-weight of small-headed, large-headed and pelagic morphs are shown in Figure 4.5. Regression of log-weight against log-length yielded a significant (P < 0.001, P < 0.001, P < 0.001 for large-headed, small-headed and pelagic charr) linear relationships. The mean fork length of fish was 19.16 cm (range 6.0 cm-26.5 cm), 19.4 cm (range 14.8-23.5 cm), 18.64 cm (range 8.0-22.5 cm) for large-headed, small-headed benthic and pelagic charr respectively. The length of most fish in the 3 morphs sample was between 16.1 cm and 22.1cm. In general, the length of large-headed benthics were greater than pelagic and in turn small-headed benthic (One-way ANOVA, $F_{49,172}$ = 4.958, P<0.001).

The mean weight of fish was 99.0 g (range 17.7-245 g), 102.4 g (range 41.7-151.4 g), 91.7 (range 9.3-171.4 g) for large-headed and small-headed benthic and pelagic charr respectively . Most fish weight in the three groups was between 50.0-125.0 g. Again, large-headed benthics were usually heavier than small-headed and pelagic (One-way ANOVA, $F_{49,172} = 7.38$, $P < 0.001$). Being longer and heavier of benthics than pelagic supports the idea of being robust and living longer of benthic than pelagic.

The condition factor of charr was observed to vary between 0.6-1.9, 0.9-1.7 and 1.0-1.9 for large-headed benthic, small-headed benthic and pelagic charr respectively (Figure 4.4). There were statistically significant differences between condition factor of three morphs (One-Way ANOVA, $F_{2,250} = 13.12$, $P < 0.001$). No statistically significant correlation was found between intensity of infections and condition factor of fish (Spearman Rank Correlation, $R_s = -0.072$ for large-headed, $R =_s -0.073$ for small-headed and $R_s = -0.281$ for pelagic), although the regression lines had negative slopes (Figure 4.6).

4.4.3 Helminth species

The species, development stages, microhabitat and intermediate hosts of the helminths recovered from the three sympatric morphs of arctic charr are given in Table 4.2. The prevalences of infections are shown in Figure 4.7. Table 4.3 gives the prevalence, intensity and range of the helminths. Overall, six helminth species were recorded in 3 morphs of charr namely *Diplostomum sp.* (Trematoda); *Diphyllobothrium dendriticum*, *D. ditremum* and *Eubothrium crassum* (Cestoda); *Echinorhynchus truttae* and *Neoechinorhynchus rutili* (Acanthocephala). *Diphyllobothrium dendriticum* and *D.*

ditremum were considered as *Diphyllobothrium* spp., but *D. ditremum* was observed more common than *D. dendriticum* in Loch Rannoch charr.

4.4.4 Prevalence and intensity of infections

Six species of helminth were found in the Arctic charr from Loch Rannoch (Table 4.2) and the prevalence of infections in the three morphs is shown in Figure 4.7. Overall 81 % of the pelagic morphs were found to harbour helminths, as compared with 56 % and 47 % of the large-headed and small-headed morphs, respectively. The prevalence of infections in the pelagic charr was significantly greater than in the benthic charr ($\chi^2 = 22.7$, df = 2, $P < 0.001$).

After adjustment for host size, pelagic morphs were found to harbour significantly more worms than large-headed benthic morphs, which in turn harboured more worms than small-headed benthic morphs (Kruskal-Wallis Anovar, H = 98.66, df = 2, P = 0.001).

Pelagic and benthic fish show different patterns of infection. Metacercariae of *Diplostomum* sp. were significantly more common in benthic than in pelagic charr (Figure 4.7; $\chi^2 = 8.06$, df = 2, $P < 0.05$). The plerocercoids of *Diphyllobothrium dendriticum* and *D. ditremum* were more common in the pelagic than benthic charr ($\chi^2 = 43.71$, df = 2, $P < 0.001$). Adult *Eubothrium salvelini* were recovered only from pelagic fish ($\chi^2 = 13.64$; df = 2, $P < 0.001$). Acanthocephalan infections were relatively uncommon (Figure 4.7), with none being found in pelagic morphs and *Neoechinorhynchus rutili* being found in only two of the small-headed benthic morphs. This may be due to the intermediate hosts of these species being relatively rare in Loch Rannoch.

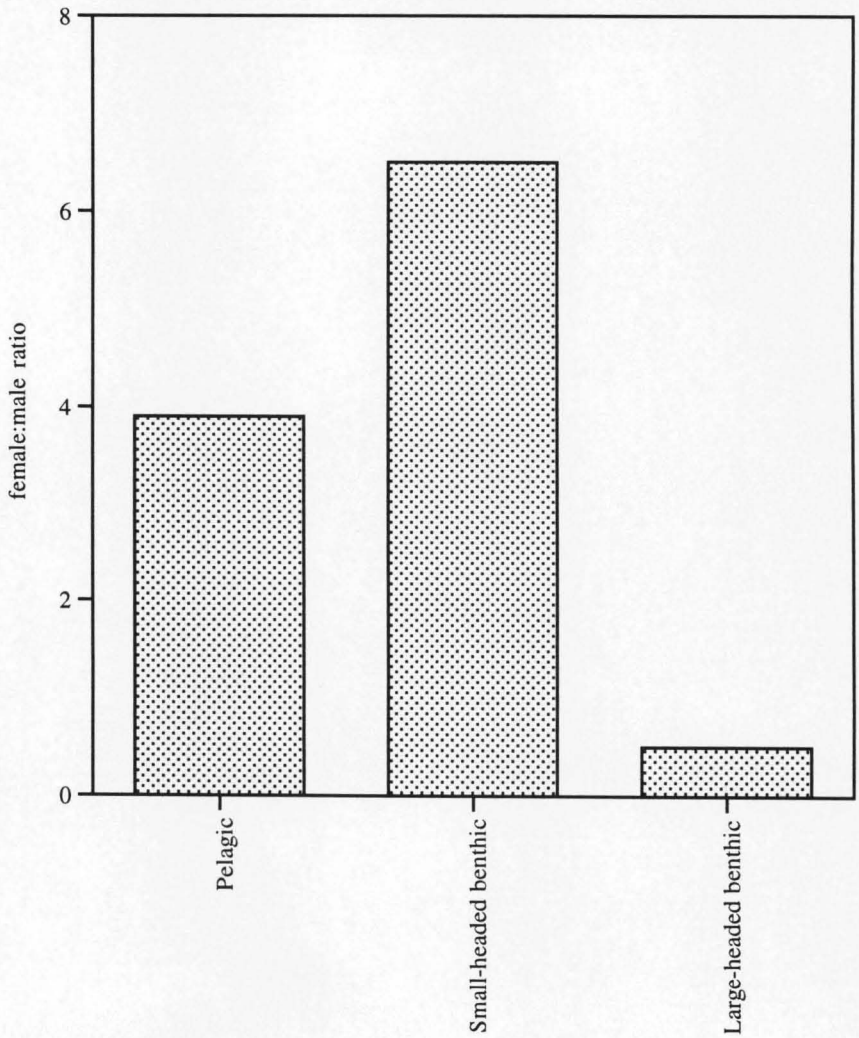


Figure 4.4 Sex ratio of three morphs of Arctic charr from Loch Rannoch

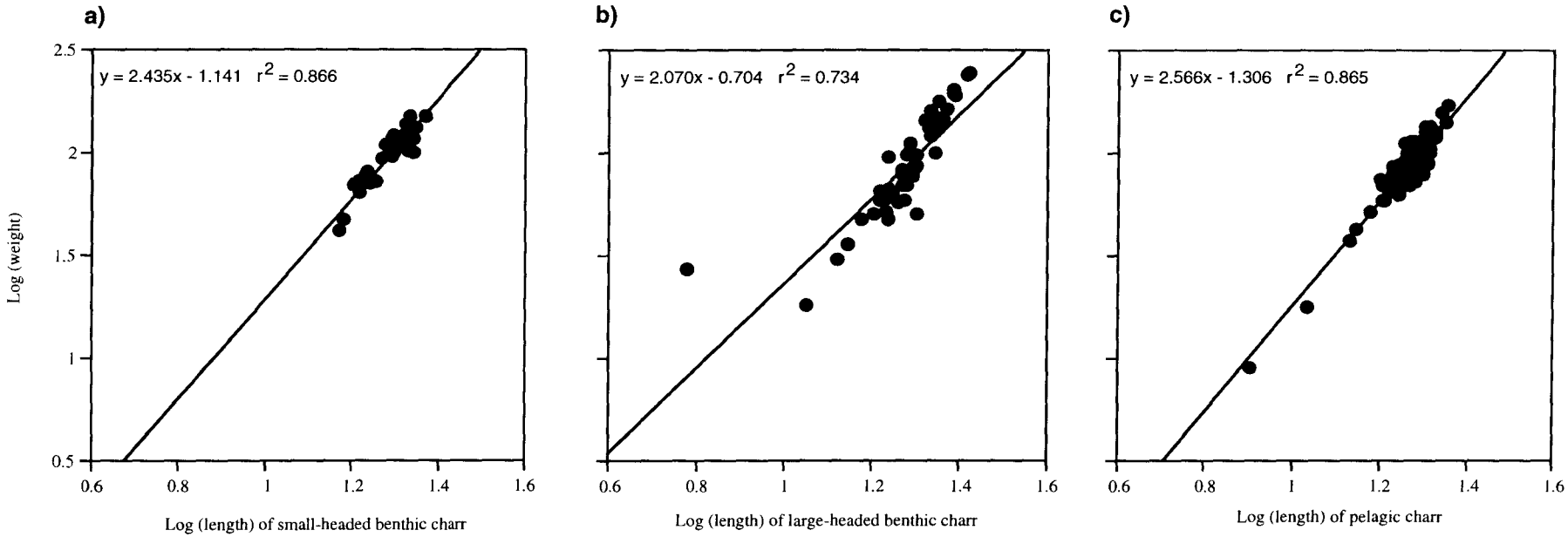


Figure 4.5 Regression of log-length for (a) small-headed, (b) large-headed and (c) pelagic morph

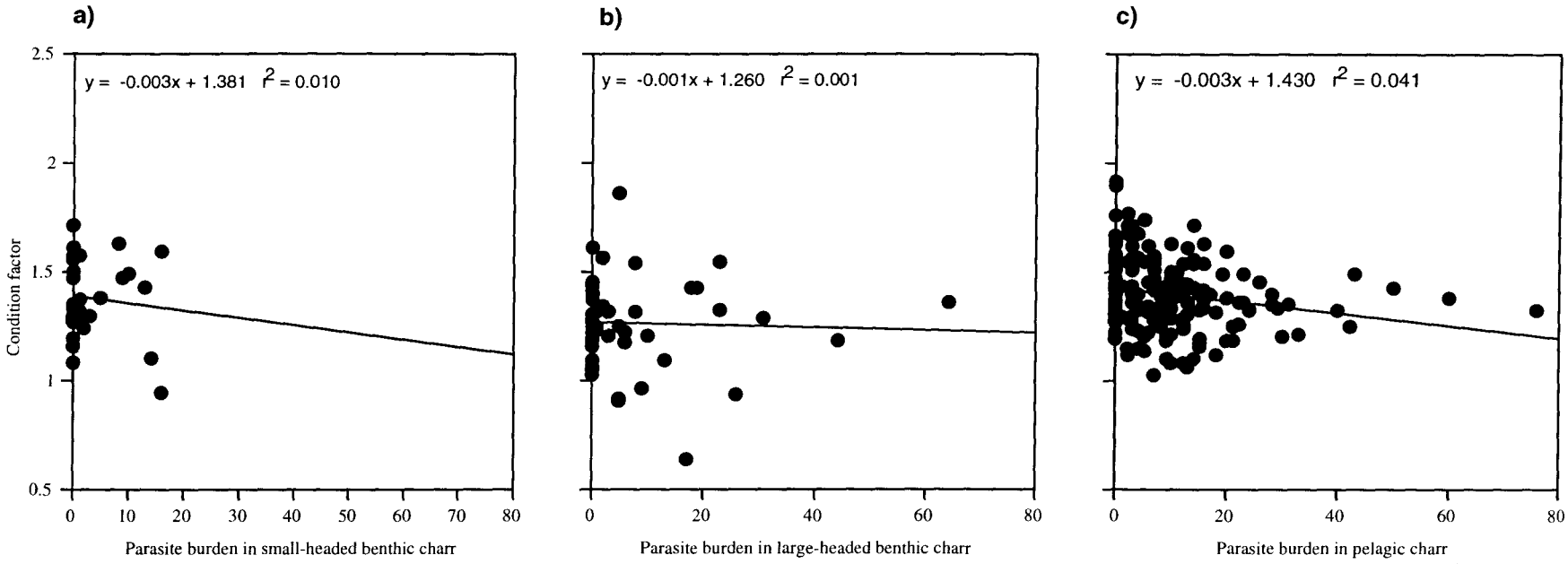


Figure 4.6 Relationships between total parasite burden and condition factor of (a) small-headed, (b) large-headed and (c) pelagic morph

Table 4.2 Endoparasitic helminths from Arctic charr (*Salvelinus alpinus*) from Loch Rannoch, Scotland.

Helminth species	Charr morph	Development stage	Microhabitat	Intermediate host
Trematoda				
Diplostomum sp.	LHB, SHB, P	Metacercaria	Lens of eyes	Snails
Cestoda				
Pseudophyllidea				
Diphyllobothrium dendriticum	LHB, SHB, P	Plerocercoid	Encysted in body cavity	Copepods
Diphyllobothrium ditremum	LHB, SHB, P	Plerocercoid	Encysted in body cavity	Copepods
Eubothrium salvelini	P	Adult	Pyloric caeca and intestine	Copepods
Acanthocephala				
Echinorhynchus truttae	LHB, P	Adult	Intestine	Amphipods
Neoechinorhynchus rutili	SHB	Adult	Intestine	Ostracods

Charr morphs: LHB, large-headed benthic; SHB, small-headed benthic; P, pelagic.

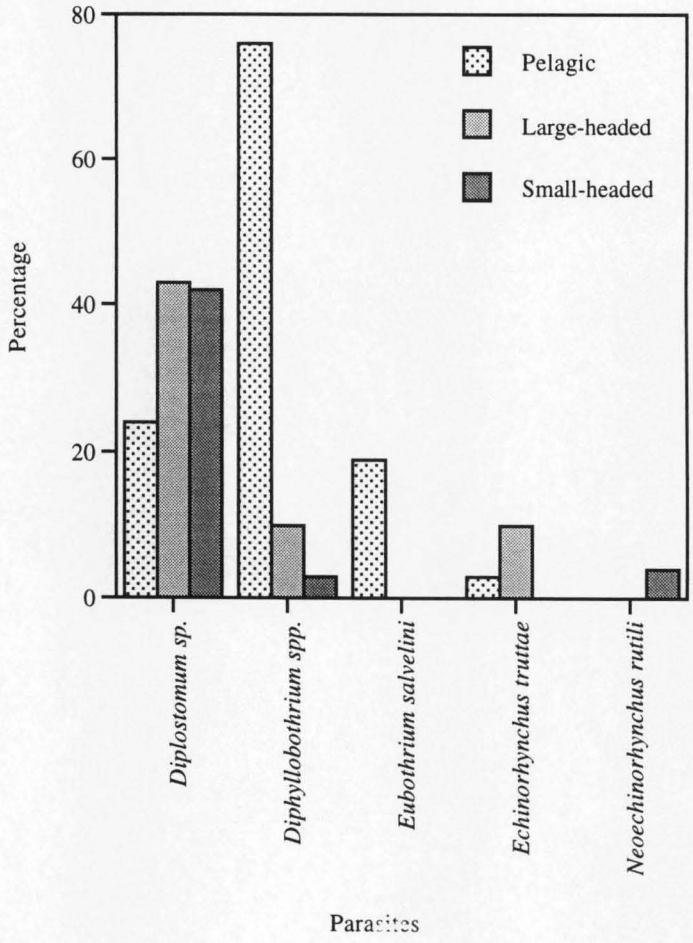


Figure 4.7. Percentage number of Arctic charr morphs infected with endoparasitic helminth species

4.5 DISCUSSION

4.5.1 Helminths as an indicator of morph segregation

Qualitative and quantitative occurrence of helminths in the host has been used to decide whether the parasite species could be use as tags (Cloutman,1976; Davis and Huffman, 1977; Moser and Hsieh, 1992). The distribution tends to reflect differences in parasite faunas, with the parasites most commonly selected as tags for a particular group of fish tending to be the most common parasites of that group. However, the dominant components of parasite faunas do not necessarily make the best tags. Species which occur less commonly or only as incidental parasites of the host being studied are probably the most convincing. These parasites can only be acquired by the host in restricted areas within its range (MacKenzie, 1987). The fact that infection of *Eubothrium salvelini* is restricted to the pelagic morph of charr in Loch Rannoch may be an example of this view.

Past evidence for the separation of charr into large-headed, small-headed and pelagic morphs has been inconclusive. Walker *et al.* (1988) studied parasites of two charr morph, one is pelagic and other benthic and suggested that differences in parasite loading of two morphs was evidence of niche segregation.

There are several factors that could cause the difference in the helminth communities of three morphs of Arctic charr from Loch Rannoch. One of these may be site segregation. Qualitative difference between the diets of the morphs is another factor which would create differences in their helminth communities. Other factors, such as physiological differences between the three morphs probably contribute to the differences in the helminth communities of morphs. This proposition would require further studies. However from the observations in this study, the host's ecology appears

as an influential factor acting to determine the structure of its helminth community. Although the three morphs are closely related and exist in the same loch, significant differences in their helminth communities indicate that the three morphs are very different ecologically as well as morphologically.

Margolis (1963) surveyed the parasite fauna of migrating freshwater sockeye salmon from western Alaska and the Kamchatka Peninsula and selected two parasites as tags. The larval cestode, *Triaenophorus crassus* was found only in fish from western Alaska and the adult nematode, *Dacnitis truttae* was restricted to Kamachatka salmon. In the current study from Loch Rannoch, *Echinorhynchus truttae* and *Neoechinorhynchus rutili* were so rare that they could not be used as tags. The parasites of freshwater origin most commonly used as tags in recent studies are plerocercoids of the cestode genus *Diphyllobothrium*. In salmonid fish of the genera *Oncorhynchus* and *Salvelinus* different levels of *Diphyllobothrium* infection have been shown to result from different feeding strategies of intraspecific groups (MacKenzie, 1987). Of the six parasite species found, only *Diplostomum* sp., *Diphyllobothrium dendriticum* and *D. ditremum* had suitable intensity of infection for their use as a biological tag. It was evident that the prevalence, intensity and abundance values of *Diphyllobothrium* spp. and *Diplostomum* sp. differed between benthic and pelagic morphs in this study. The small-headed benthic morph exists in shallow water and the large-headed benthic morph in relatively deeper water. Calm water probably favours high concentrations of swimming cercariae (Davis and Huffman, 1977) therefore benthic morphs are more exposed to *Diplostomum* sp. than pelagic in Loch Rannoch. In contrast, pelagic morphs are more exposed to *Diphyllobothrium dendriticum* and *D. ditremum* than benthics. Despite these high intensities of *Diplostomum* sp. which occurred in the eyes of the

host, vision in these charr did not appear to be affected (Bouillon and Curtis, 1987; Bouillon and Dempson, 1989).

The differences between the helminth communities of the benthics and pelagic morphs were attributed mainly to differences between morphological appearances, the size and diet of the fishes and to the spatial niche that each morphs occupies in the Loch. Also observed differences between the helminth communities of large-headed, small-headed and pelagic morphs in Loch Rannoch indicate that these three sympatric morphs are very different ecologically.

4.5.2 Prevalence and intensity of helminths and effects of feeding specialisation on parasite transmission

Of the 5 species of parasites collected in this study all, with the exception of *Echinorhynchus truttae*, have previously been reported from Arctic charr in Loch Rannoch (Walker *et al.* 1988).

It has been shown in other studies that morphs of Arctic charr, *Salvelinus alpinus*, living sympatrically also differ in their parasite prevalence. For instance, it was found that *Diphyllobothrium ditremum* and *D. dendriticum* were more prevalent in pelagic charr while *Crepidostomum metoeus* and *Cyathocephalus truncatus*, a cestode using *Gammarus* as an intermediate host, were more prevalent in benthic charr (Henricson and Nyman, 1976). In another study, Frandsen *et al.* (1989) described quantitative differences between the endoparasitic associations of five Arctic charr morphs in Thingvallavatn, Iceland, again relating these to habitat and feeding preferences. Also analyses of Arctic charr parasites have often been utilised in the past

to get information about fish foraging behaviour and to separate lake-resident from migratory forms (Eddy and Lankester, 1978; Dick and Belosevic, 1981).

The presence of helminth infections in three morphologically distinct forms of Arctic charr living in sympatry in Loch Rannoch supports the finding that the three morphs have became specialised to exploit different diets (Walker *et al.*, 1988; Adams, unpublished). The clearest difference in parasite fauna is between the pelagic and the two benthic morphs; this is associated with the distinction between the diet of these types. However there are also qualitative differences between the two benthic morphs confirming more subtle differences in foraging specialisation between these two types. These observations are important because they provide evidence about how host helminth associations might become established and how parasite life cycles evolve. The three populations of Arctic charr morphs in Loch Rannoch are being exposed to different parasite faunas as predicted by Combes's (1991) theoretical encounter filter.

The diets of four sympatric Arctic charr morphs (small-benthic, large-benthic, small-limnetic and large-limnetic) from Thingvallavatn were analysed by Malmquist *et al.* (1982). Benthic morphs foraged on mainly the molluscs *Lymnaea peregra*. Small limnetics preyed mainly upon three-spined stickleback, *Gasterosteus aculeatus*. A strong correlation between ecology and morphology suggested that the benthic and limnetic morphs have become adapted to littoral benthic and pelagic niches respectively in Thingvalavatn. Malmquist *et al.* (1986) examined a total of 1299 charr and found clear differences in frequency of *Diphyllobothrium* infections between the four morphs. Only 0.3 % of the small benthic fish were infected and just 6.2 % of the large benthic charr. On the other hand 76.9 % of the small limnetic charr were infected, as were 90.1 % of the large limnetic charr.

The prevalence of helminth infections in Arctic charr from Loch Rannoch is high. The markedly higher prevalence of the plerocercoid stage of *Diphyllobothrium* spp. in pelagic morphs, which also accords with data presented by Malmquist *et al.* (1992) and Walker *et al.* (1988), and the restriction of *Eubothrium salvelini* to the pelagic morph reflects the fact that the intermediate hosts of these worms are planktonic copepods. In contrast, the bottom-feeding habit of the benthic morphs makes them more likely to be exposed to trematode cercaria released from snails. Thus the difference in feeding ecology between benthic and pelagic charr from Loch Rannoch has profound effects on their vulnerability to infection by parasite helminths and result in clear differences between morphs in their parasites fauna. Overall prevalence and intensity of parasites in the pelagic morph is much greater than in their benthics, especially the small-headed benthic even though benthic fish may live longer (Walker *et al.*, 1988). So, either their foraging habits increase infection rate or their infection protection mechanisms are less well developed. This system will permit a test of the wider applicability of the ideas advanced by Combes (1991) in his explanation of how parasite life cycles are initiated.

A number of authors have suggested a relationship between host foraging habits and their subsequent exposure to infection by particular species of parasite. For example, Kennedy and Burrough (1978) found low helminth richness, high abundance and low density in *Salmo trutta* in Malham Tarn apparently due to presence of limited numbers of prey species. Conversely, Kennedy *et al.* (1992) used the fact that eels are infected by helminth species such as *Proteocephalus macrocephalus*, for which planktonic copepods are the intermediate host to deduce that the eels were feeding on plankton. Due and Curtis (1995) found that Arctic charr feeding on chironomids were

free of parasites, but those eating copepods were more infected with the cestodes. Similarly, our findings showed that the number of parasite species in benthics (especially large-headed benthic) which mainly feed on tricoptera and chironomid larvae were less compared to pelagic which feed preliminary on cladocera and copepoda.

The findings of the present study that *Diphyllobothrium ditremum* is more common than *D. dendriticum* in Arctic charr from Loch Rannoch agrees with those of Curtis *et al.*, (1995), who found prevalence of 74.9 % *D. ditremum* and 13.7 % *D. dendriticum* in Arctic charr from a small lake in northern Quebec.

4.6 SUMMARY

1. Three sympatric morphs of Arctic charr, *Salvelinus alpinus*, occur in Loch Rannoch; these are the small-headed benthic, large-headed benthic and pelagic morphs.

2. Six species of endoparasitic helminth were found in the fish, and the morphs had different patterns of infection.

3. Overall infections in pelagic charr were heavier than in large-headed benthics, which were in turn heavier than in small-headed benthics.

4. Pelagic fish had high prevalences and intensities of pseudophyllidean tapeworms, the intermediate host of which are copepods; the prevalence and intensity of metacercariae of *Diplostomum* sp. (the intermediate host of which are snails) were high in the benthic morphs.

5. Thus, it is suggested that pseudophyllidean tapeworms can be choosen as biological indicators for pelagic morphs and *Diplostomum* sp. for benthic morphs of Arctic charr from Loch Rannoch.

Chapter 5 Annual pattern of infections of pseudophyllidean cestodes in *Cyclops strenuus abyssorum* (Copepoda) in relation to the abundance of zooplankton species in Loch Lomond, Rowardennan.

5.1 INTRODUCTION

5.1.1 Life-cycle of the parasite

Pseudophyllidean cestodes usually require three hosts (Schmidt and Roberts, 1989). For example, eggs of *Diphyllobothrium dendriticum* are released by means of birds' faeces and hatch in the water (Figure 5.4). The coracidia are ingested by copepods and develop to procercoids within this host (Figure 5.3) and fish become infected by ingesting copepods carrying procercoids. In the fish, the parasite develops to the plerocercoid stage which is infective to birds (Figure 5.1). The eggs from the hermaphroditic adult worm in the intestine of the definitive host must enter water in order to undergo further development. Time of embryonic development varies with respect to external condition. Completion of development to coracidium takes from 8 days to several weeks, depending on the temperature (Schmidt and Roberts, 1989). After leaving the egg shell the larva is now called a coracidium which swims by means of its beating cilia. The direction of ciliary movement changes every 5 to 15 seconds. The coracidium swims in a circle finally settling on the bottom and eventually dies. For further development the coracidium must be swallowed by the first intermediate host, a copepod (Henricson, 1978). Soon after being eaten, the coracidium loses its ciliated epithelium and immediately begins to attack the wall of the midgut with its six tiny hooks and penetrates the copepod's body cavity (Figure 5.2). The larva begins to grow there and on the 10th to 11th day the cercomer begins to form. Differentiation is completed on the 14th day, where the parasite enters to the procercoid phase. Further

development occurs after the infected copepod is swallowed by a fish. In the fish stomach, the procercoid leaves the copepod, perforates the stomach wall and develops into the plerocercoid stage in the internal organs or in the muscles. Adult *Diphyllobothrium* mature and produce eggs in the avian definitive host within 2 weeks after the plerocercoids are ingested by susceptible bird. The adult tapeworm may survive 2-4 months in the intestine of the bird; they produce a vast number of operculated eggs which hatch on reaching water (Cheng, 1986).

Previous investigations showed the most common first intermediate host for *Diphyllobothium* spp. are *Cyclops abyssorum*, *Diaptomus gracilis*, *D. vulgaris*, *D. gracilioides*, *Mesocyclops oithonoides*, *Cyclops strenuus*, *C. vicinus*, *C. scutifer* and *C. serrulatus* (see Appendix II).

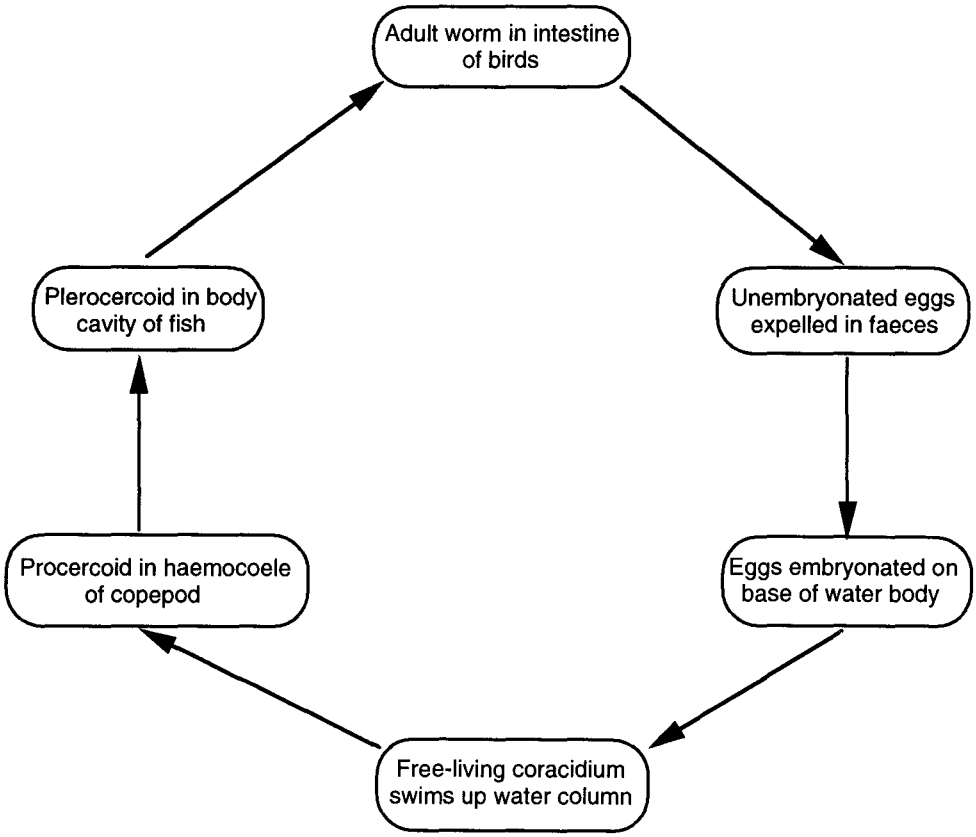


Figure 5.1 Outline life-cycle of *Diphyllobothrium* spp.

Figure 5.2 The *Cyclops strenuus abyssorum* infected with procercoid of *Diphyllobothrium* spp. (p) procercoid. Bar represents 0.3 mm.

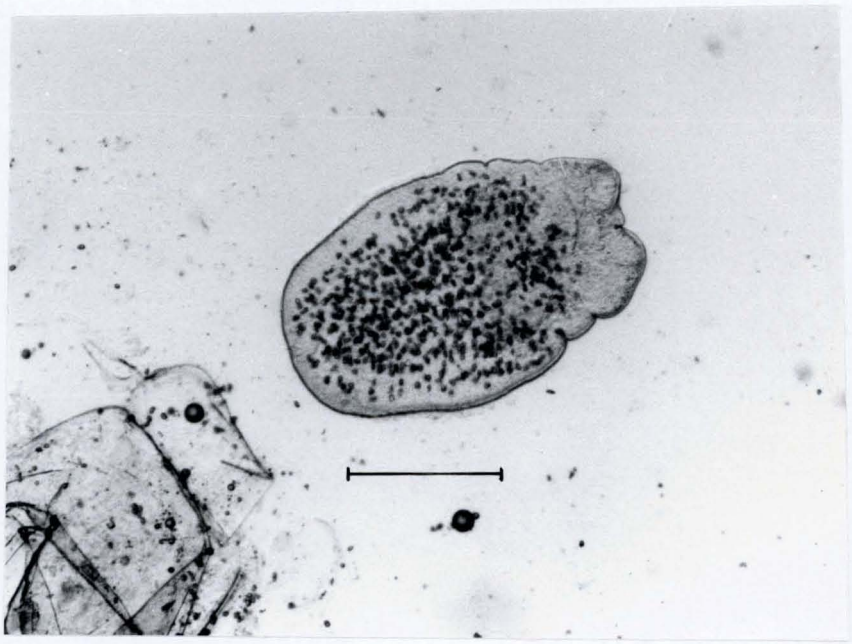


Figure 5.3 Plerocercoid of *Diphyllobothrium* spp. from infected *Cyclops strenuus abyssorum*. Bar represents 0.12 mm.

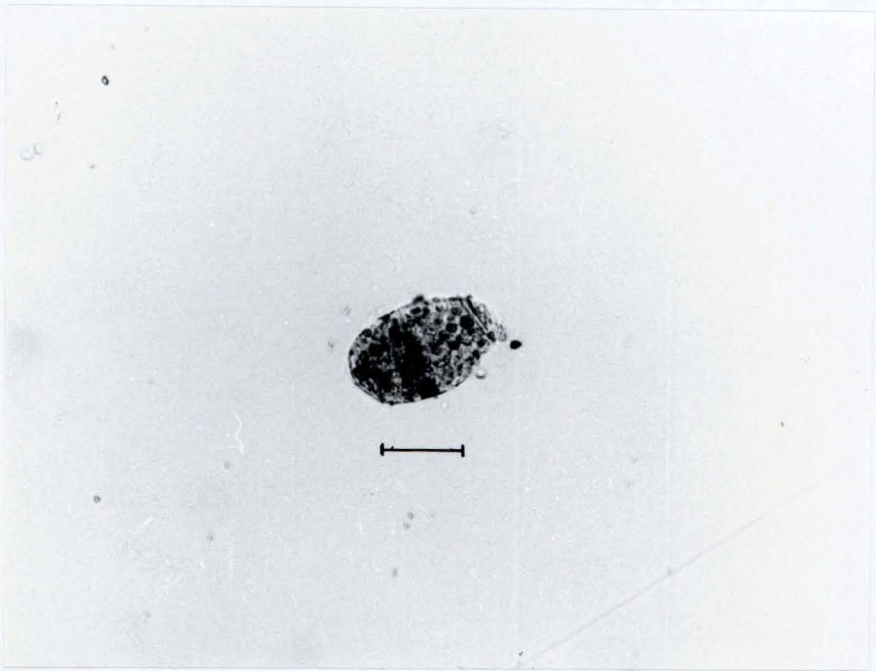


Figure 5.4 Egg of *Diphyllobothrium* spp. found in the sample of plankton from Loch Lomond. Bar represents 20 μm.

5.1.2 Evidence for *Diphyllobothrium* procercoids in *Cyclops strenuus abyssorum* in Loch Lomond

It was assumed that procercoids found in *C. str. abyssorum* were probably of the genus *Diphyllobothrium* for the following reasons: plerocercoids of the genus *Diphyllobothrium* are common and widely distributed cestode parasites of fish, especially salmonid fish in the British Isles (Kennedy, 1970; Dorucu *et al.*, 1995). Plerocercoids of this genus are commonly found encysted on stomach of powan from Loch Lomond (Slack 1957, see Chapter 6). Furthermore, powan in Loch Lomond chiefly feed on zooplankton (Pomeroy, 1991; Maitland and Campbell, 1992; Pomeroy, 1994) of which *Cyclops str. abyssorum* was identified as a major component (see below). Also on one occasion, an egg of *Diphyllobothrium* spp. (Figure 5.4) was found in the samples of plankton. Moreover, Pasternak *et al.* (1995) studied the metabolism and behaviour of the freshwater copepod *Cyclops strenuus abyssorum* from Loch Lomond infected with procercoids considered to be *Diphyllobothrium* spp.

5.1.3 Zooplankton community of Loch Lomond

Loch Lomond's zooplankton community has recently been evaluated from the results of zooplankton surveys carried out by Slack and Hamilton (1955-1957), Chapman (1965, 1969, 1972), Maitland *et al.* (1981) and Pomeroy (1987). Annual cycles of *Eudiaptomus gracilis* (Sara), *Cyclops strenuus abyssorum* (Sars) and *Mesocyclops leuckarti* (Claus) were described by Pomeroy (1994) for the mid-basin of Loch Lomond. According to Pomeroy (1994), *E. gracilis* overwinter as adults with variable egg production during their lifespan of 5-8 months. Cyclopoid copepods have

variable life history characteristics. *Cyclops str. abyssorum* produces a single generation each year and overwinters by means of resting eggs and a small population of planktonic adults. *Mesocyclops leckuarti* is present in the plankton throughout the year, but in low numbers during winter. Pomeroy (1994) also classified species found in zooplankton samples from Loch Lomond by limnological origin. *Eudiaptomus gracilis*, *C. str. abyssorum* and *Mesocyclops leuckarti* were described as pelagic, *Macrocyclops* spp. as littoral and *Acanthocyclops viridis* (Jurine) as profundal.

The copepod species known to exist in Loch Lomond are *Cyclops strenuus abyssorum*. *Diaptomus gracialis and Mesocyclops leuckarti* details being given Chapman (1965). *Cyclops strenuus abyssorum* is infected with *Diphyllobothrium* spp. (Pasternak *et al.* 1995).

5.1.4 Seasonal variation in copepod abundance

The copepod community changes not only in different years but also during the course of a single year. For example, Hanzelova (1992) observed that different species were dominant at different times of the year in Dobsina dam, Czechoslovakia. In years with a regime of decreasing annual temperature, with a cool spring and warm autumn *Eudiaptomus zacharias* dominated. On the other hand, a regime of decreasing annual temperature favoured the development of copepods of the genus of *Cyclops*. In that study, positive correlation was also recorded between the number of copepods and the water temperature. With increasing temperature the number of copepods in the Dobsina dam tended to increase, and conversely, in the years with a warm spring and cool autumn the number of copepods decreased in the autumn. During the three years of study, it was reported that in the second half of the second year of the study two of the

copepod species (*Cyclops bohater* and *Mesocyclops crassus*) that occurred in the lake had not been seen there before.

5.1.5 Seasonal variation in the intensity of infection in copepods with pseudophyllidean procercoids

Although the seasonal dynamics of infection of intermediate hosts is an important factor in the cestode life cycle in natural waters, relatively few studies have been carried out on this aspect of the life cycle. The development of pseudophyllidean eggs and coracidia is affected by a variety of environmental factors of which water temperature is the most important (Williams and Jones, 1994). In other words, as fish are poikilotherms, the effects of abiotic factors are pronounced on their tapeworms where growth and maturation of these parasites are tied to temperature. Most infections of fish occur during the summer and early autumn and adult tapeworms grow and mature during late spring and early summer. In spring and early summer most tapeworms reach sexual maturity and release eggs; this is the time when populations of the invertebrate hosts reach their peak. Differentation into procercoids is also rapid due to high water temperatures and there is sufficient time for infections of fish to occur prior to winter (Dick and Choudhury, 1995). Previous studies indicated that natural infections of cyclopoid copepods are greater in summer than in winter (Guttowa, 1963; Sysoev, 1987; Hanzelova, 1992). For example, Halvorsen (1966) found a seasonal variation in the incidence and intensity in infection of female *Eudiaptomus gracilis* with procercoid of *Diphyllobothrium dendriticum*. The infection level was minimum in March, higher in January and April, but maximum in June, July and October. However, in *Cyclops strenuus* no seasonal variation has been observed.

Guttowa (1963) examined a total of 1187 specimens from 11 species of Copepoda (*Cyclops strenuus* (Fischer), *Thermocyclops oithonoides* (Sars), *Mesocyclops leuckarti* (Claus) *Macrocyclops albidus* (Jurine), *Macrocyclops distinctus* (Richard), *Acanthocyclops viridis* (Jurine), *Eucyclops macrurus* (Sars), *Eucyclops macruroides* (Lillj.), *Macrocyclops bicolor* (Sars), *Heterocyclops appendiculata* (Sars), and *Eudiaptomus gracilis* (Sars)) between May and July in Lake Karpero, Finland. 40 % of *Cyclops strenuus* and 18.5 % of *Thermocyclops oithonoides* were found to be infected with procercoids of *Diphyllobothrium latum*. Infection peaked with a prevalence of 52.3 % in mid June followed by a fall the end of June. Grabiec *et al.* (1963) found a positive effect of light intensity and time of exposure, and negative effect of wavelength of light on the hatching of coracidia from eggs of *Diphyllobothrium latum*. It has been suggested that the critical temperature for the hatching of *Diphyllobothrium latum* eggs is 8 °C and that such conditions would occur in spring and summer, mainly in the littoral region where the majority of *Diphyllobothrium latum* eggs would accumulate from sewage and animal excreta (Kuhlow, 1953). In a recent study carried out by Hanzelova (1992), infections usually with a single procercoid of *Proteocephalus* were found in four copepod species namely *Eudiaptomus zachariasi*, *Cyclops vicinus*, *C. strenuus* and *Mesocyclops albidus*. The intensity of *Proteocephalus neglectus* procercoids in copepods had two peaks detected in the summer season. A typical short-term increase in infection intensity was observed in September.

5.2 AIMS

The aims of the study were (1) to investigate seasonal variation in abundance of copepod species (2) to investigate the natural levels of infection with procercoids of pseudophyllidean cestodes (assumed to belong to *Diphyllobothrium* spp.).

5.3 MATERIALS and METHODS

5.3.1 Study area

Loch Lomond lies just north of Glasgow, at 56°05' N and 04°35' and has the largest surface area (7112.5 ha) of any of the British (excluding Ireland) lakes. It is of glacial origin and this is clearly indicated by the steep-sided narrow and deep upper part (Figure 5.5). The sampling area chosen for the study is situated near the University Field Station at Rowardennan (Figure 5.5).

5.3.2 The fauna of Loch Lomond

Although, there are differences between the results of previous surveys on the abundance and occurrence of zooplankton species, *Cyclops strenuus abyssorum*, *Cyclops leuckarti*, *Diaptomus gracilis*, *Daphnia hyalina* and *Bosmina coregoni* are principal species that occur in zooplankton in Loch Lomond (Table 5.1).

Loch Lomond probably has the most diverse fish fauna of any of the Scottish freshwater lochs. Maitland (1972) recorded fifteen species namely the sea lamprey, *Petromyzon marinus*; river lamprey, *Lampetra fluviatilis*; brook lamprey, *Lampetra planeri*; Atlantic salmon, *Salmo salar*; brown trout, *Salmo trutta*; powan, *Coregonus lavaretus*; pike, *Esox lucius*; minnow, *Phoxinus phoxinus*; roach, *Rutilus rutilus*; stone loach, *Noemacheilus barbatulus*; eel, *Anguilla anguilla*; three-spined stickleback; ten-

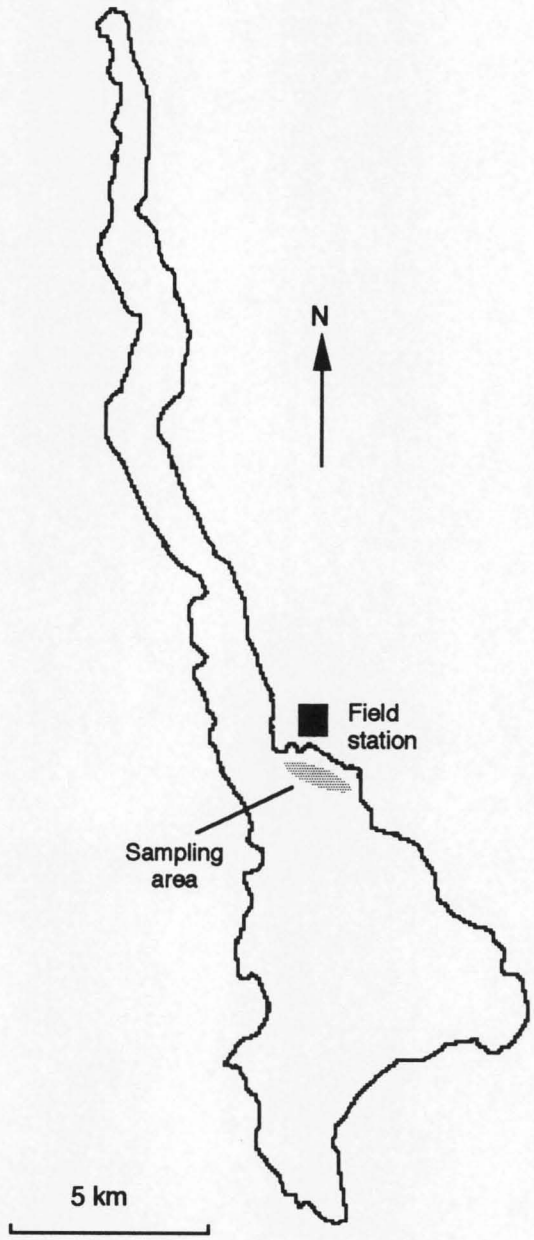


Figure 5.5 Map of Loch Lomond showing zooplankton sampling area

spined stickleback; perch, *Perca fluviatilis*; and the flounder, *Platichthys flesus*. Lyle and Maitland (1994) listed 19 species of fish with recently introduced species: gudgeon, *Gobio gobio*; chub, *Leuciscus cephalus*; dace, *Leuciscus leuciscus* and ruff, *Gymnocephalus cernua* (Giles, 1981).

The avian fauna associated with Loch Lomond is also very diverse with some 200 species recorded to date (Richmond, 1974). Of these species, the following occur in reasonable numbers on the loch and are potential fish predators: Great Crested Grebe, *Podiceps cristatus*; Little Grebe, *Podiceps ruficollis ruficollis*; Heron, *Ardea cinerea*; Tufted Duck, *Aythya fuligula*; Goldeneye, *Bucephala clangula*; Red-breasted Merganser, *Mergus serrator*; Common Gull, *Larus canus*; Black-headed Gull, *Larus ridibundus*; Common Tern, *Sterna hirunda hirundo* and Arctic Tern, *Sterna macrura*.

5.3.3 Sample collection

Samples of zooplankton were collected with nets (Figure 5.6; frame diameter 250 mm; length 0.5 m; mesh 250 μm) from the surface water of Loch Lomond, at the University Field Station, near Rowardennan (Figure 5.5), each month from March 1993 to February 1994. The nets were pulled (Figure 5.6) a distance of approximately 500 m and samples were poured into 400 ml jars. Copepods were counted in 5 ml samples (repeated 5 times) and the number in the whole sample and the number of each species in per cubic metre were then estimated as follows.

$$V = \pi.(r)^2.h$$

Where:

V = total volume of water filtered

r = half-diameter of circle of plankton nets

h = distance which nets were pulled

$$N = \frac{400 \times n}{5}$$

Where:

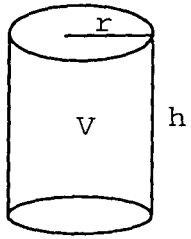
n = individual number of zooplankton species in 5 ml

N = individual numbers of zooplankton species in 400 ml consequently in total volume of water filtered.

Quantitative plankton samples were collected simultaneously for establishing the infection level, especially when number of copepods were low. *Cyclops strenuus abyssorum* was identified by using the keys in Harding and Smith (1974) and individual copepods were examined under the light microscope for infection status. Water temperature (°C) was recorded at the time of sampling. Living copepods had to be immobilised to facilitate examination with a compound microscope. This was achieved by placing a copepod on a cavity slide, withdrawing the water and adding a drop of carbonated water to the slide, thereby anaesthetising the copepods.

5.3.4 Statistical analysis

Differences in prevalence of *Diphyllobothrium* spp. infection in *Cyclops strenuus abyssorum* between seasons was tested by the χ^2 test. Prevalence of infection was correlated with water temperature using the Spearman Rank Correlation Coefficient. Changes in abundance of *Cyclops strenuus abyssorum* between monthly sample in the year was investigated by One-Way ANOVA.



Where:
V = volume of water sieved
h = distance which nets were pulled
r = half diameter of net's circle

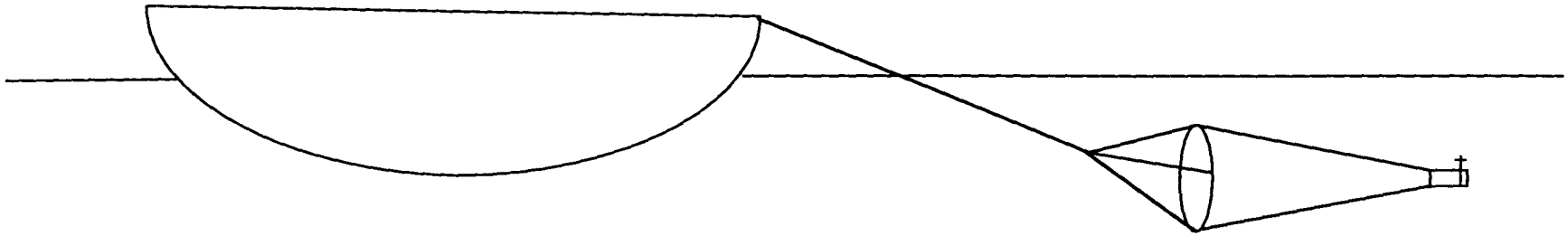


Figure 5.6. Zooplankton sampling technique

5.4 RESULTS

5.4.1 Abundance of species in the zooplankton community

The numbers of zooplankton species per cubic metre each month between March 1993 and February 1994 are given in Table 5.1 and illustrated in Figure 5.7. Overall, the small calanoid copepod *Diaptomus gracilis* (Sars) was found to be the most abundant species in the zooplankton for the most of the year. *Cyclops strenuus abyssorum* (Sars) and *C. leuckarti* (Claus) are common members of the plankton community in late spring and summer until mid-autumn. *Daphnia hyalina* and *Bosmina coregoni* are the main phytoplankton-grazing cladocerans in Loch Lomond (Pomeroy, 1994).

Cyclops strenuus abyssorum occur in plankton throughout the year but its abundance however changes with season (Table 5.1 Figure 5.7; one-way Anova; $F_{11,48} = 463.62$, $p < 0.001$). In general, the number of individuals was higher during the warm months as compared with the cold months ($x^2 = 24.57$, df = 5, $p < 0.001$). A seasonal peak occurred in September and second highest number was recorded late spring with lowest levels in November and January.

Table 5.1 The numbers of zooplankton species per cubic metre in Loch Lomond near Rowardenan.

Months	*C. abyssorum*	*C. leucarti*	*D. gracilis*	*D. hyalina*	*B. coregoni*
March	23	-	2946	8	6
April	32	-	623	12	15
May	173	228	45	6	116
June	111	496	158	163	8
July	85	731	167	264	7
August	27	56	119	99	8
September	276	-	80	8	4
October	52	189	1975	30	-
November	150	7	1025	17	30
December	36	-	1306	75	-
January	8	10	55	2	2
February	6	8	287	10	7

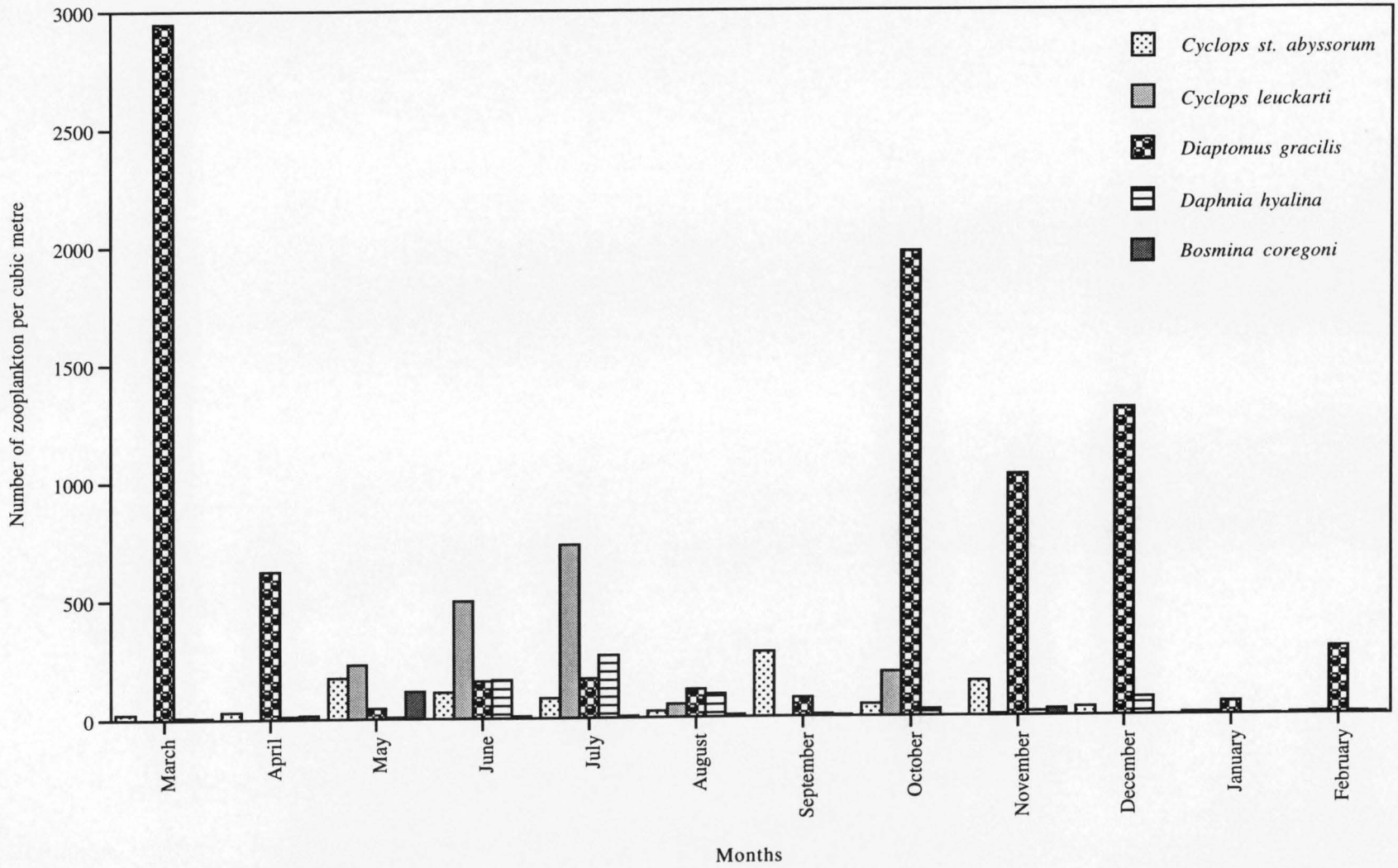


Figure 5.7. Seasonal pattern of the abundance of zooplankton species in Loch Lomond.

Table 5.2 Annual aspects of *Cyclops strenuus abyssorum* infected with procercoids of *Diphyllobothrium* spp. in Loch Lomond near Rowardenan.

Months	No of cyclops examined	Infected	Percentage of infection	Water temperature (C^{o})
March	116	-	-	3.7
April	141	5	3.5	4.3
May	413	16	3.8	10.7
June	290	12	4.1	15.1
July	273	9	3.2	16.0
August	333	8	2.4	15.0
September	534	8	1.5	13.9
October	210	5	2.3	11.0
November	136	4	2.9	8.2
December	38	-	-	6.1
January	63	-	-	3.2
February	55	1	1.8	4.3

5.4.2 Prevalence of *Diphyllobothrium* spp. in *Cyclops strenuus abyssorum*

The proportion of *C. str. abyssorum* infected with *Diphyllobothrium* spp. is given in Table 5.2. The annual cycle of infection level is also shown in Figure 5.8a.

Overall, 68 out of 2602 (2.6%) *Cyclops strenuus abyssorum* were found to harbour the procercoid stage of *Diphyllobothrium* spp. (Figures 5.2 and 5.3). Water temperature at the sampling site varied from about 3-4 °C between January and April and reached seasonal high of 16 °C during June, July and August (Figure 5.8b). The prevalence of a *Diphyllobothrium* spp. in copepods increased with water temperature (Spearman Rank Correlation, $R_s = 0.729$, $n = 12$, $p < 0.01$), reaching its maximum in early spring when the water temperature was rising steeply. The maximum prevalence of infection was recorded in June with 4.1% and the infection was either low or undetectable from December to March ($\chi^2 = 15.45$, $df = 5$, $p < 0.01$). No *Cyclops strenuus abyssorum* was found to be infected with more than one procercoid (Figure 5.2).

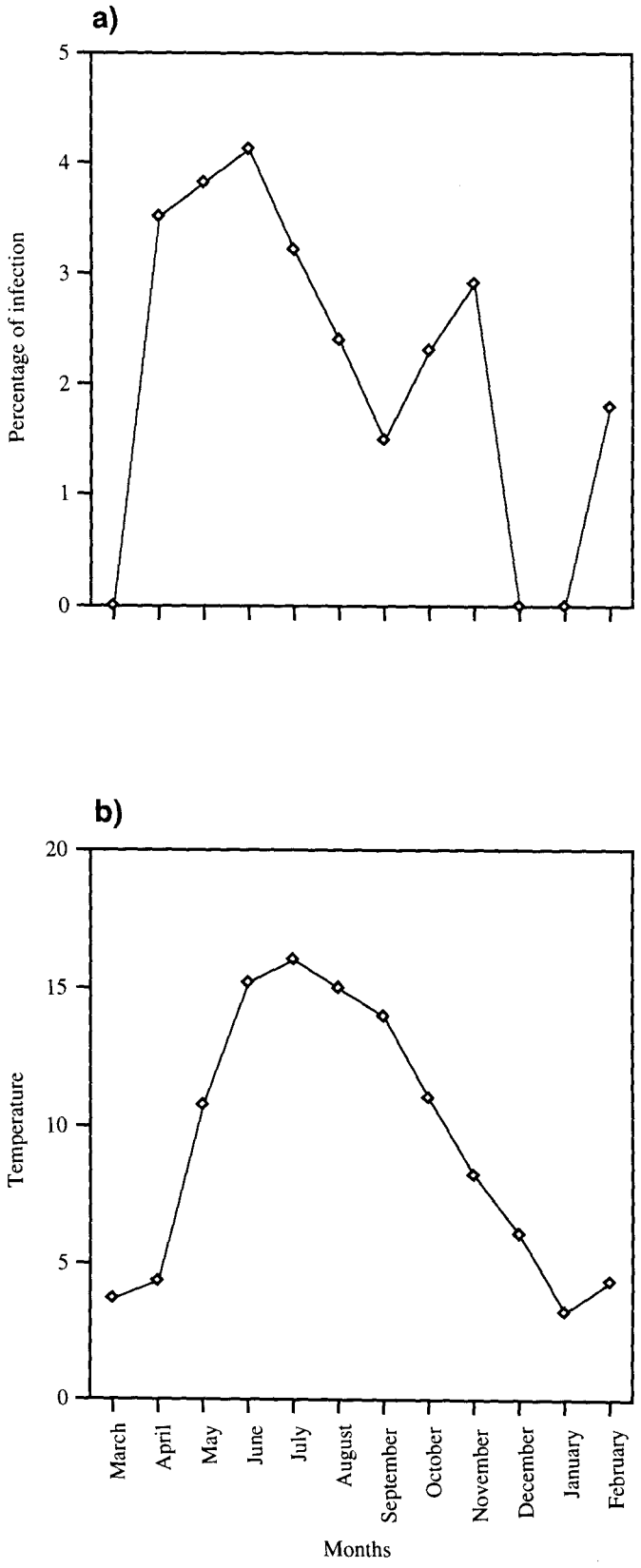


Figure 5.8 Seasonal changes of (a) the prevalence of infected *Cyclops strenuus abyssorum* and (b) surface water temperature of Loch Lomond at Field Station Bay.

5.5 DISCUSSION

5.5.1 Abundance of zooplankton

The results of previous studies on seasonal cycles of cyclopoid copepods in Loch Lomond have been variable. For example, Chapman (1972) affirmed that *Cyclops strenuus abyssorum* was absent from the winter plankton. By contrast, *C. str. abyssorum* was recorded by Maitland *et al.* (1981) and Pomeroy (1987) throughout the winter in the plankton, although in low numbers. Differences in abundance of the other zooplankton species are also evident in the data recorded by Slack and Hamilton (1957); Maitland *et al.* (1981) and Pomeroy (1987). The abundance of *Daphnia* was found to be variable between surveys, with low numbers recorded by Pomeroy (1987). *Holopedium gibberum* was a relatively common seasonal member of the plankton in the loch in the 1970s and 1980s, although not recorded by Slack, 1957b; Hamilton (1958) or Chapman (1965, 1969, 1972). The periodicity of the larger Cladocera in the loch is distinct. Numbers of zooplankton taken in hauls in May 1985 were particularly low compared to those of May in the previous two years (Pomeroy, 1987). It can be concluded that abundance of zooplankton may vary from year to year as in the year.

5.5.2 Infections of *Diphyllobothrium* spp. in first intermediate host

Cyclops strenuus abyssorum and *Diaptomus gracilis* serve as potential first intermediate hosts of *Diphyllobothrium* spp. in Loch Lomond. The work carried out by Hanzelova *et al.* (1989) showed that the intensity of infection of *Proteocephalus neglectus* in its first intermediate hosts did not exceed a value of one procercoid. Only in four *Eudiaptomus zachariasi* and in one *C. vicinus* were two procercoids observed.

In agreement in this study, no *C. str. abyssorum* was found to be infected with more than one plerocercoid of *Diphyllobothrium* spp. This may be due to the low numbers of infective coracidia in Loch Lomond's water.

5.5.3 Effects of temperature on life-cycle of pseudophyllidean cestodes and abundances of zooplankton

The onset of the infection process is connected with two factors. These are the time of discharge of eggs into water and the effect of water temperature on rate of the procercoid development (Hanzelova *et al.*, 1989). The changed temperature regime and the presence of extraneous substances in the natural environment affect the parasite fauna of animals associated with the aquatic environment or modify seasonal cycles of occurrence and development of helminths (Hanzelova, 1992).

The abundance of cyclopoids, the prevalence and intensity of infection with the procercoid and the rate of development of the cestode depend on ecological and climatic conditions. Annual changes in the pattern of copepod infection with *Diphyllobothrium* spp. were primarily dependent on climatic factors (Sysoev, 1987; Hanzelova, 1992). Grabiec *et al.* (1963) demonstrated the positive effects of light and temperature on the hatching of eggs of *D. latum* under experimental condition. According to Kuhlow (1953) and Halvorsen (1966), *Diphyllobothrium* spp. eggs hatched in 8 days at 23 ° C, 10-11 days at 20 ° C and in 48 days at 7 °C; there was no development at 0 °C. The low levels of *Diphyllobothrium* infections in *Cyclops strenuus abyssorum* during cold months in Loch Lomond may therefore be due to low water temperature and also the presence of definitive hosts, as a consequence of the availability of parasite eggs.

The findings reported here agree with data presented by Guttowa (1963), Watson and Lawler (1965), Sergeeva and Freze (1981) and Sysoev (1987) in that copepods infection with procercoids of *D. latum*, *D. dendriticum*, *D. ditremum*, *Triaenophorus nodulosus*, *Schistocephalus pungitii*, *Proteocephalus filicollis* and *Bothriocephalus gowkongensis* occur in high numbers in the plankton during the warm period of the year. The seasonal changes of abundance of cyclopoid copepods can also be explained by alterations in the ecological and climatic conditions in the lake. Adalsteinsson (1979) found seasonal variations in abundance and frequency of crustaceans in Lake Myvatn. The herbivorous cyclopoids, *Paracyclops fimbriatus* and *Eucyclops serrulatus* peaked in June and July-August respectively. Cladocerans peaked in July-August and carnivorous *Cyclops abyssorum* had peaks in May, June and September. *Megacyclops viridis* peaked in July-August. Sergeeva and Freze (1981) identified the procercoid in copepods by examining of the definitive and second intermediate hosts. Infections of *D. dendriticum* and *D. ditremum* in those hosts encouraged them to conclude that procercoids in the copepod from Karelia Lake are of *D. dendriticum* and *D. ditremum*. Thus the seasonal pattern of abundance and level of infection in *C. str. abyssorum* with *Diphyllobothrium* spp. in Loch Lomond can be explained in terms of seasonal patterns of temperature changes.

No seasonal variation of infection of *C. str. abyssorum* with *D. dendriticum* suggested that susceptibility to infection varied with season only in some copepod species (Halvorsen, 1966). It can be assumed that procercoids could occur in all months of the year in natural waters, but the incidence of the procercoid is likely to be minimal during the cold months and maximal during the warm months. In colder areas the definitive hosts have seasonal migrations (Henricson, 1978). It is clear that these hosts

would only be infected, and would only release eggs of parasites into the ecosystem on a restricted seasonal basis. However, in more temperate regions, especially in coastal areas, Laridae may be present all year. In the Hamburg area, Kuhlow (1953) found 5.2 % of *Larus ridibundus* infected with *D. ditremum* in the winter months. However, from November to January, no *Diphyllobothrian* adults were found, but infection occurred in February (6%), March and April (about 20 %). The reason for the appearance of adult worms during these latter months was related to the presence of plerocercoids in *Gasterosteus aculeatus*, and the fact that these fishes became available as food for the gulls from February onward.

5.6 SUMMARY

1. Water temperature in Loch Lomond near Rowardennan varied from 3.2 to 16 °C between March 1993 and February 1994. The lowest value was recorded in January and the highest in July.

2. *Diaptomus gracilis*, *C. str. abyssorum* and *C. leuckarti* are common copepod species and *Daphnia hyalina* and *Bosmina coregoni* the main cladoceran members in the zooplankton in Loch Lomond's shallow water near Rowardennan.

3. Infection of *C. str. abyssorum* with procercoids of *Diphyllobothrium* spp. occurred throughout the year, but infection levels were low in winter compared to the summer months. This may be related to temperature in that eggs may fail to hatch or hatching might be delayed at low temperature.

4. Transmission of *Diphyllobothrium* spp. procercoids in Loch Lomond is provided by *C. str. abyssorum*, although other suitable species may be present.

Chapter 6 Prevalence and intensity of infections of *Diphyllobothrium* spp. in powan, *Coregonus lavaretus*, from Loch Lomond*; relation to host size, sex and growth rate.

6.1 INTRODUCTION

6.1.1 Classification of *Diphyllobothrium spp.*

The difficulty of identifying plerocercoids of *Diphyllobothrium* spp. is well appreciated by many parasitologists (Hillard, 1960; Fraser, 1960; Hoffman and Dunbar, 1960; Vik,1964; Chubb 1968; Andersen, 1971; Andersen, 1975; Andersen and Halvorsen,1978; Chubb 1980 and Andersen *et al.*, 1987). Most authors have used morphological criteria for their assessment of the taxonomic status of different species. Recent papers published by Chubb, 1980; Chubb *et al.*, 1987; Anderson *et al.*, 1987; Andersen and Gibson, 1989 and are all in agreement that three species (*Diphyllobothrium dendriticum*, *D. ditremum* and *D. latum*) predominate in the northern hemisphere. Sharp (1990) indicated that *Diphyllobothrium canadense*, *D. cordiceps*, *D. exile*, *D. fissiceps*, *D. laruei*, *D. oblongatum*, *D. strictum*, *D. medium*, *D. norvegicum*, *D. sebago* and *D. ursi* could now be considered as synonymous with *D. dendriticum* and *D. osmeri* as synonymous with *D. ditremum*. The life cycles (Chapter 5) of *D. dendriticum* and *D. ditremum* are almost identical, copepods acting as the first intermediate host. However, salmonid fish are the second intermediate host and birds are the final hosts; the life cycle of *D. latum* is slightly different in that non-salmonid fish usually act as second intermediate host (although it is found in salmonid fish) and mammals as the final host (see Appendix II).

(*) Powan become available for study during my efforts to sample brown trout and other fish from Loch Lomond. When a net is set for trout in Loch Lomond, it is inevitable that powan will be caught. Since most powan die once netted, it seemed reasonable to examine them for parasites.

Diphyllobothrium dendriticum plerocercoids are usually found in cysts adhering to or encapsulated within the visceral organs of their host fish (Curtis, 1984). This situation is in contrast with *D. latum* which frequently lies unencysted in fish musculature (von Bosdorff, 1977). Plerocercoids of *Diphyllobothrium dendriticum* most often occurs within a creamy-white cyst 2-10 mm in diameter, adhering to or embedded in the stomach or liver. Less commonly, a plerocercoid may be found in or on the intestine wall, gonads or kidney or on the parietal peritoneum adjacent to the body wall (Curtis and Bylund, 1991). The fact that the intermediate host of *Diphyllobothrium latum* is usually a non-salmonid and the particular site of infection mean that it is relatively easy to distinguish it from other species.

According to Chubb (1968) seven species of *Diphyllobothrium* (*D. latum, D. dendriticum, D. ditremum, D. osmeri, D. vogeli, D. medium* and *D. norvegicum*) have been reported in western and northern Europe since 1949 and only five of these *D. latum, D. dendriticum, D. ditremum, D. medium* and *D. norvegicum*) have been reported in the British Isles. This may be an overestimate; examination of adult worms from natural and experimental infections and plerocercoids from natural infections showed that only three of these species are valid, namely *D. latum, D. dendriticum* and *D. ditremum* (Chubb, 1968). The worms described as *D. osmeri* and *D. vogeli* by Kuhlow (1953) were considered the primary strobila of *D. ditremum*. *Diphyllobothrium medium* as redescribed by Fraser (1960) and *D. norvegicum* as described by Vik (1957) are believed by Chubb (1968) to be *D. dendriticum*.

6.1.2 The formal classification of *Diphyllobothrium* spp. is as follows:

Phylum	**Platyhelminthes**
Class	**Cestoidea**
Order	**Pseudophyllidea**
Family	**Diphyllobothriidae** Luhe, 1910
Genus	***Diphyllobothrium*** Cobbold, 1858
Species	***dendriticum*** (Nitzsch, 1824)
	ditremum (Creplin, 1825)
	latum (Linnaeus, 1758)

6.1.3 Distribution of *Diphyllobothrium* spp.

According to Sharp (1990) plerocercoids belonging to the genus *Diphyllobothrium* were first reported in the British Isles by Chaloner (1912) from an epizootic in brown trout, *Salmo trutta*, in Loch Morar, Scotland (Sharp, 1990). Later, Hickey and Harris (1947) and Fraser (1950) described the distribution of *Diphyllobothrium* plerocercoids in relation to length and age of fish in two outbreaks in Ireland and England. In Norway, Vik (1957) investigated the distribution of *D. norvegicum* (=*D. dendriticum*) in trout, *Salmo trutta*, and char, *Salvelinus alpinus*, in relation to age size and sex. Halvorsen (1970) provided information on the relationships between length and intensity of infection with *Diphyllobothrium* sp. in three-spined stickleback, *Gasterosteus aculeatus*, from Lake Storvatn. Bylund (1972) in Finland

investigated the correlation between length of whitefish, *Coregonus lavaretus*, and the number of cysts of *D. dendriticum*. Henricson (1977) studied the abundance and distribution of *D. dendriticum* and *D. ditremum* in the char in Sweden with regard to age, size and lake regions, and noted that *Diphyllobothrium ditremum* was more common than *D. dendriticum*, and that infection increased as the age and size of the fish host increased. Revenga (1993) studied the occurrence and co-occurrence of *D. dendriticum* and *D. latum* and their association, abundance, distribution, pathogenic effects and risk of human infection in Lake Moreno, southern Argentina. According to this study, brook trout, *Salvelinus fontinalis*, and rainbow trout, *Oncorhynchus mykiss*, harboured both species, perch, *Percichthys* sp., harboured only *D. latum* and pejerrey, *Patagonina hatcheri*, were not infected. *Diphyllobothrium latum* was less abundant than *D. dendriticum*. No evidence were observed to indicate that either of the species is harmful to the host. *Diphyllobothrium dendriticum* was found in the liver, pyloric caeca and stomach; *D. latum* was found only in the liver. The most recent literature concerning the distribution of *Diphyllobothrium* species indicated that *D. dendriticum* and *D. ditremum* appear to be widely distributed in the British Isles (Kennedy, 1974 and Chubb *et al.*, 1987)

6.1.4 Biology of the powan

Of the four species of Coregonine that live in the British Isles, three are glacial relict populations isolated in freshwater lakes. *Coregonus pollan* occurs in Loughs Neagh, Erne, Ree and Derg in Ireland. This species is more closely related to the ciscoes of North America than to other British coregonines (Ferguson, 1974; Ferguson *et al.*, 1978; Brown and Scott, 1994). *Coregonus albula* is now restricted to Bassenthwaite and

Derwentwater in Cumbria, having recently become extinct in Scotland. *Coregonus lavaretus* occurs in Red Tarn , Ullswater and Haweswater in Cumbria, in Llyn Tegid in Wales and in two lochs in Scotland, Loch Lomond and Loch Eck.

6.1.5 Parasites of powan

Hickey and Harris (1947) demonstrated that the degree of infection of trout with *Diphyllobothrium* increased in proportion to the size of the trout; fish less than 250 mm long being uninfected. They found a positive correlation between infection with *Diphyllobothrium* spp. and length of fish. Apart from fish killed by infection with *Diphyllobothrium dendriticum*, they examined 146 live trout and of these 18 were less than 250 mm in length and were all uninfected, 28 were over 400 mm in length and were all infected while the remaining 100 fish showed increasing infection with increasing length.

Although Chubb (1980) did not mention in his list Loch Lomond as a site for *Diphyllobothrium* spp., Copland (1957) studied the parasites of Loch Lomond fishes and observed plerocercoids encysted around the powan stomach. He identified the plerocercoids from those cysts as *D. dendriticum* and *D. ditremum.* Copland (1957) also concluded that the fishes of Loch Lomond harbour a wide variety of helminth parasites and that for many, little is known concerning life histories, habitats and their effects on the health of their fish hosts. Thus, he indicated that there was abundance of material which is available for the study of the ecology of these parasitic helminths. Most recently, Sharp (1990) examined 4 brown trout and 16 powan from Loch Lomond and found 50 % and 31 % infection of *Diphyllobothrium dendriticum* in brown trout and in powan respectively. To my knowledge, no detailed study has been carried out on

helminth parasites of fish in Loch Lomond since Copland (1957). The main aim of this study was therefore to examine the ecology of parasitic helminths in the Loch.

6.1.6 Powan in Loch Lomond

According to Brown and Scott (1994), the first scientific studies of the ecology of powan in Loch Lomond were carried out by Lamond (1922). Slack (1957) studied the biology of powan in Loch Lomond. Maitland (1969) extended these studies, with particular emphasis on reproduction and fecundity, predation and conservation. Recently Adams (1994) has investigated the effect of the introduction of alien species into Loch Lomond on the powan population. Apart from short term fluctuations, the length of powan seems to have been consistent for a long time. According to Brown and Scott (1994), MacGibbon and Parnell (1938) described a specimen 355 mm long and stated that they occasionally grew to 406 mm. The largest specimen seen by Lamond (1911) was 432 mm in total length, which suggested that maximum size has not changed much over the last 80 years.

The population size of powan in Loch Lomond is not known, but they are very numerous. Brown and Scott (1994) cited from Lamond (1922), who remarked that culling a quarter of a million powan from Loch Lomond had no appreciable effect on the total population. The only documented change in abundance of powan occurred in 1968, when the population suffered massive mortality associated with fungal infection and an epidemic of "bald spot disease" (Roberts *et al.*, 1970).

Powan feed mainly on plankton, especially on cladocerans and copepods (Bauer, 1970). In Loch Lomond, powan consume zooplankton as the main part of their diet (Slack, 1957; Pomeroy, 1987, 1991; Maitland and Campbell, 1992; Pomeroy 1994;

Brown and Scott, 1994; Figure 6.1) from May to September. For the rest of the year take only small amounts of benthic species (mainly *Pisidium* sp. and chironomid larvae). From October to April many individuals have empty alimentary canals. Slack (1957) found that powan stomach contents consisted of 90 % zooplankton, especially Cladocera and Copepoda. Later, Maitland and Campbell (1992) and Pomeroy (1994) made a similar observation.

6.2 AIMS

The aim of the work described in this chapter was to extend knowledge of the ecology of *Diphyllobothrium* spp. (Cestoda: Pseudophyllidea) infection in Loch Lomond by investigating (1) the prevalence and intensity of *Diphyllobothrium* infections in relation to host size and sex and (2) the relationship between host size and degree of infection.

6.3 MATERIALS AND METHODS

Powan were caught in gill nets of various length and mesh sizes ranging from 10 mm to 40 mm knot to knot. The nets which had been set to catch trout and other fish, were set at a depth of approximately 10m in Loch Lomond, near Rowardennan between 1992 and 1995. Rowardennan is on the eastern shore of the middle basin of Loch Lomond (Fuller *et al.* 1976). Nets were set around the midday, lifted 24 hr later and any fish found to be still alive were anaesthetised by exposure to an overdose of benzocaine where upon they died immediately. They were transferred to the laboratory in Glasgow and *post-mortem* examination was carried out. The worms recovered were fixed in A.F.A (see Chapter 2). Morphological features were studied by scanning

electron microscopy and histological techniques. The publications by Andersen *et al.* (1987); Chubb *et al.* (1987); Yamane *et al.* (1989) and Chubb (pers. comm.) were used to identify the plerocercoids as *D. dendriticum* and *D. ditremum*.

Fork length (nearest mm) and weight (nearest g) were recorded and sex of the fish determined (Table 6.1). The fork length of powan used in the present study ranged from 180 mm to 360 mm, from 127 mm to 360 mm, from 175 mm to 360 mm and from 150 mm to 353 mm for 1992, 1993, 1994 and 1995 respectively. Overall, the length of most of powan examined during this study were between 260 mm and 330 mm (Figure 6.3).

6.3.1 Statistical analysis

The data were checked for normality and transformed where necessary. For normally distributed variables parametric statistics were used and for non-normally distributed variables non-parametric statistics were used. Differences in prevalence of infection between and within years were tested by the χ^2 test. Seasonality of *Diphyllobothrium* spp. infection were tested by One-Way ANOVA. Length classes were correlated with parasite burden in fish using the Product Moment Correlation Coefficient.

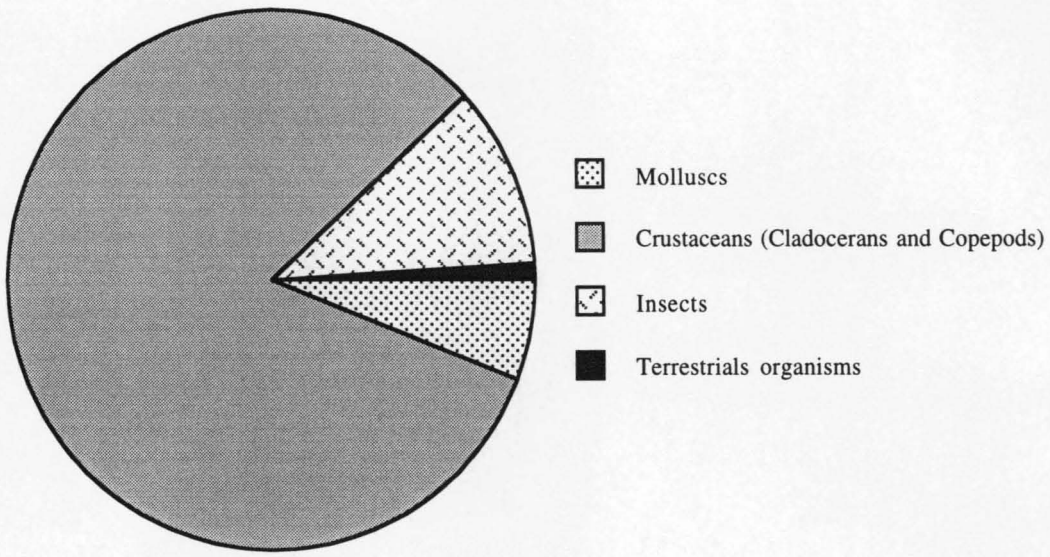


Figure 6.1. Stomach contents of powan from Loch Lomond as percentage of volume (data taken from Maitland and Campbell).

Table 6.1 Prevalence and intensity of *Diphyllobothrium* spp. infections in powan of Loch Lomond from October 1992 to August 1995.

Date	No fish examined	Infected	% infection	M	F	M:F ratio	Total parasite	Mean intensity	SE	% infec. in M	% infec. in (F)	Mean inten.M	Mean inten. F	s^2/x
Oct. 1992	19	11	58	14	5	2.8:1	155	14.0	3.5	50	80	12.4	17.0	29.1
Oct. 1993	17	13	76	11	6	1.8:1	168	12.9	2.5	90.9	50	13.4	11.3	10.9
Mar. 1994	19	6	32	10	9	1.1:1	19	3.2	0.4	40	22.2	2.0	5.5	4.1
June 1994	8	2	25	7	1	7:1	19	8.0	1.7	14.2	-	5.0	14.0	10.5
July 1994	7	1	14	5	2	2.5:1	1	1.0	0.1	20	0	1.0	0.0	1.0
Sept. 1994	8	6	75	3	5	0.6:1	26	4.3	1.1	66.6	80	2.6	4.5	3.2
Oct. 1994	22	11	50	10	12	0.8:1	242	22.0	4.4	40	58.3	20.7	22.7	38.6
Dec. 1994	25	7	28	9	16	0.6:1	73	10.4	1.3	22.2	31.2	3.5	13.2	14.8
Nov. 1994	17	9	53	5	12	0.4:1	75	8.3	2.0	60	50	7.0	9.0	15.6
Jan. 1995	19	10	53	11	8	1.4:1	153	15.3	5.5	70	37.5	6.1	36.6	72.5
April 1995	8	2	25	4	4	1:1	3	1.5	0.2	25	25	2.0	1.0	1.4
May 1995	12	10	83	5	7	0.7:1	94	9.4	4.5	100	71.4	2.2	16.6	31.2
June 1995	10	3	30	4	6	0.6:1	10	3.3	0.6	25	25	2.0	4.0	4.8
Aug. 1995	18	8	44	9	9	1:1	30	3.7	0.5	33.3	55.5	3.6	3.8	2.7

Figure 6.2 Powan infected with *Diphyllobothrium* spp.

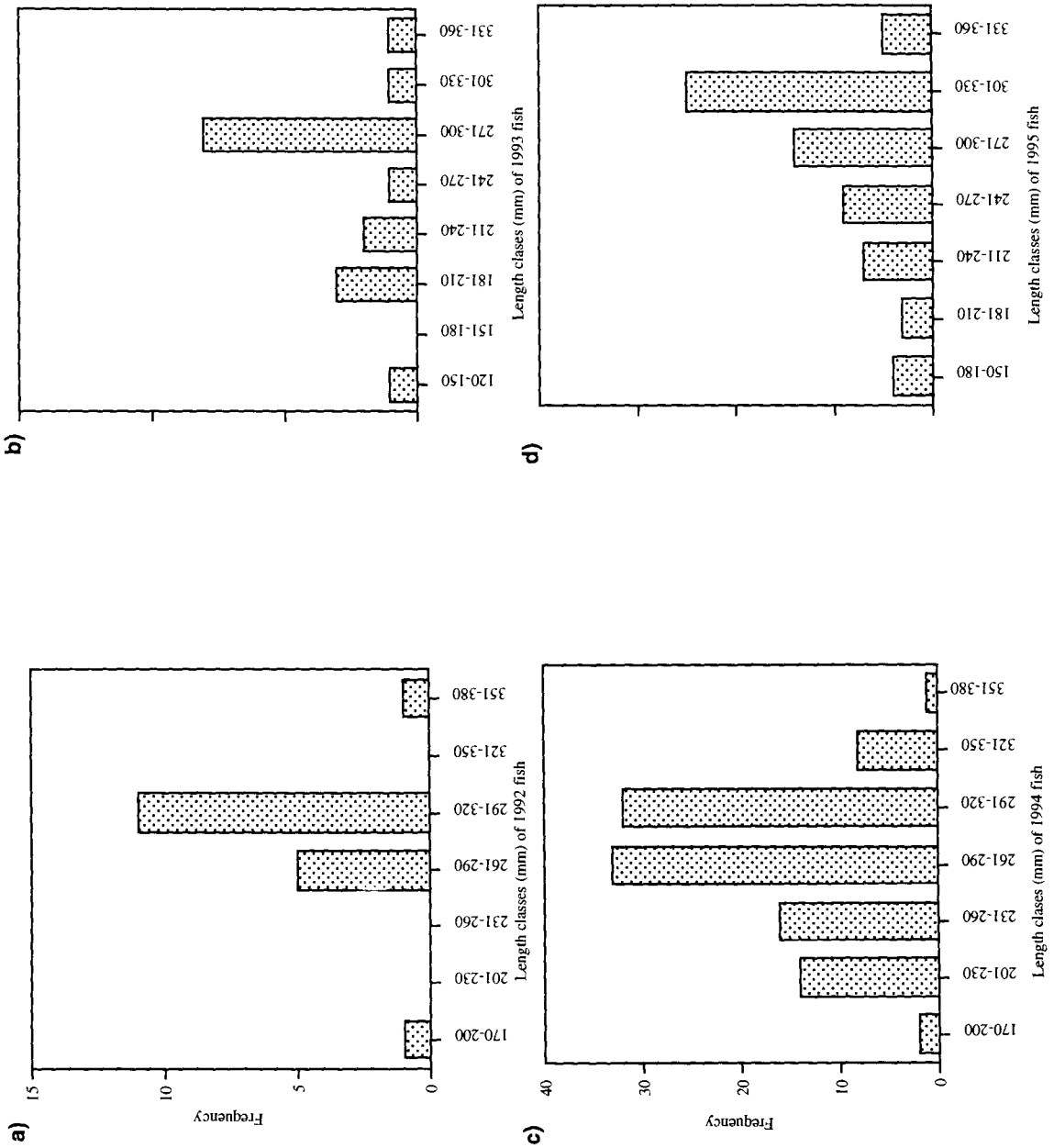


Figure 6.3 Frequency of length classes of powan caught in (a) 1992, (b) 1993, (c) 1994 and (d) 1995

6.4 RESULTS

6.4.1 *Diphyllobothrium* spp. infections in powan of Loch Lomond

Post-mortem examination showed that the powan in Loch Lomond are frequently infected with plerocercoids of *Diphyllobothrium* spp. Both *D. dendriticum* and *D. ditremum* were abundant in the samples of powan from the population in Loch Lomond. Of the 209 powan examined, 99 (47.3%) were found to be infected with plerocercoids of *D. dendriticum* and *D. ditremum*. The cysts found around the stomach of powan were smooth, round creamy and in colour (Figure 6.2). The size of the cysts varied with the size of the encysted plerocercoids reaching a maximum diameter of 10 mm. Each cyst contained one plerocercoid. No evidence of migration of plerocercoids which have been encysted was obtained in the present investigation.

6.4.2 Prevalence of infection

Information about the number of powan examined and the prevalence of *Diphyllobothrium* spp. infection in powan from Loch Lomond between October 1992 and August 1995 is given in Table 6.1. Prevalence of infection in each sampling month is shown in Figure 6.7. Powan in Loch Lomond, are concurrently infected with plerocercoids of both *D. dendriticum* and *D. ditremum* (Figures 6.5 and 6.6, Chubb pers. comm.). The highest prevalence of infection was found in May 1995 with 83% and the lowest in July 1994 with 14%. There were significant differences in the number of infected and uninfected fish between years (χ^2 = 9.26, df = 3, $p<0.05$), as well as between sampling months during the four year study (χ^2 = 28.99, df = 13, $p<0.01$). For example, the highest prevalence of infection was recorded from fish caught in May

Table 6.2 Abundance of *Diphyllobothrium* and mean condition factor of infected and uninfected fish in four years in Loch Lomond.

Years	No of infected	No of uninfected	Total no of parasites	Mean intensity	Infected condition $\bar{X}$ ±SD	Uninfected condition $\bar{X}$ ±SD
1992	11	8	155	14.0	1.31±0.27	1.36±0.24
1993	13	4	168	12.9	1.26±0.30	1.49±0.18
1994	42	64	455	10.8	1.31±0.35	1.30±0.26
1995	33	34	290	8.7	1.12±0.42	1.37±0.80

Table 6.3 Results from Mann-Whitney test between number of parasites in individual male and female fish.

Years	W	n (m,f)	p=	
1992	131.0	14,5	0.41	n.s
1993	112.0	11,6	0.20	n.s
1994	2423.5	49,57	0.15	n.s
1995	1116.5	33,34	0.94	n.s

Table 6.4 Results from Mann-Whitney test between condition factor of infected and uninfected powan from Loch Lomond.

Years	W	n	p=	
1992	73.0	11,8	0.59	n.s
1993	46.0	13,8	0.28	n.s
1994	3483.0	42,64	0.70	n.s
1995	1341.0	33,34	0.02	s.n

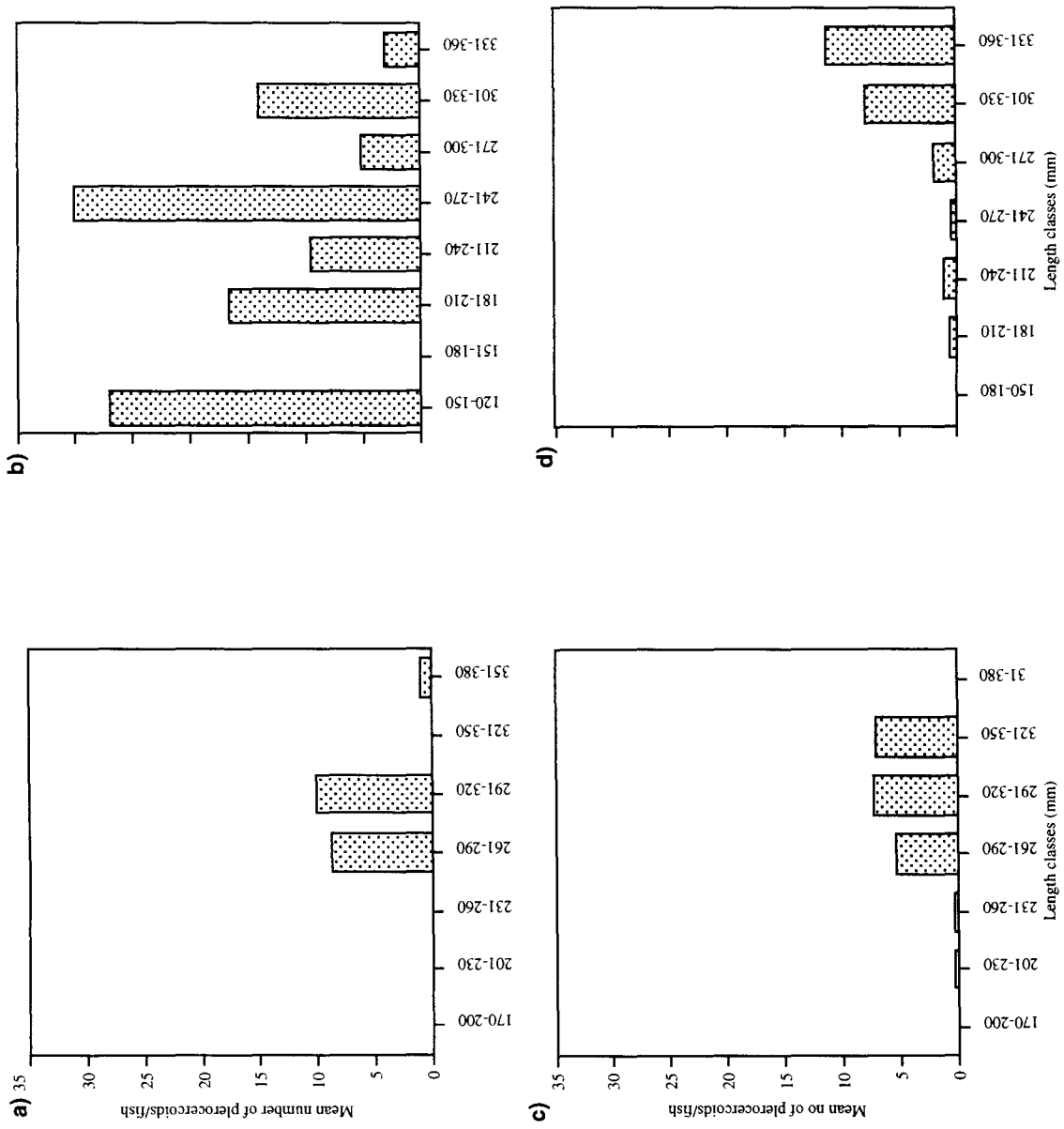


Figure 6.4 Mean number of plerocercoids in each length-classes for (a) 1992, (b) 1993, (c) 1994 and (d) 1995

Figure 6.5 Scanning electron micrograph of *Diphyllobothrium dendriticum*. Magnification x 105.

Figure 6.6 Scanning electron micrograph of *Diphyllobothrium ditremum*. Magnification x 105.

1995 and the lowest in July 1994. Overall, the number of male powan examined were higher than female (Table 6.1). No statistically significant differences were observed between the prevalence in male and female fish during the four year period (Table 6.3). Hence, It can be assumed that the gender of the host has no influence on infection of *Diphyllobothrium* spp. in powan of Loch Lomond.

6.4.3 Intensity of infection

The mean intensity (number of plerocercoids in each infected fish) and overdispersion ($s^2/\bar{x}$) were calculated for each sampling period and are given Table 6.1. The frequency distribution of numbers of plerocercoids of *D. dendriticum* and *D. ditremum* in the powan in Loch Lomond was found to be overdispersed. The mean intensity of infection varied from 1.0 to 22.0 plerocercoids/per infected fish during the four year study period. Interestingly, both high and low intensity of infections were recorded in 1994 samples. For example, the highest intensity was recorded in October 1994 and lowest July 1994. By comparing years, the intensity of infection was found to be markedly higher in 1992 and 1993 than in 1994 and especially than in 1995 (Table 6.2). As may be expected for parasites that survive for many years in their hosts, there was no evidence of seasonal periodicity in intensity of plerocercoids in powan from Loch Lomond (one-way ANOVA, $F_{13,\ 195} = 1.48$, $P = 0.129$). For the exception 1995 ($P = 0.02$), there was no evidence to indicate that *Diphyllobothrium* plerocercoids had any negative effect on the condition of powan in Loch Lomond (Table 6.4). Also the intensity of plerocercoids of *Diphyllobothrium* spp. had no effect on fish condition.

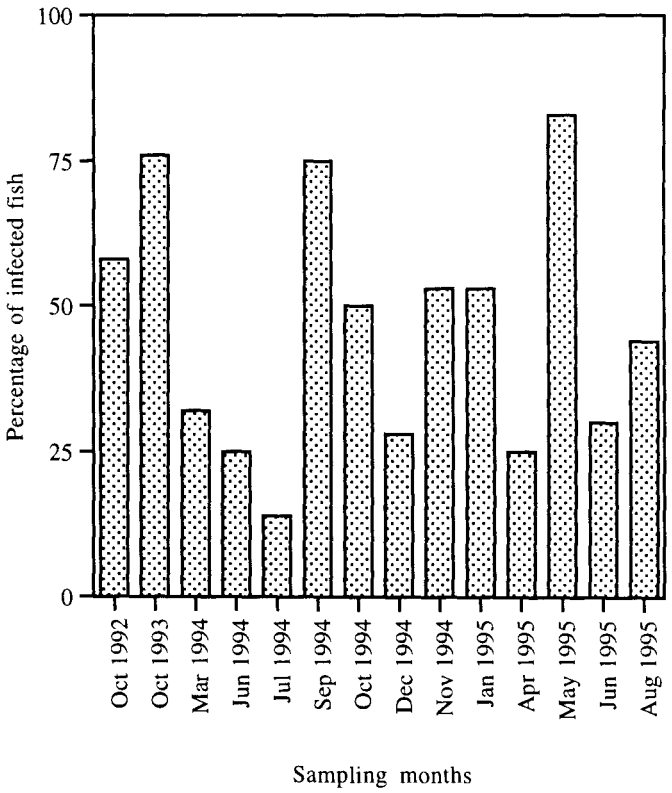


Figure 6.7 Percentage of infected fish in each sampling month between October 1992 and August 1995.

6.4.4 Host size and parasite intensity interaction

The overall intensity of infection was high in fish with a length range from 260 mm to 360 mm with the exception of 1993 fish (Figure 6.4). The degree of infection in the powan also showed a variation with the size of the fish, there being an increase with increasing length (Figure 6.4). Correlation between length classes from all fish and total parasite burden showed that bigger fish had more parasites ($R = 0.965$, $P > 0.001$). The greatest number of plerocercoids recovered from a powan in Loch Lomond during the study period was 105.

6.5 DISCUSSION

6.5.1 Prevalence and intensity of infection

The investigation revealed extensive infection of the powan in Loch Lomond, with plerocercoids of *Diphyllobothrium dendriticum* and *D. ditremum* often occurring concurrently. This is contrary to Sharp (1990) who found that powan in Loch Lomond were only infected with *D. dendriticum*. It was noted that *D. dendriticum* is more common in powan of Loch Lomond than *D. ditremum*. The intensity of infection varied with size of the fish, generally being absent or rare in small fish and heavy in bigger fish. As found by Andersen *et al.* (1987), the plerocercoids examined in this study were in irregular shaped cysts of varying size located on the viscera.

6.5.2 Impact of *Diphyllobothrium* plerocercoids on fish host

It has been reported that *Diphyllobothrium* spp. are potentially among the most pathogenic of fish parasites and serious losses of salmonid fish (Duguid and Sheppard, 1944; Hickey and Harris, 1947; Fraser, 1960) which occurred in certain reservoirs in

British Isles were due to heavy infections with the plerocercoid stage of this tapeworm. Migrating larvae of *Diphyllobothrim*, particularly in small fish, can cause much tissue damage and even mortality (Hoffman and Dunbar, 1961). Most recently, Dick and Choudhury (1995) put *Diphyllobothrium dendriticum*, *D. latum* and *Eubothrium* spp. (see Chapter 7) into pathogenic fish tapeworms for salmonid fish in their table. Almost all the outbreaks have occurred or else have increased in intensity in the summer months. According to Hickey and Harris (1947), the majority of fish in the Poulaphouca Reservoir, Ireland, died between May and September. Fraser (1960) recorded that the greater number of trout died during the months of July and August. The existence of seasonal fluctuation led Hickey and Harris to suggest that high temperature increases the activity of the plerocercoids causing them to leave the cysts and migrate through the tissues, giving rise to a peritonitis. An effect of temperature on worm activity was demonstrated by placing plerocercoids into physiological saline at different temperatures; the worms in saline were non-motile at 10 °C, sluggishly motile at 12 °C and active at 14 °C. Plerocercoids in the viscera of a heavily-infected trout kept for several days in saline at 5 °C; remained within their cysts and showed no evidence of activity. When the temperature of the viscera raised to 15 °C many of the plerocercoids immediately became active and within a few minutes had left their cysts. Such effects of temperature readily explains why *Diphyllobothrium* induced mortality is greatest in the warmer months.

Becker and Brunson (1967) stressed that infections with helminth parasites may contribute to the mortality of fish directly by mechanical injury to organs and tissues and indirectly through traumatic stresses that lower host resistance to environmental factors. Henricson (1977, 1978) recorded mortality of *Salvelinus alpinus* in Lake

Bjellojaure, Sweden due to heavy infection of *D. dendriticum* between May and July. Duguid and Sheppard (1944) recovered as many as 300 plerocercoids of *Diphyllobothrium* in each dead trout from South Wales and Hickey and Harris (1947) from 100 to 300 in Poulaphouca Reservoir, Ireland. Sharp (1990) pointed out that plerocercoid worm burdens vary but it is not uncommon to find fish containing between 500 and 1000 plerocercoids of *Diphyllobothrium* spp. In the present study, the highest worm burden was 105 plerocercoids. In Loch Lomond, no evidence was observed to indicate the mortality or even the serious loss of condition of the fish caused by *Diphyllobothrium* infections. It may therefore be concluded either that the numbers of plerocercoids of *Diphyllobothrium* are insufficient to cause mortality or even serious loss of condition of the fish or water temperature of Loch Lomond (see Chapter 5) does not stay warm enough to induce activity of plerocercoid even during summer months. Also powan, *Coregonus lavaretus* has a well-developed defence mechanism, the plerocercoid rapidly enclosed in cysts and even a heavy infection does not cause any external symptoms (Bylund, 1972). Therefore, the harmful effects of *Diphyllobothrim* spp. may be inversely related to the resistance of the host and the ability of the host to inactive the plerocercoids through encystment.

A predominant feature of the host-parasite interaction in all *Diphyllobothrium* species is an intense inflammatory response around developing plerocercoids leading to complete encapsulation of the parasites (Sharp, 1990). Fraser (1960) noted haemorrhage from the abdominal pore in heavily infected trout found in a dying condition in the west of England. It has also stated that some parasites of whitefish cause a delay of growth rate, decrease of the fish condition, the fat content and other physiological indices which negatively influence the productivity of fishes (Bauer, 1970).

Previous studies (e.g. Fraser, 1960) indicated that in heavy infections of trout with *Diphyllobothrium*, cysts exist on liver, gonads, surface of swim-bladder, spleen and occasionally the abdominal musculature apart from stomach surface and pyloric caeca. In this study, cysts of *Diphyllobothrium* spp. were found only on the stomach surface and pyloric caeca of powan (Figure 6.2). The above description might be valid for trout infected with *Diphyllobothrium dendriticum* cysts.

6.5.3 Cellular response of powan to *Diphyllobothrium* spp. infections

The host cellular response to tapeworm infections may result in total encapsulation of parasite as in *Diphyllobothrium* spp. in powan (Figure 6.8) or partial encapsulation as in *Ligula* (Dick and Choudhury, 1995). Encapsulation is a general phenomenon and occurs on the stomach of powan with *Diphyllobothrium* spp. (Figure 6.2). With time, cellular activity decreases as the parasite is encapsulated and isolated. This host response may reduce the impact of the parasite on the host. In this respect, Bylund (1972) suggested that the pathogenicity of *Diphyllobothrium dendriticum* depends on the host's defence mechanism. Fish with weak or no cellular response , for example, *Coregonus albula*, have more lesions which lead to greater mortality than fish, such as *C. lavaretus*, which produces a strong cellular encapsulation response. Although the parasites are encapsulated, they often live as long as their hosts. Adhesions,

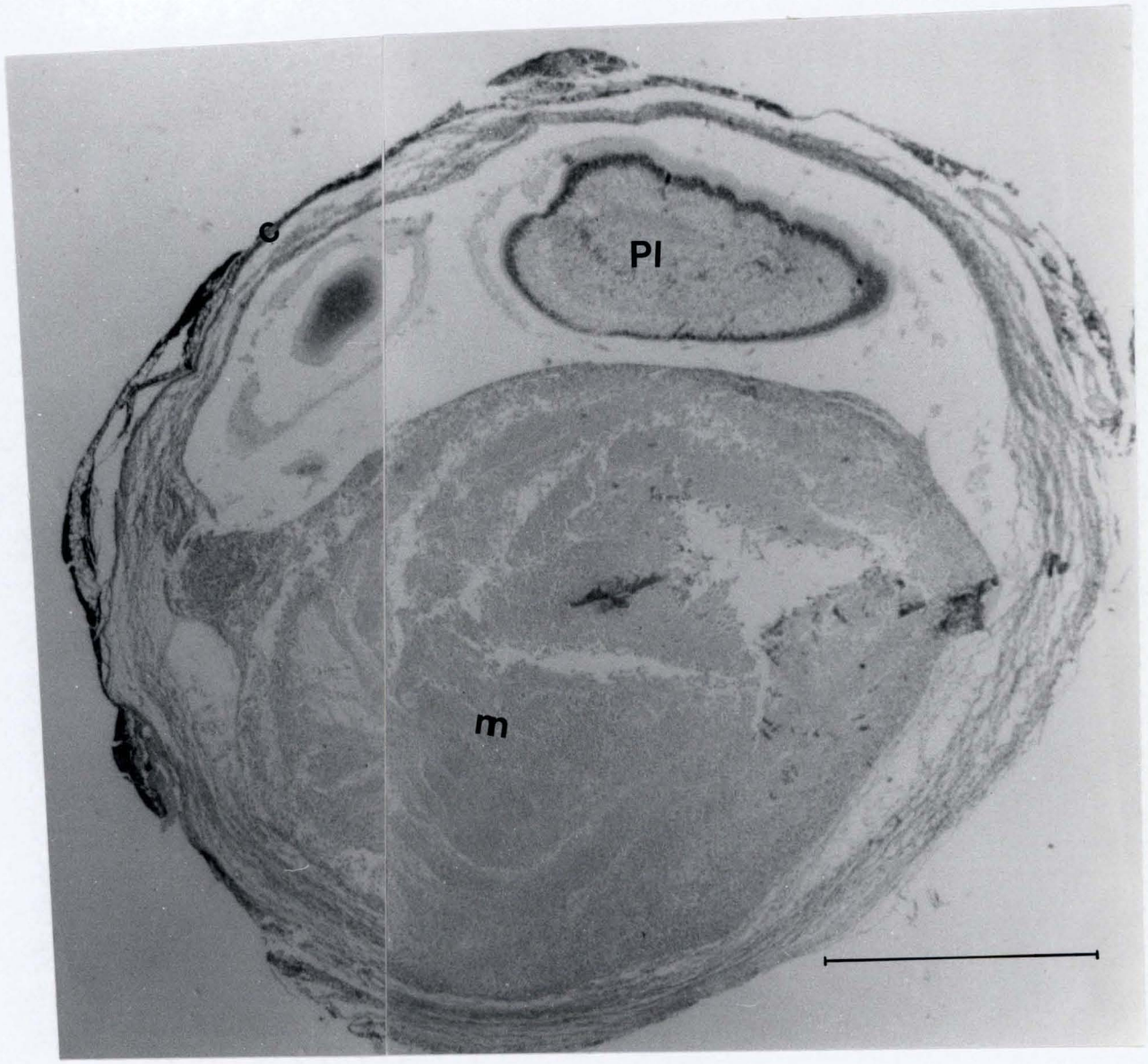


Figure 6.8 Cross section of a *Diphyllobothrium* spp. cysts. (Pl) plerocercoid, (m) non-cellular mass, (c) connective tissue. Bar represents 1mm.

haemorrhaging, discolorations of visceral tissues, reduced adipose tissue in parasitized fish could not be observed in infected powan with *Diphyllobothrium* spp. from Loch Lomond. This does not agree with observations by Weiland and Meyer (1989) in coho salmon, *Onchorhynchus kisutch* infected with *Diphyllobothrium ditremum*, 1989. Along with previous studies, observations on gross pathology of infected powan showed that *Diphyllobothrium* spp. is not as harmful in powan as it is in other salmonids.

6.6 SUMMARY

1. Powan, *Coregonus lavaretus*, from Loch Lomond was found to be infected with plerocercoids of *Diphyllobothrium* spp.

2. These high prevalence and intensities are related to the fact that powan feed mainly on zooplankton.

3. The sex of the fish has no influence on the prevalence or intensity of infection.

4. There was no evidence that infection with *Diphyllobothrium* plerocercoids was harmful to powan in Loch Lomond, in terms of causing major tissue damage.

Chapter 7 The occurrence of *Eubothrium crassum* (Bloch, 1779) (Cestoda: Pseudophyllidea) in farmed salmon, *Salmo salar.*

7.1 INTRODUCTION

7.1.1 Distribution and life-cycle of *Eubothrium crassum*

The adult stage of *E. crassum* is a common parasite of salmonid fish from Europe and North America (Wardle and Mcleod, 1952; Hoffman, 1967; Wootten, 1972; Kennedy, 1974, 1978a; Dorucu *et al.* 1995 (see also Chapter 3)). According to Wardle and Mcleod (1952) the length of *E. crassum* in Atlantic salmon, *Salmo salar* and trout, *Salmo trutta*, in Europe and Canada measures between 120-600mm, but Wootten (1972) recorded the length of the worms from 10mm to 996mm in trout, *Salmo trutta*, from Hanningfield Reservoir. Two races of *E. crassum* are found in the British Isles, one in freshwater *Salmo trutta* and the other in anadromous *Salmo salar* and *Salmo trutta trutta* (Andersen and Kennedy, 1983).

Mature segments at the posterior end of adult worms discharge thousand of eggs into the environment. These hatch soon after reaching water, to release free swimming coracidia. The coracida are ingested by cyclopoid copepods which act as the first and possibly only intermediate host for this parasite. In the copepod, the coracidium sheds its coat to become a procercoid. This then develops within the body cavity of the copepods to a plerocercoid, a more advanced larval stage of tapeworm (Hoffman, 1967; Wardle and Mcleod, 1957). Plerocercoids infect their definitive host either through direct ingestion of the copepod intermediate host, or through transmission via a paratenic host. In the latter case, the copepod might be ingested by a perch or a stickleback which is subsequently

preyed upon by the final host. In the final host, the plerocercoid lodges itself in the wall of a pyloric caecum by means of its scolex and develops to a mature tapeworm (Mitchell, 1993; Figure 7.1).

7.1.2 Classification of *Eubothrium crassum*

Phylum	**Platyhelminthes**
Class	**Cestoda**
Order	**Pseudophyllidea**
Family	**Amphicotylidae** Nybelin, 1922
Subfamily	**Amphicotylinae** Luhe, 1900
Genus	***Eubothrium*** Nybelin, 1922
Species	***crassum*** (Bloch, 1779)

7.1.3 Origin of *Eubothrium* infections in sea-run salmon

Tapeworms of the genus *Eubothrium* have been of special interest to parasitologists and fishery biologists because some races infect marine hosts, some freshwater hosts and some are capable of living in hosts from both habitats (Kennedy, 1978a). *Eubothrium crassum* is of particular interest; its marine intermediate host is unknown. There are different theories about the origin of *Eubothrium crassum* infection in sea-run salmon. One is that the fish are infected as smolts or parr in freshwater or estuaries and the parasites are carried to sea

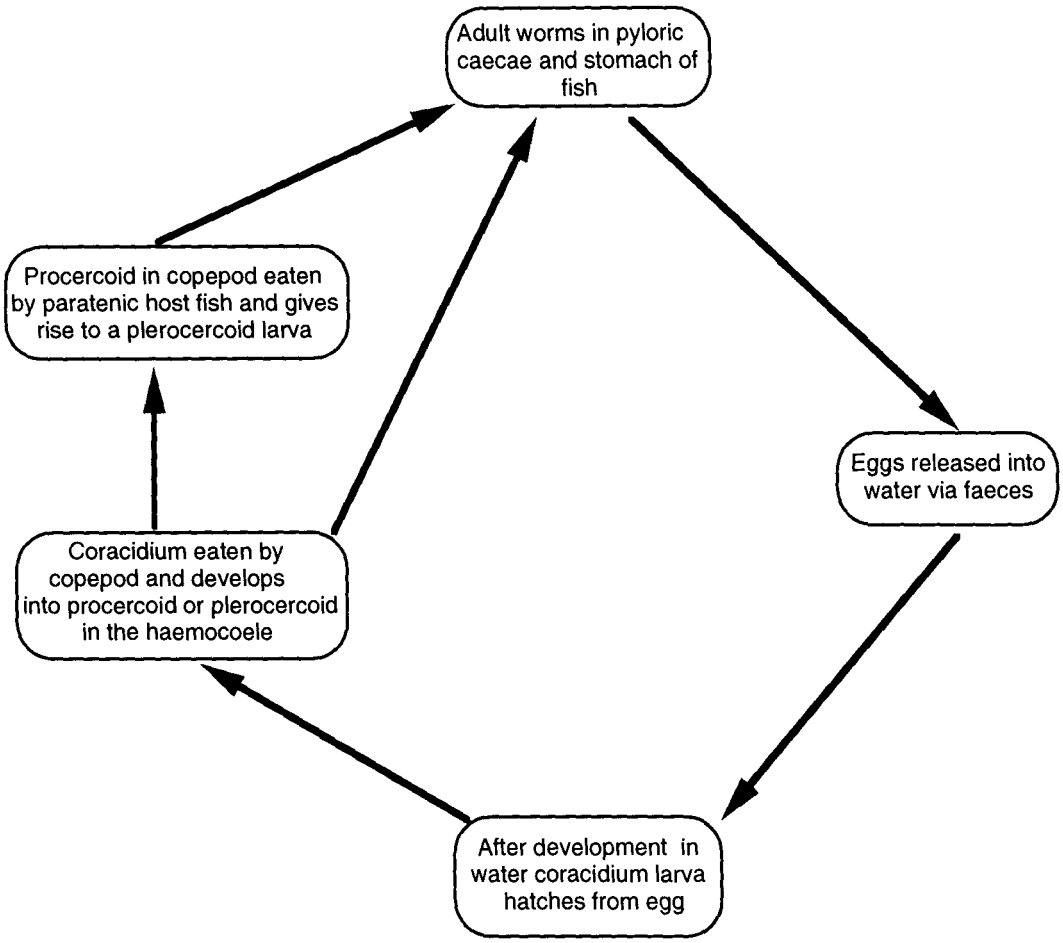


Figure 7.1. Outline life-cycle of *Eubothrium crassum*

during migration of host fish and surviving in this habitat to return with the adult fish (Vik, 1963; Kennedy, 1978a). The other is that the parasites are transported to sea in smolts are then lost at sea and that the *E. crassum* in returning adult salmon and sea trout are acquired at sea and represent the residue of a marine life-cycle (Kennedy, 1969, 1978a). Kennedy (1969) indicated that salmon and trout newly arrived in rivers from the sea or caught in coastal waters of Europe or Greenland often contain both large and sexually mature *E. crassum* as well as small and hence recently acquired ones. This together with the fact that *E. crassum* is found in sea trout, which do not travel far from coastal waters, suggests that salmonids become infected in coastal rather than oceanic waters.

7.1.4 Intensity of *Eubothrium crassum* infection

Intensity of a parasitic infection in nature may vary according to both environmental factors and the availability of host species. These factors may limit transmission success. In other words, the hypothesis of compatibility and encounter filters put forward by Combes (1991) may well explain this variation. Intensity of *Eubothrium* sp. infections have been found to vary in previous studies. For example, Bristow and Berland (1991a) recorded a mean intensity of 8.1 *Eubothrium* in *Salmo salar* ranging from 1 to 78 in the west coast of Norway. Kennedy (1978a) examined 45 adult salmon taken at sea off Soroya, northern Norway, and found 53.8 % of the fish to be infected with *Eubothrium*, the maximum number of parasites per fish being 81. The mean intensity of infection of *Eubothrium crassum* was recorded as 84.3 per infected fish by Wootten (1972) in brown trout and rainbow trout from Hanningfield Reservoir, Essex. The mean length of the

worms varied from 13.8 mm to 365 mm. There was no evidence to suggest that the size of individual worms was affected by the numbers of *E. crassum* present. Rawson (1957) recorded 100 % of infection of *E. crassum* with an intensity of 20 worms/per infected trout in Lake Windermere.

7.1.5 Effects of *Eubothrium* on fish host

Eubothrium infection in salmonid fish has been reported in Scandinavia (Bristow and Berland, 1991a,b;), in Canada (Smith and Margolis, 1970), and in the British Isles (Rawson 1957; Kennedy, 1974, 1978; Mitchell, 1993). Bristow and Berland (1991b) found 10 % loss of growth resulting from low level *Eubothrium* sp. infection in farmed salmon, *Salmo salar* by comparing infected and uninfected fish. This was more marked in male fish (approximately 800 g loss per fish) than female fish (approximately 450 g per fish). They concluded that the direct value of this loss may be millions of dollars each year in Norway. Other effects of *Eubothrium* on fish hosts may include loss of food and susceptibility to other diseases (Bristow and Bernard, 1991b; Mitchell, 1993).

Studies of *E. salvelini* infection in wild Arctic charr have shown that even at the site of attachment, where the scolices of the parasites interface with the host tissue, inflammation of the pyloric caecal epithelium is not evident (Hoffman *et al.* 1986). However, Hoffman *et al.* (1986) recorded some deleterious effects on the host, including a reduction in condition factor, reduced levels of circulating red blood cells and reduced haemoglobin levels. In heavy infections the intestinal tract may become blocked, resulting in death of the host.

Smith and Margolis (1970) studied effects of *E. salvelini* on sockeye salmon, *Onchorhynchus nerka*, in Babine lake, British Columbia and found from 20 % to 30 % infection of *E. salvelini* in yearling smolts. Infected smolts were 2-4 g and carried 5-10 or more worms each weighing 40 mg and averaging 50 mm in length. It was noted in this study that escape from predators and the procurement of food would be likely to be more difficult for heavily-infected fish and their survival wo ıld be lessened accordingly.

7.2 AIMS

This chapter is concerned with describing the prevalence, intensity and distribution of *E. crassum* in a population of salmon held at a Scottish fish farm, Otterferry, Argyll. Specifically, the following questions were addressed:

1. What is the prevalence and intensity of *E. crassum* infection in this population of farmed salmon?

2. What is the relationship between size of fish and infection of *E. crassum*?

3. What is the relationship between *E. crassum* infection and body condition?

4. What is the relationship between intensity of *E. crassum* infection and mesenteric fat, hepatosomatic and gonadosomatic index?

5. Do any of these effects depend on host gender?

7.3 MATERIALS AND METHODS

The commercial production of salmonids takes place in fresh and salt water. Immature salmon are held in fresh water in containers such as tanks and troughs for

varying periods before the salt water phase of their life cycle. The smolts are then moved into net pens or tanks through which sea water is pumped or flows. Salmon mature in sea water but are generally moved into fresh water to spawn (Bruno and Poppe, 1996).

Seventy eight Atlantic salmon, *Salmo salar*, were obtained from Otter Ferry Farm, Argyll, Scotland, in August 1993. After hatching, the parr were kept in freshwater cages between February 1991-May 1992, then transferred to seawater and kept 15 months in the tanks. All fish were weighed and their fork length measured, dissected in order to determine gender (13 males, 65 females). Their livers and gonads were also weighed. Routine *post-mortem* examinations were carried out on the salmon to search for *Eubothrium crassum*, which occur in pyloric caeca and in the anterior intestine. The length and weight of all the worms recovered from each fish were recorded.

Parasite Index (PI), Gonadosomatic index (GSI), Hepatosomatic Index (HSI) and Condition Factor (C_f) were calculated as follows

HSI = Liver weight / Fish weight x 100

GSI = Gonad weight / Fish weight x 100

$C_f = W/L^k$ (Ricker, 1979; Bolger and Connolly, 1989; Kadri *et al.*, 1995)

where

W = weight, L = Length and k = the slope of the regression of ln (W) on ln (L).

Parasite Index = weight of parasite / weight of fish x 100

7.3.1 Statistical analysis

The data were checked for normality and transformed where necessary. For normally distributed variables parametric statistic were used and for non-normally distributed variables non-parametric statistic were used.

Relationships between length and weight of fish were investigated by means of Regression Analysis. Differences in intensity of infection between male and female fish were tested by the Mann-Whitney U-test. Spearman Rank Correlation Coefficient was used to investigate relationships between gonadosomatic index and parasite index, hepatosomatic index and parasite index and between % mesenteric fat and parasite index. Differences in condition factor, hepatosomatic index, gonadosomatic index, % mesenteric fat between infected and uninfected fish for both sex were examined using Kruskal-Wallis test.

A Mutiple Comparison test was then employed to investigate differences in condition factor between infected and uninfected female, and infected female-uninfected male; differences in hepatosomatic index between infected male-infected female, infected female-uninfected male and uninfected male and-uninfected female. Condition factor of infected female fish was correlated with parasite index using Spearman Rank Correlation Coefficient. Differences in mean weight of infected and uninfected fish and male and female fish were examined by means of the t-test. The relationship between length and weight for infected and uninfected fish was investigated Regression Analysis.

7.4 RESULTS

7.4.1 The prevalence and intensity of infection

The overall prevalence of *E. crassum* (Figure 7.2) infection in the sample of farmed salmon was found to be 37.2 % (29/78 fish). The frequency of number of worms found in individual fish is given in Figure 7.3. The degree of overdispersion was not high in this study (variance: mean ratio = 1.36). Mean intensity (number of worms/per infected fish) was 1.34, ranged from 1 to 5, with only one fish harboured the highest number. Most of the fish examined had one or two worms. Because only one fish had 3 worms and one fish had 5 worms of *Eubothrium crassum*, the effects of multiple infection on growth of parasite could not judged. Although Figure 7.4a shows slight positive correlation between fish length and parasite weight, no statistically significant relationship was found between fork length of the host fish and parasite weight ($F_{1,27} = 2.66$, $R^2 = 9.0$, $P = 0.115$). There was no evidence to indicate that parasite burden is different in male and female salmon from Otter Ferry Farm (Mann-Whitney U-test; $U = 505$, $P = 0.89$). Thus, it can be concluded that gender has not influence susceptibility to *E. crassum* infection.

Body measurements of farmed salmon infected with *Eubothrium crassum* are given in Table 7.1. The length of *E. crassum* recovered from the salmon from Otter Ferry Farm was 234 mm and ranged from 35 mm-500 mm. The mean weight of the worms was 573 mg and ranged from 34 g to 2180 mg. A positive relation between length of worms and weight of worms is shown in (Figure 7.4b).

A significant negative correlation was detected between gonadosomatic index and parasite index (Spearman Rank Correlation Coefficients, $R_s = - 0.370$, $n= 29$, $P < 0.05$).

Table 7.1 Body measurements of farmed salmon infected with *Eubothrium crassum*.

	Infected				Uninfected			
	Male (n=5)		Female (n=24)		Male (n=8)		Female (n=41)	
	Mean (Range)	SE	Mean (Range)	SE	Mean (Range)	SE	Mean (Range)	SE
Length	596 (563-640)	22.9	532.7 (474-595)	7.29	604 (520-660)	15.6	545 (460-623)	6.2
Weight	2116 (1800-2960)	213	1747 (1200-2600)	83.8	2349 (1620-3000)	168	1771 (1080-3060)	67.8
CF	1.035 (0.949-1.129)	0.029	1.141 (1.016-1.29)	0.016	1.04 (0.98-1.11)	0.01	1.07 (0.89-1.28)	0.01
HSI	1.001 (0.693-1.326)	0.134	1.477 (0.398-2.071)	0.067	0.859 (0.71-1.06)	0.04	1.37 (0.61-1.97)	0.04
GSI	3.72 (0.072-5.871)	0.993	2.925 (0.192-5.657)	0.356	3.905 (1.12-7.50)	0.65	2.98 (0.20-5.67)	0.25
FAT	7.31 (4.72-11.02)	1.23	6.850 (3.30-12.05)	0.484	7.234 (5.07-10.3)	0.72	6.82 (2.67-13.5)	0.40

Figure 7.2 Scanning electron micrograph of *Eubothrium crassum.*

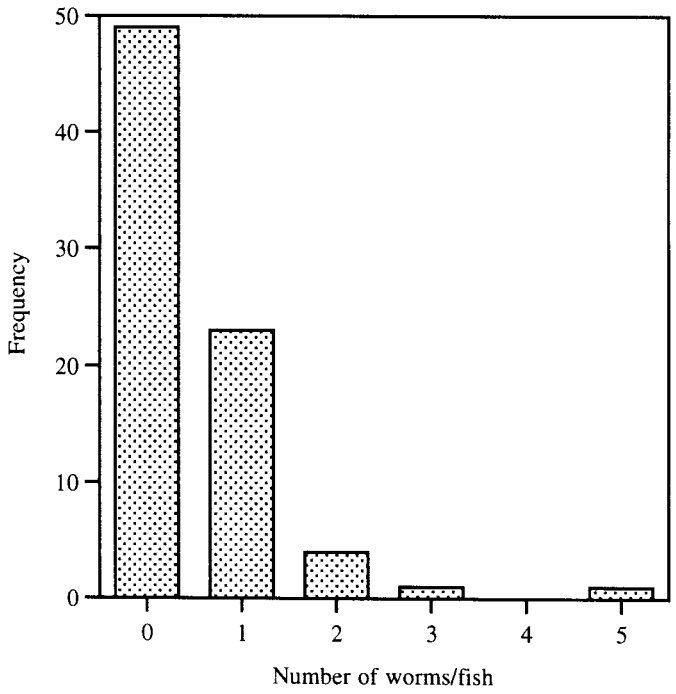


Figure 7.3. The frequency distribution of *Eubothrium crassum* in pyloric caecae and stomach of farmed salmon

However, there appeared to be no particular relationship between parasite index and either hepatosomatic index (R_s = 0.225, n = 29, n.s.) and % mesenteric fat (R_s = 0.117, n = 29, n.s.).

Condition factor and hepatosomatic index of infected and uninfected male and female fish were found to be different (Kruskal-Wallis Anovar, H=14.03, df = 3, P = 0.03; H = 25.2, df = 3, P = 0.001) for condition factor and hepatosomatic index repectively. There were no statistically significant differences in gonadosomatic index and mesenteric fat of infected and uninfected male and female fish (Kruskal-Wallis Anovar, H = 1.87, df = 3, P = 0.60; H = 0.37, df = 3, P = 0.94) for gonadosomatic index and mesenteric fat. Further statistical analysis were applied and a Multiple Comparison Test (Siegel and Castellan, 1988) showed that condition factor appeared to be different between infected female and uninfected female; infected female and uninfected male ($P < 0.05$). Hepatosomatic index infected male and infected female, infected female and uninfected male, uninfected male and uninfected female were also found to be different ($P < 0.05$).

A negative correlation was found between condition factor of infected female fish and parasite index (Spearman Rank Correlation Coefficient; R_s = -0.54, n = 24, $P < 0.05$; Figure 7.5a) and between gonadosomatic index of infected female fish and parasite index (R_s = -0.64, n = 24, $P < 0.01$; Figure 7.5b).

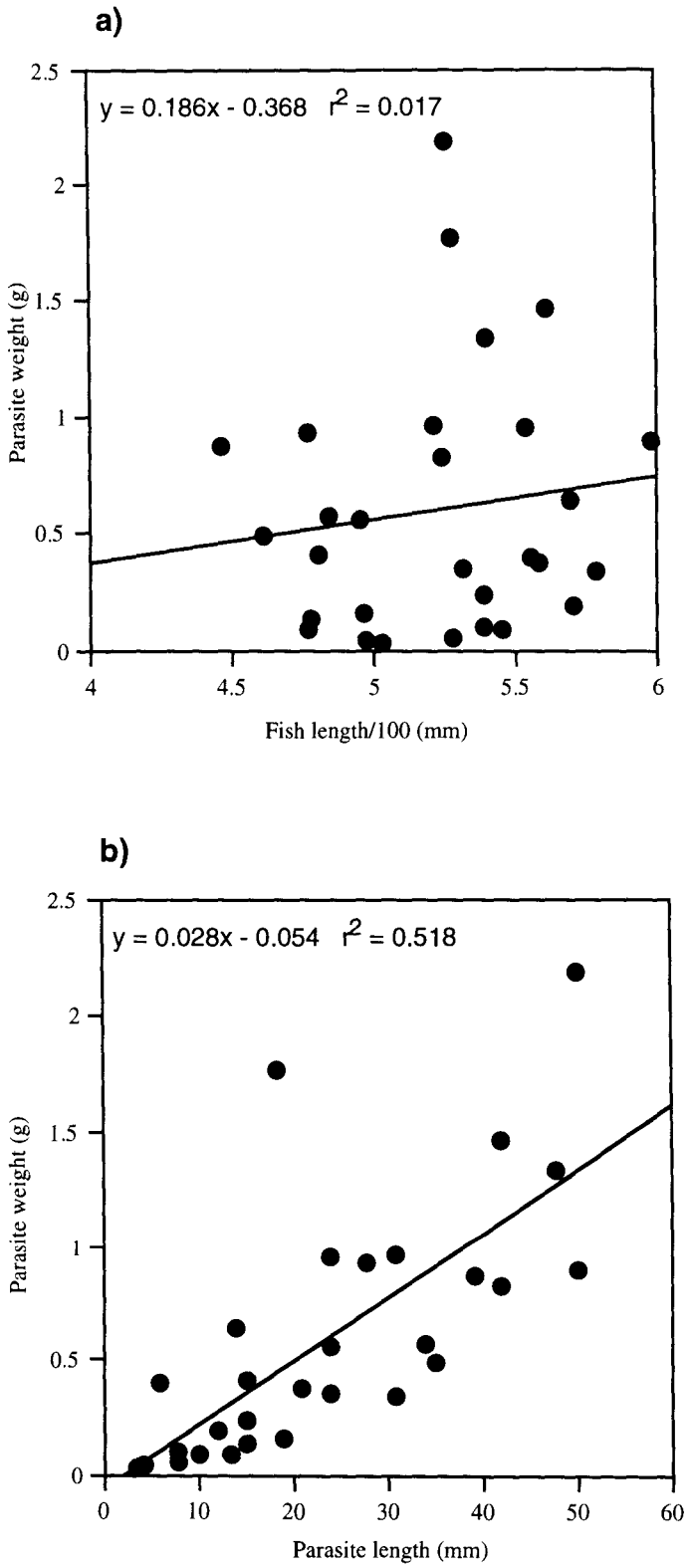


Figure 7.4 Relationship between (a) fish length and parasite weight and (b) parasite length and parasite weight

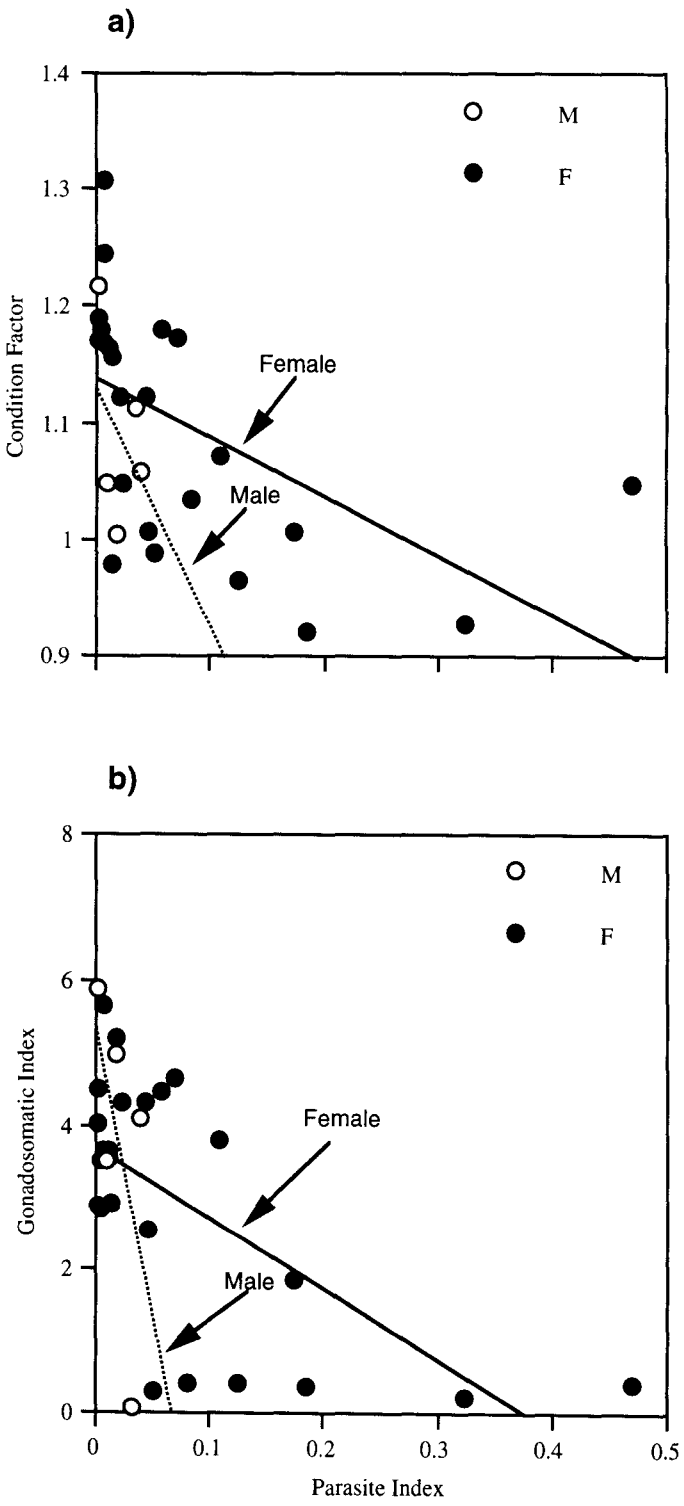


Figure 7.5 Relationship between (a) parasite index and condition factor and (b) parasite length and parasite weight.

The length- frequency distribution of infected and uninfected salmon taken from the Otter Ferry Farm is shown Figure 7.6a, and the weight-frequency distribution is shown in Figure 7.6b. The size of fish examined ranged from 460 mm to 660 mm. A large number of fish appeared to be between 520 mm and 580 mm. The weight of fish examined in this study ranged between 1080-3080 g and most infected and uninfected fish were between 1280-2280 g in weight. The total number and mean intensity of *Eubothrium crassum* were found to be randomly distributed in length classes (Figure 7.7).

The overall regression equation describing the relationship between the length and weight of infected and uninfected salmon for both sexes from Otter Ferry Farm is given in Figure 7.8a and Figure 7.8b. The regression equation describing the relationship between length and weight of both infected and uninfected fish is given in Figure 7.9. Although it was not statistically significant, the slope for infected fish appeared slightly lower than uninfected ($F_{1,74} = 0.01$, $P = 0.932$).

7.5 DISCUSSION

7.5.1 The prevalence and intensity of infection

The prevalence of infection with *Eubothrium crassum* found in this study (37.2 %) is quite high for cultured fish. The overall prevalence of *E. crassum* infection in the salmon from Otter Ferry Fish Farm was lower than those observed by Kennedy (1978a) and Rawson (1957). Although numbers of *E. crassum* were found to be highly overdispersed in the salmon in previous studies (see above), the degree of overdispersion was not so high in this study. Adult worms were recovered from individuals salmon, demonstrating that they

may be infected with the parasite very early in its life. Moreover, it is possible that salmon examined in this study may acquire the infection when they were stocked at the parr stage in freshwater. This is perhaps unsurprising since salmon feed solely on plankton for the first few months of life, switching to more energetically profitable benthic and pelagic macro-invertebrates after this period (Maitland and Campbell,1982). Buchmann *et al.* (1995) recorded *Eubothrium crassum* infection in rainbow trout only in one farm in their study which was carried out in 5 fish farms. The farm received natural lake waters. The results from the present study indicate a lower intensity of infection and range of intensity than the work of Bristow and Berland (1991a) and Kennedy (1978a). The inclusion of a fish paratenic host in the life-cycle of *E. crassum* would seem to be unlikely in farmed salmon.

7.5.2 Effects of parasites on host fish

The pathology caused by tapeworms in the gut may cause tissue alteration or destruction, mechanical blockage and nutrient absorption at the expense of the host.

The effect of parasites on gonad development of fish has been noted by other workers (Arme and Owen, 1967; Wilson 1971; Bean and Winfield, 1989, 1992 and Tierney, 1991). However, this is in contrast with the findings of McPhail and Peacock (1983) who found no significant changes in the gonads of infected sticklebacks. Mean liver weight has also

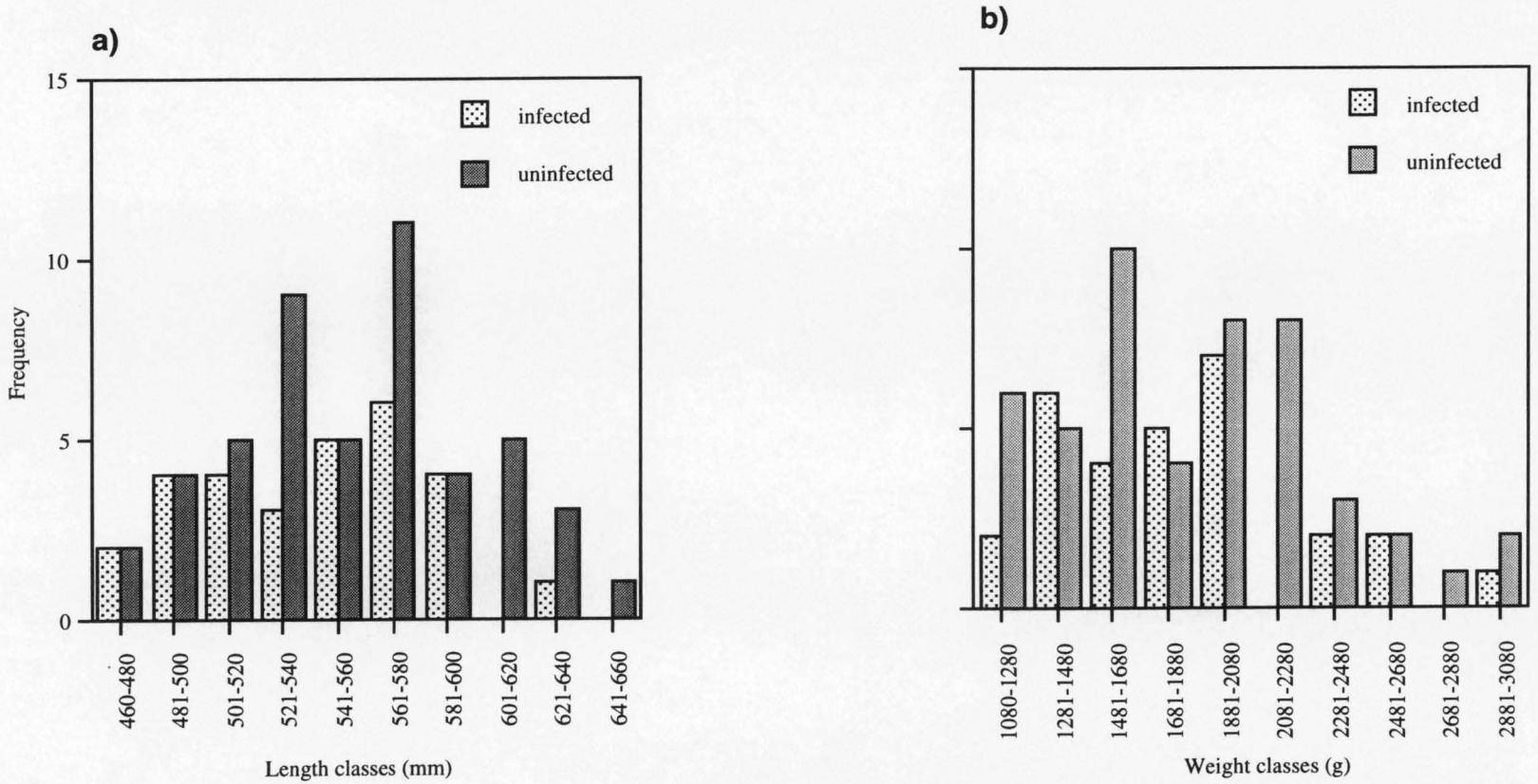


Figure 7.6 Frequency of (a) length classes and (b) weight classes of infected and uninfected farmed salmon

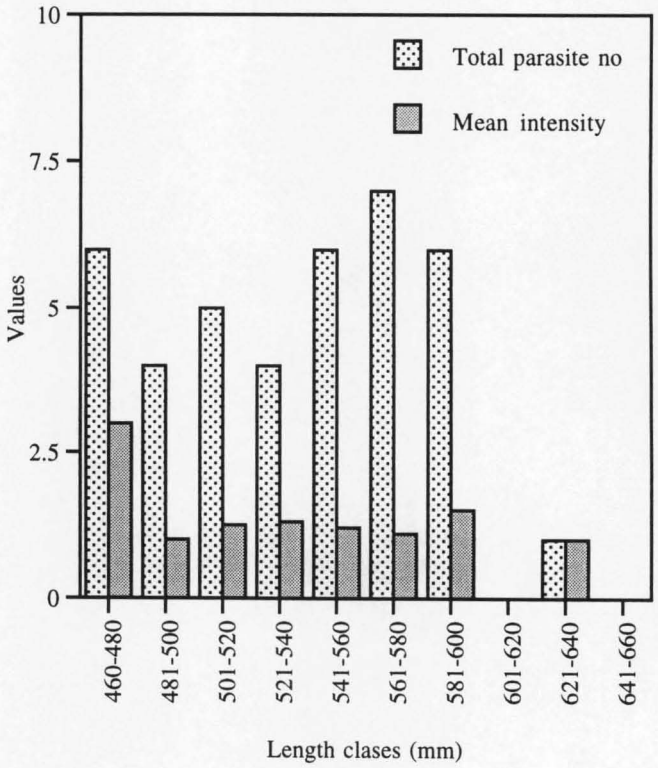


Figure 7.7 Total number and mean intensity of worms in size classes of farmed salmon

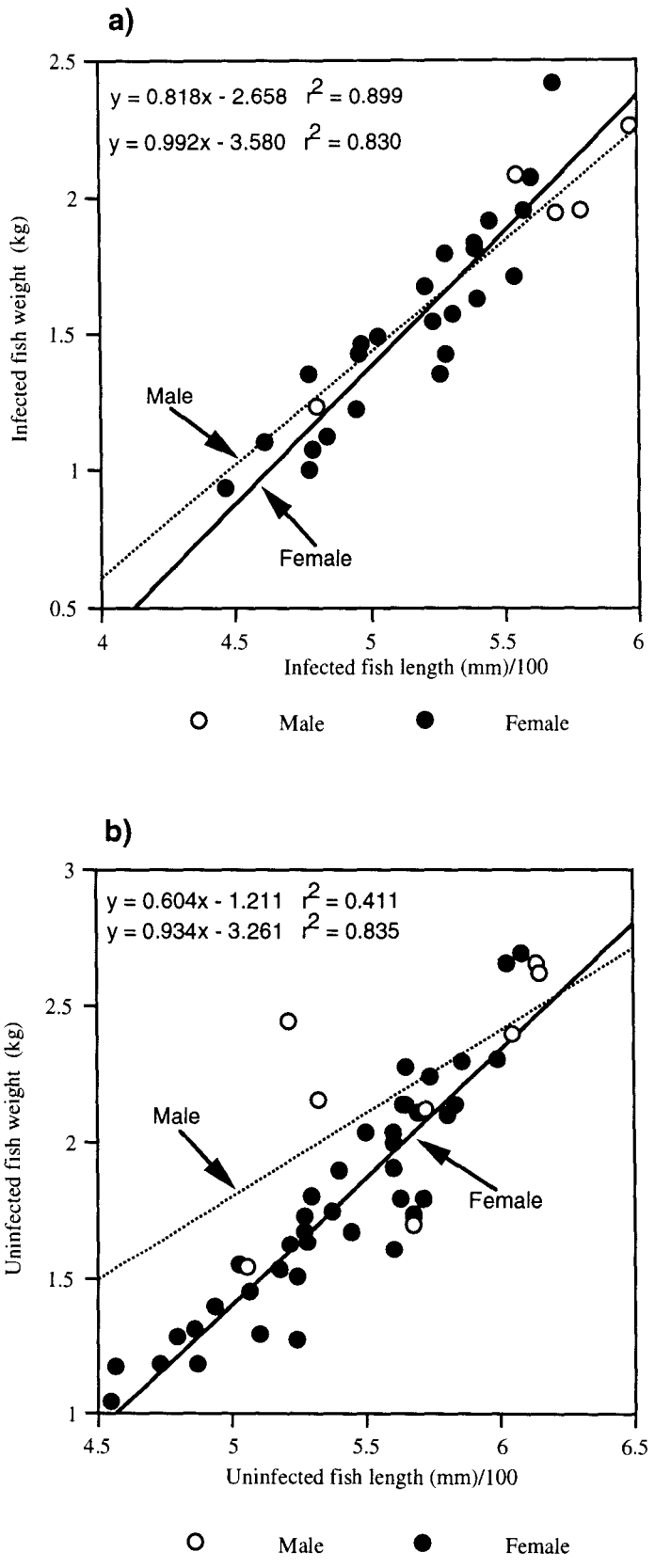


Figure 7.8 Relationship between length and weight of (a) infected and (b) uninfected male and female salmon.

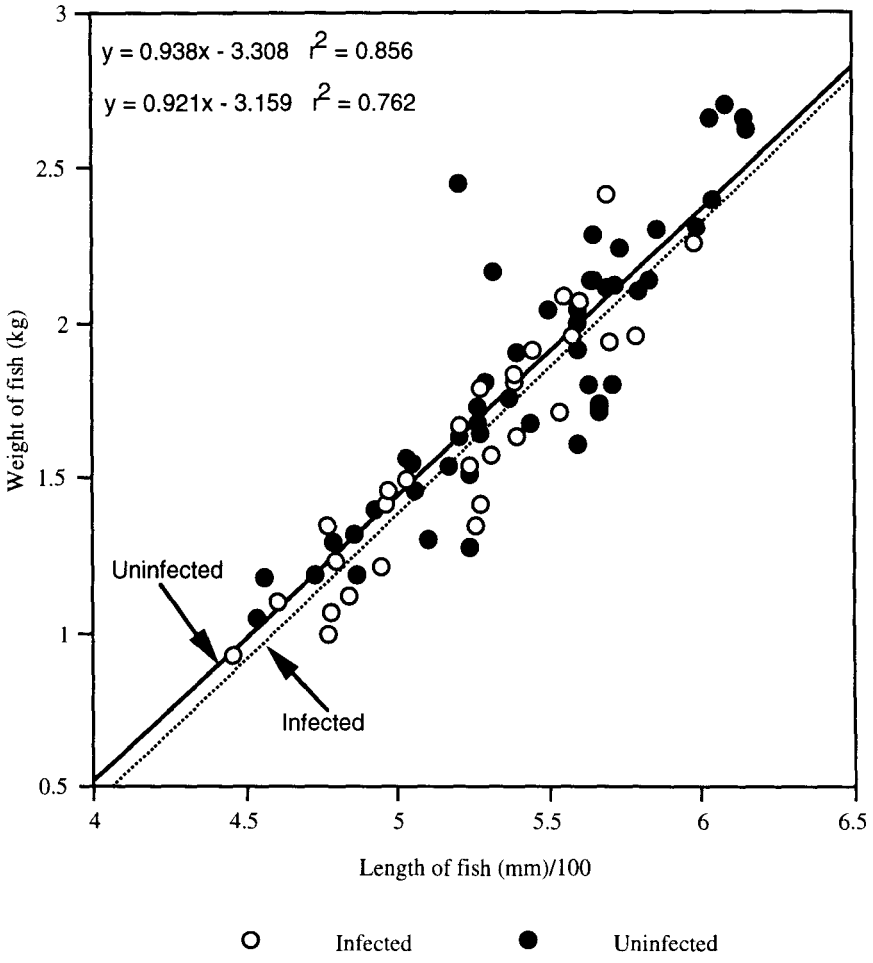


Figure 7.9 Combined length-weight profiles for all uninfected and infected fish.

been shown to be significantly reduced in infected fish and the structure of the liver has been noted to become more diffuse (Arme and Owen, 1967; Sweeting, 1977). My findings agreed with the above that *E. crassum* had negative effects on condition of fish and gonadosomatic index. It is evident that helminths can not be as harmful to salmon as they are to small fish like stickleback. There was no evidence to indicate that parasite burden is different in male and female fish. Thus it can be concluded that gender is not related to *E. crassum* infection in salmon from Otter Ferry Farm

7.6 SUMMARY

(1) The epidemiology of the adult stage of *E. crassum* in its final host, *Salmo salar,* in a sea-based salmon farm is described.

(2) The overall prevalence of the parasite was 37.2 % and this was not influence by gender.

(3) Frequency distribution was weakly overdispersed ($s^2/\bar{x}=1.36$) within the farmed salmon population.

(4) Although negative effects of *Eubothrium crassum* infection were recorded on the condition and gonadosomatic index of the fish host, there was no evidence to suggest an effect of infection on the hepatosomatic index and percentage body fat of host fish.

(5) It was concluded that salmon parr acquire infection when they are stocked in freshwater.

Chapter 8 *In vivo* and *in vitro* cultivation of *Diphyllobothrium dendriticum*

8.1 INTRODUCTION

The studies reported so far in this thesis all concern fish that were naturally infected with *Diphyllobothrium* spp. Deductions concerning, for example, the effect of *D. dendriticum* on body condition and gonadal status, are therefore based on correlations, so that cause and effect can not be separated. To probe further several aspects of the interactions between *D. dendriticum* and fish hosts it is necessary to study the effects of experimental infections. This requires a supply of infected copepods, which in turn requires a supply of eggs. This chapter describes a series of studies aimed at generating a supply of *D. dendriticum* eggs.

8.1.1 *In vivo* culture of *Diphyllobothrium dendriticum*

Recently, the laboratory maintenance of the life cycle of *Diphyllobothrium dendriticum* (Sharp, 1990) and *Schistocephalus solidus* (Tierney, 1991) have been achieved. Sharp *et al.* (1990) established *Diphyllobothrium dendritium* (30-60 mm long) infection by feeding herring gulls, *Larus argentatus*, with plerocercoids. In their experiments, of 4 birds initially infected, 1 bird lost its infection within 3 weeks but infection survived up to 6 months in the remaining birds. Adult *D. dendriticum* produced operculate, tanned eggs which were easily identified in the faeces of experimentally infected birds. Eggs first appeared in the faeces 6 days post-infection,

initially in low numbers but increasing to tens of thousands/day by week 3 post-infection.

From the data of Archer and Hopkins (1958) and Sharp *et al.* (1990), it would appear that plerocercoids of *D. dendriticum* are very likely to establish as adults in herring gulls, *Larus argentatus*; black-headed gulls, *Larus ridibundus*; hamster, *Mesocricetus auratus*; cat, *Felis domestica* and rat, *Rattus norvegicus*, but that the probability of establishing decreases through ducklings, *Anas boschas*; chickens, *Gallus gallus* and pigeons, *Columba livia*. An initial aim was to establish *in* vivo culture of *D. dendriticum*. We first chose domestic chickens, *Gallus gallus* as the laboratory host for adult *D. dendriticum* because they are easy to obtain and house and because parasite eggs can also be readily recovered from their faeces (Tierney, 1991). We also used ducklings.

8.1.2 *In vitro* culture

The absence of an intestine in cestodes has made them an interesting challenge for culture studies (Taylor and Baker, 1987; Smyth and McManus, 1989; Dick and Choudhury, 1995). A great deal of progress has been made on *in vitro* development of cestodes (Smyth, 1947; Smyth, 1959; Taylor and Baker, 1987). The criteria for development and maturation usually accepted are segmentation, organogeny, gametogenesis and egg shell formation (Smyth and MacManus, 1989).

It is generally realised that the axenic culture (a system in which only one type of organism is growing) of parasitic helminths involves the solution of specific

problems not encountered for free-living organisms (Smyth, 1959). These are summarised by Smyth (1959) as follows:

1. Helminths depend on living biological habitats (for example, the intestine, the liver and the blood stream) whose physicochemical properties are often imperfectly known and whose characteristics may be difficult to reproduce *in vitro*.

2. Many adult helminths live in nonsterile habitats; consequently treatment by antiseptic procedures is necessary before axenic culture can be attempted. This difficulty may be overcome by starting with larval stages obtained from a sterile environment instead of adult forms.

3. Helminths normally feed on biological materials such as blood, mucus, tissue exudates and intestinal contents that are complex in nature and origin and with nutritional properties which are difficult to replace by artificial media.

4. In the normal habitat, diffusion of waste materials from the site of metabolism can readily take place. Any *in vitro* method must similarly provide conditions for the efficient removal of waste materials of a possible toxic nature.

5. Finally, detailed accounts of relatively few helminth life cycles are available. Although the broader aspects of many life-cycles are well established, detailed morphological, cytological, histochemical and biochemical pictures of the entire processes of maturation from egg to adult, even for such a well-known species as *Schistosoma mansoni*, are rare. Without such detailed information it is difficult to establish satisfactory criteria by which development *in vitro* can be assessed.

Smyth (1959) pointed out that the most advanced development of pseudophyllidean plerocercoids had been achieved in medium concentrated embryo

extract (EE_{20}) between techniques used, which are EE_{20}, dilute embryo extract (EE_{10}), serum, Earl's solution, chick yolk sac. He divide the ontogeny of cestodes into several developmental stages which were designated as follows: stage (0) undifferentiated larvae; stage (1) cell multiplication; stage (2) segmentation; stage (3) organogeny; stage (4) early gametogeny; stage (5) late gametogeny; stage (6) egg-shell formation stage and (7) oviposition. Smyth's (1959) most successful experiment using EE_{20}, fragments of plerocercoids of *Diphyllobothrium* spp. became segmented by the second day, developed uterine and testis anlagen by the third day and by the sixth day had differentiated into proglottids containing the cirrus, cirrus sac, testes, uterus and ovaries. It was observed that even in the most successful experiments, autolysis set in on the seventh to eighth day, which is at a time when *in vivo* the vitellaria mature and eggs having shells begin to appear in the uterus. Smyth (1947) assumed that autolysis of these worms *in vitro* was probably linked to the stage of intense protein synthesis related to egg formation. Smyth (1947) also suggested that glucose, vitamins and hormones present in culture medium are necessary for normal growth and development.

The pseudophyllidean group of cestodes contains many well-known parasites of fish-eating birds and mammals with intermediate stages growing in copepods and fish. Plerocercoid larvae are common in fish and their localisation in sterile habitats such as the coelom or muscles makes them suitable for axenic culture attempts. The majority of plerocercoids reach only the undifferentiated stage in their most advanced larval condition. Certain forms, however, notably *Schistocephalus solidus* and *Ligula intestinalis*, have progenetic plerocercoids in which anlagen of the genitalia are formed while still within the coelom of the fish host. Maturation *in vivo* and *in vitro*, it is only

involves further differentiation from stage (4), early gametogeny to stage (7), oviposition (see above).

8.2 AIMS

The overall objective of the study was to get fertile eggs, starting from *Diphyllobothrium dendriticum* plerocercoids. The first aim was to grow *Diphyllobothrium dendriticum in vivo* until mature and get eggs from them for further studies. Then this proved unsuccessful, a second aim was, to generate eggs from *in vitro* cultivation of worms. A subsidiary aim, independent of success in rearing worms to maturity, was to characterise growth and development of *D. dendriticum in vitro* culture. A final aim (related to lack of success in the previous aims) was to use a cestode with a more developed plerocercoid, *Schistocephalus solidus* to assess our technique and medium.

8.3 MATERIALS AND METHODS

8.3.1 Obtaining plerocercoids from fish

The larva of *Diphyllobothrium dendriticum* used were recovered from the body cavity of powan, *Coregonus lavaretus*, collected from Loch Lomond by means of gill nets. Plerocercoids ranging from 10-30 mm in length occurred encysted on body-cavity side of the stomach of fish. The larvae were removed carefully with forceps and placed in 0.6 % saline. Plerocercoids of *Schistocephalus solidus* were recovered from the body cavities of the 3-spined stickleback, *Gasterosteus aculeatus*, obtained from Inverleith Pond, Edinburgh, and kept alive in the department's aquaria until required. The removal of both parasite species from their fish host was carried out under sterile

condition within a flow cabinet. All work surfaces were swabbed regularly with 70 % alcohol as was the fish's surface prior to dissection. All instruments, culture tubes, petri and culture dishes were autoclaved at 120°C and 12 lb/in^2 for an hour before use.

8.3.2 Infection of definitive host and collection of faeces

Initially, attempts were made to set up the life history of *D. dendriticum* by adopting the methods of Sharp *et al.* (1990), for infecting the definitive host (herring gulls) and for harvesting and embryonating the eggs. Details about *in vivo* trials are given in Table 8.1. For *in vivo* experiments, 24 day-old chicks were obtained from Marshall's hatcheries (Whitburn, East Lothian) and maintained in the licensed animal house of the Royal Infirmary, Glasgow. Later, day-old ducklings were obtained from Dickson, Little Auldmir Farm, Dalry, Ayrshire. They were reared in cages with constant heat, light, water and food for 9 days before plerocercoids were administered. The larvae from powan from Loch Lomond were placed in 0.6 % sodium chloride solution. Two to four plerocercoids were fed to each chicken and duckling (Table 8.1) by placing them individually at the back of the throat and then closing the bill and gently rubbing the throat, thus promoting a swallowing reflex. After checking for regurgitation the chickens were placed in a wire-bottomed cage, under which trays of wet paper towelling were placed to catch faeces (Tierney, 1991).

Date of experiments, number of chicks and ducklings used and number of worms exposed are given in Table 8.1. At the beginning 3 chicks were fed with 4 pleroceroids of *D. dendriticum* each. Faeces of chicks was collected between day 2 and 14, and examined for *D. dendriticum* eggs. The 24 hour output of faeces was collected from each host and suspended in water. The suspension was passed through a series of

sieves (38, 53, 100, 150 and 250 meshes/inch). The remaining material in the smallest mesh size sieve was examined under the microscope for eggs of *Diphyllobothrium* spp. No eggs were found in the faeces. Chicks were killed and dissected 15 days after feeding plerocercoids. Stomach and whole intestine were examined, but no sign of any worm was observed. In the second trial, 3 chicks were fed with 2 and 1 chick with 3 plerocercoids. The infection of *D. dendriticum* did not establish. In the third experiment, 6 ducklings fed with 2 and other 6 with 1 plerocercoids. Infection did not establish.

8.3.3 Materials and methods for *in vitro* culture of *Diphyllobothrium dendriticum*

Fertile duck eggs (EDS-free), obtained from Cherry Valley Farms, Lincoln were incubated at 40 ^{o}C, which is the body temperature of ducks, for 11 days by agreement with the Home Office. Then 4 g of homogenised embryonic tissue from the eggs were mixed in 10ml Tyrode's solution under sterile conditions. After standing for about 1 hour, the supernatant fluid was removed, the remaining portion was centrifuged and the supernatant from that was used as the culture medium. Plerocercoids of *D. dendriticum* were cultured in various number according to availability of worms and medium in screw cap vials respectively at 40 ^{o}C in a non-shaking incubator. The culture medium was changed at 48 h intervals under sterile condition (Smyth, 1958, 1959).

The supplements used were non-essential amino acids, fetal calf serum 206, glucose and vitamin B_{12}. Amino acid was added at a 1:100 ratio, amino acid to medium; serum at a 10:100 ratio, serum to medium; glucose at a 1:100 ratio, glucose to medium and Vit. B_{12} at a ratio of 1:10,000 Vit. B_{12} to medium.

Tyrode's solution

The Tyrode's solution used in this work consisted of:

	Gram/litre
NaCl	8.0
KCl	0.2
$CaCl_2$	0.2
$MgCl_2 . 6H_2O$	0.1
$NaH_2PO_4 . H_2O$	0.05
$NaHCO_3$	1.0
Glucose	1.0

Dissolved in 1litre double distilled water and sterilised by autoclave.

Solution A

$CaCl_2$	0.2 g
$MgCl_2\ 6H_2O$	0.1 g

dissolve in 100 ml of deionised water.

Solution B

NaCl	8.0
KCl	0.2
NaH_2PO_4	0.05
D-Glucose	1.0

Dissolve in 800 ml deionised water, add solution A to Solution B stir vigorously. Pour into a glass bottle, autoclave and then store in the fridge.

Solution C

$NaHCO_3$	1.0 g

Dissolve in 100 ml deionised water.

Final Tyrode Solution

Before using, added 55 ml of $NaHCO_3$ to 350 ml tyrode solution, working Tyrode is now ready for use.

8.3.4 Histological preparations and comparisons

Table 8.2. shows the timetable of experimentation work *in vitro* cultivation, and the aims of each trial. In order to monitor the *in vitro* development of *Diphyllobothrium dendriticum* and *Schistocephalus solidus*, specimens were taken for histological studies at the start of the experiments and at intervals throughout culturing. Due to small sample size, it was not always possible to take specimens every day for analysis. Specimens from both species were fixed in AFA for 24 hours prior to processing through graded alcohols (30 %-100 %). The dehydrated specimens were then blocked in wax (56°C) before sectioning on a microtome. The degree of differentiation and development of worms was determined by cutting sections of the proglottids. Sections were cut at 6 μm and stained with haematoxylin and eosin. In order to differentiate host-derived products, tissues and immunological cells from parasite tissues, cysts removed from powan containing *D. dendriticum* plerocercoids were processed in AFA for 24 hours and then dehydrated through graded alcohols as above. Specimens taken for scanning electron microscopy were fixed in AFA for 24 hours and then dehydrated through graded alcohols before being placed in 100 % acetone for 24 hours.

8.3.5 Satatistical analysis

Relationship between initial size and growth rate (weight gain) for *Diphyllobothrium dendriticum* and *Schistocephalus solidus* was investigated by means of Spearman Rank Correlation. χ^2 test was employed to examine differences in survival of *Diphyllobothrium dendriticum* plerocercoids between medium with supplement and medium without supplement.

8.4 RESULTS

8.4.1 *In vivo* cultivation of *Diphyllobothrium dendriticum*

In none of the trials with chicks and ducklings was there any evidence of establishment of *Diphyllobothrium dendriticum* maturity in the animal host, either from the presence of eggs in the faeces or from the presence of worms in the gut at *post-mortem* examination.

8.4.2 Observations of plerocercoids prior to *in vitro* cultivation

Plerocercoids of *Diphyllobothrium dendriticum* (1-2 mm wide and 10-30 mm long), become active immediately upon release from their cysts and being placed in 0.6 % saline solution. Rapid contraction and relaxation of muscles along the entire length of the plerocercoids, (each cycle lasting about one second) continued for around 30 seconds and was followed by a period of less exaggerated contraction/relaxation. Activity was restored by gently touching the plerocercoids with a sterile pointer. Similarly, activity was restored by introducing the plerocercoids to pre-warmed culture

medium. Bothria appeared to be well differentiated (see Chapter 6), but proglottids were not visible (Figure 8.2). No organogenesis was seen (Figure 8.3).

Schistocephalus solidus plerocercoids also showed activity upon removal from their intermediate host environment. Muscular contractions were not as regular as in the plerocercoids of *D. dendriticum* but responses to stimuli (touching and temperature change) were very similar. Morphologically, *S. solidus* plerocercoids were very different, being almond-shape (Figure 8.4) with a tapering posterior end. Individuals ranged from 5 to 10 mm wide and from 15 to 30 mm long. Proglottids were clearly defined with segmentation and well developed genital primordia seen in histological preparations (Figure 8.5). Due to the progenetic nature of *S. solidus* plerocercoids, developmental stage 4 (early gametogeny) is reached whilst still within the second intermediate host. Individual proglottids remained well defined and the maximum length that any worm reached was around 50 mm.

8.4.3 Histological investigation of *Diphyllobothrium dendriticum* cysts

The majority of the cysts extended into the coelom of the powan and were attached to the wall by only 15 or 20 % of its surface area (see Chapter 6). Less commonly, the cysts were found more deeply embedded within the stomach wall. Diameter of cysts ranged from around 3 mm to 6 mm and they were creamy-white in colour. The spatial arrangement of both parasite- and host-derived tissue can be seen in Figure 8.6. Host connective tissue (stained dark blue) is found at the extreme periphery of the cysts; this is probably collagen, giving the cyst its overall structural stability. Plerocercoids appeared tightly folded within their cysts and were invariably

accompanied by a body of non-cellular material which, when the cysts were opened, was released as a single solid mass (Figure 8.6).

8.4.4 *In vitro* cultivation of *Diphyllobothrium dendriticum*

The number of *Diphyllobothrium dendriticum* plerocercoids surviving each day after establishing culture are given in Tables 8.2 , Table 8.3. and Figure 8.7.

Experiment 1. In contrast to Smyth's (1947) findings, the plerocercoids died next day in EE_{10} (diluted embryo extract) in this study.

Experiment 2. After an unsuccessful first experiment in EE_{10}, EE_{20} was used as the culture medium. In this experiment, 20.8 % (10 out of 48) worms survived until 10 days *in vitro*, but they were very fragile because autolysis started after 6 day.

Experiment 3. All worms replaced in medium were alive and active (Figure 8.7) after 2 days *in vitro*, but they died by day 8.

Survival of worms in EE_{20} after 6 days *in vitro* was higher than worms cultured in EE_{20} + supplement ($\chi^2 = 14.44$, df = 1, $P < 0.001$).

Experiment 4. Growth data on *Diphyllobothrium dendriticum* plerocercoids *in vitro* is given Table 8.4. All worms gained weight after 2 days *in vitro* in medium with supplement but growth rate of smaller plerocercoids were greater than larger plerocercoids (Figure 8.8, Spearman Rank Correlation; $R_s = - 0.814$, $P < 0.001$). Worms did grow from 1-3 cm to 15-17 cm in length (Table 8.4). All worms died after day 4.

Experiment 5. Strobila became clearly segmented by the second day (Figure 8.9). Worms attached to the walls of the culture tubes by means of their bothria (Figure 8.1). By the sixth day all worms were alive, the strobila become greatly elongated and

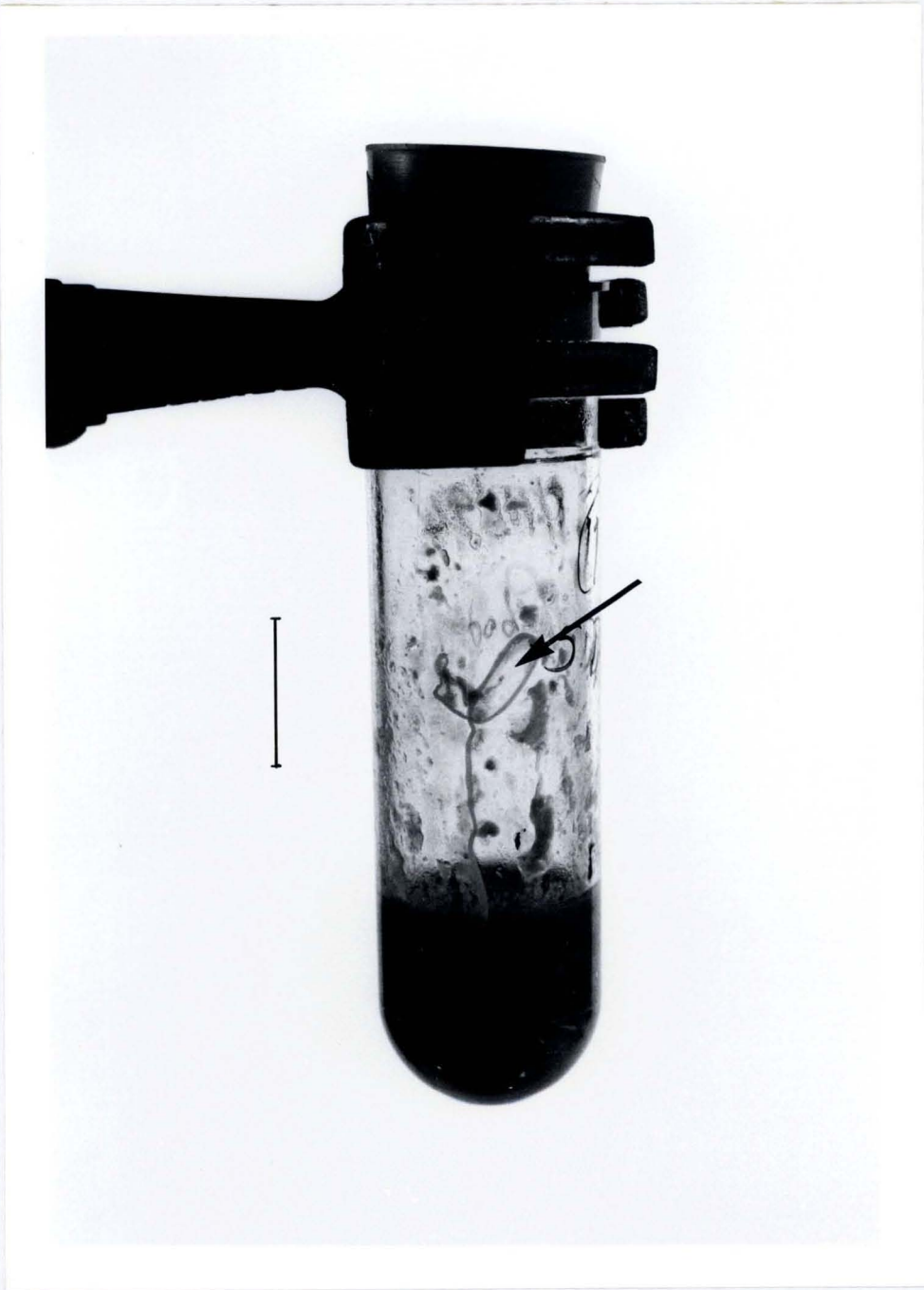


Figure 8.1 Picture of culture tube showing active worm (*Diphyllobothrium dendriticum*), attached to the tube's wall. Bar represents 1.6 cm.

Figure 8.2 Longitudinal section of plerocercoid of *Diphyllobothrium dendriticum*. Bar represents 0.5 mm.

Figure 8.3 Cross section of plerocercoid of *Diphyllobothrium dendriticum*. Bar represents 0.35 mm.

segmented, the scolex also elongated and bothridia became deeper and distinct (Figure 8.10). This gives some indication of the powerful muscular action of the bothria. No definite organogenesis could be detected, but individual proglottids and an increased number of nuclei could be seen (Figures 8.9 and 8.11). The degree of development of the bothridia in cestodes can thus be correlated approximately with duration of life within the definitive host.

8.4.5 *In vitro* culture of *Schistocephalus solidus*

Growth data for *S. solidus in vitro* is shown in Table 8.5. Four worms lost weight during day 1 and gained weight by day 2, while two worms gained weight from the beginning of experiment. There was no change in weight of one worm (Table 8.5 and Figure 8.12). All worms remained alive on the first day and 2 worms died second day.

There was no correlation between initial plerocercoid size and weight gain *in vitro* (Spearman Rank Correlation; R_s = 0.00, n.s.). Four days was the longest period of cultivation definitely achieved with this species, one worm lived for 5 days (Figure 8.13), but movement on day 4 was not well defined.

Eggs were recovered from a single tube containing one *S. solidus* worm grown *in vitro*. These were produced after 4 and 5 days in culture, although eggs were still gathered on day 5. Approximately 50 eggs were found in 1 ml medium from the 4-day-old worm and less than half that number from the same worm one day later. Eggs were approximately 50-70 μm (Figure 8.14) and the line of the operculum was just visible in outline.

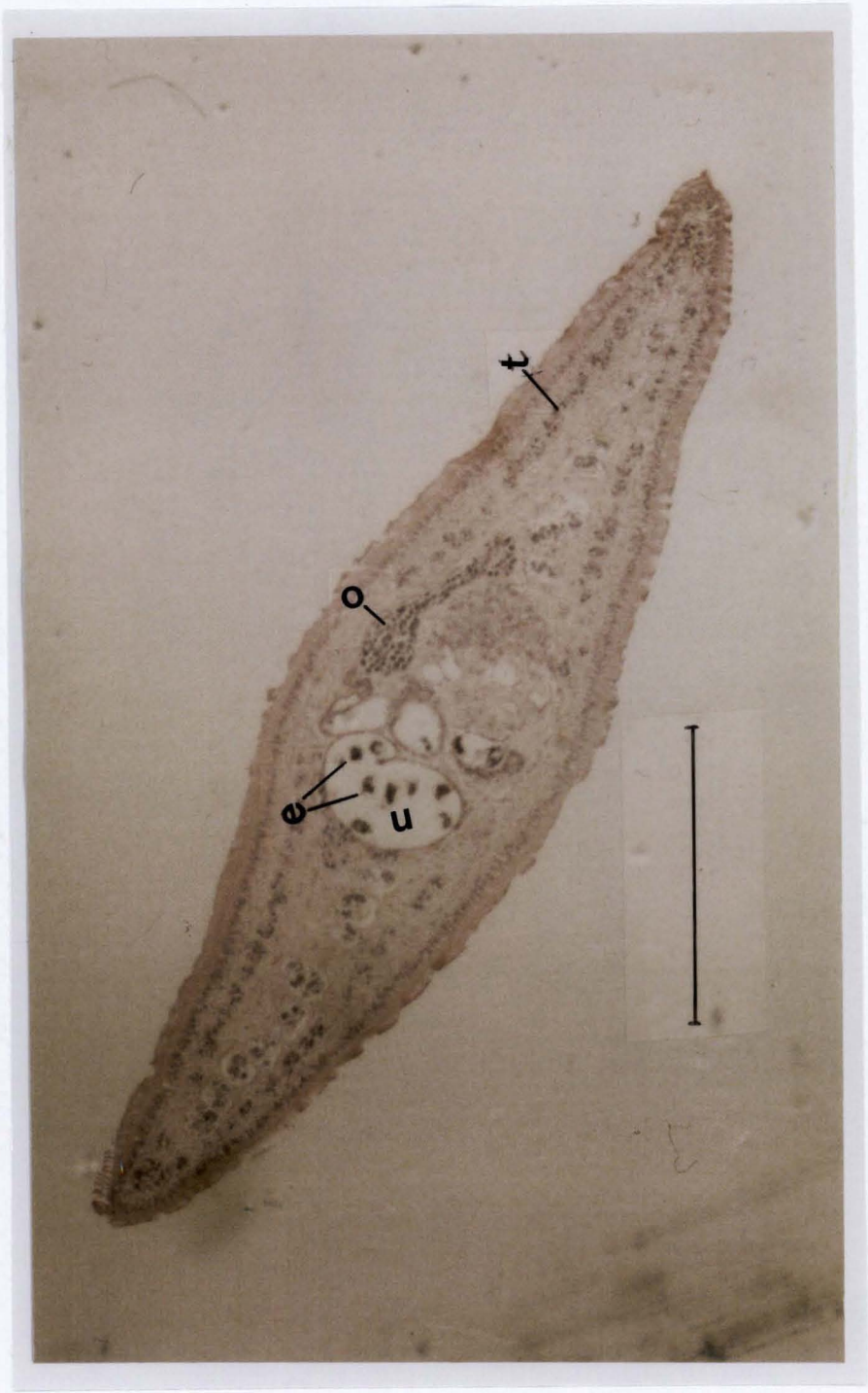


Figure 8.4 Photomicrograph showing *Schistocephalus solidus* worm after 5 days *in vitro*. Notice tapering distal edges and swollen central region. Groups of darkly stained cells labelled (t) are probably testes or possibly the vitellaria. (o) ovary, (e) eggs within the uterus and (u) uterus. Bar represents 0.5 mm.

Table 8.1 *In vivo* experiments of *Diphyllobothrium dendriticum*.

Date	Chick no	No of plerocercoids fed	Parasite eggs	Worms in intestine
20/9/1993	1	4	n.f.	n.f.
	2	4	n.f.	n.f.
	3	4	n.f.	n.f.
2/12/1993	1	2	n.f.	n.f.
	2	2	n.f.	n.f.
	3	3	n.f.	n.f.
	4	2	n.f.	n.f.
	Duckling no			
30/3/1994	1	2	n.f.	n.f.
	2	2	n.f.	n.f.
	3	2	n.f.	n.f.
	4	2	n.f.	n.f.
	5	2	n.f.	n.f.
	6	2	n.f.	n.f.
	7	1	n.f.	n.f.
	8	1	n.f.	n.f.
	9	1	n.f.	n.f.
	10	1	n.f.	n.f.
	11	1	n.f.	n.f.
	12	1	n.f.	n.f.

n.f.= not found.

Table 8.2 *In vitro* experiments of *Diphyllobothrium dendriticum*.

Date	Experiment no	No of tube	Medium	No of worms	Tested for:
10/5/1995	1	3	EE_{10}	18	survival and maturation
14/5/1995	2	4	EE_{20}	48	survival and maturation
27/6/1995	3	5	EE_{20}+supplement	25	survival and maturation
16/8/1995	4	6	EE_{20}+supplement	30	measurement
28/8/1995	5	5	EE_{20}	25	histological experiment

Compared to *D. dendriticum*, histological specimens from *S. solidus* were easier to produce and showed more marked development (Figure 8.4). In transverse section, the distal edges of worms in culture become more pointed and less wide compared to newly emerged worms. There was also a distinct increase in width at the centre of these worms. This central region corresponded to an area that was interpreted as being responsible for egg production, as many large egg-like structures were found (Figure 8.4). If this central region is the egg production area, then (u) in Figures 8.4 may well be the (u) uterus and (o) the ovary. What was interpreted as a median genital pore can be seen in Figures 8.15. Immediately below it lay a circular structure occupying a central position (x). This was probably either the ovary, uterus or some other reproductive structure such as the receptaculum seminalis, the cirrus sac or the Mehlis's gland.

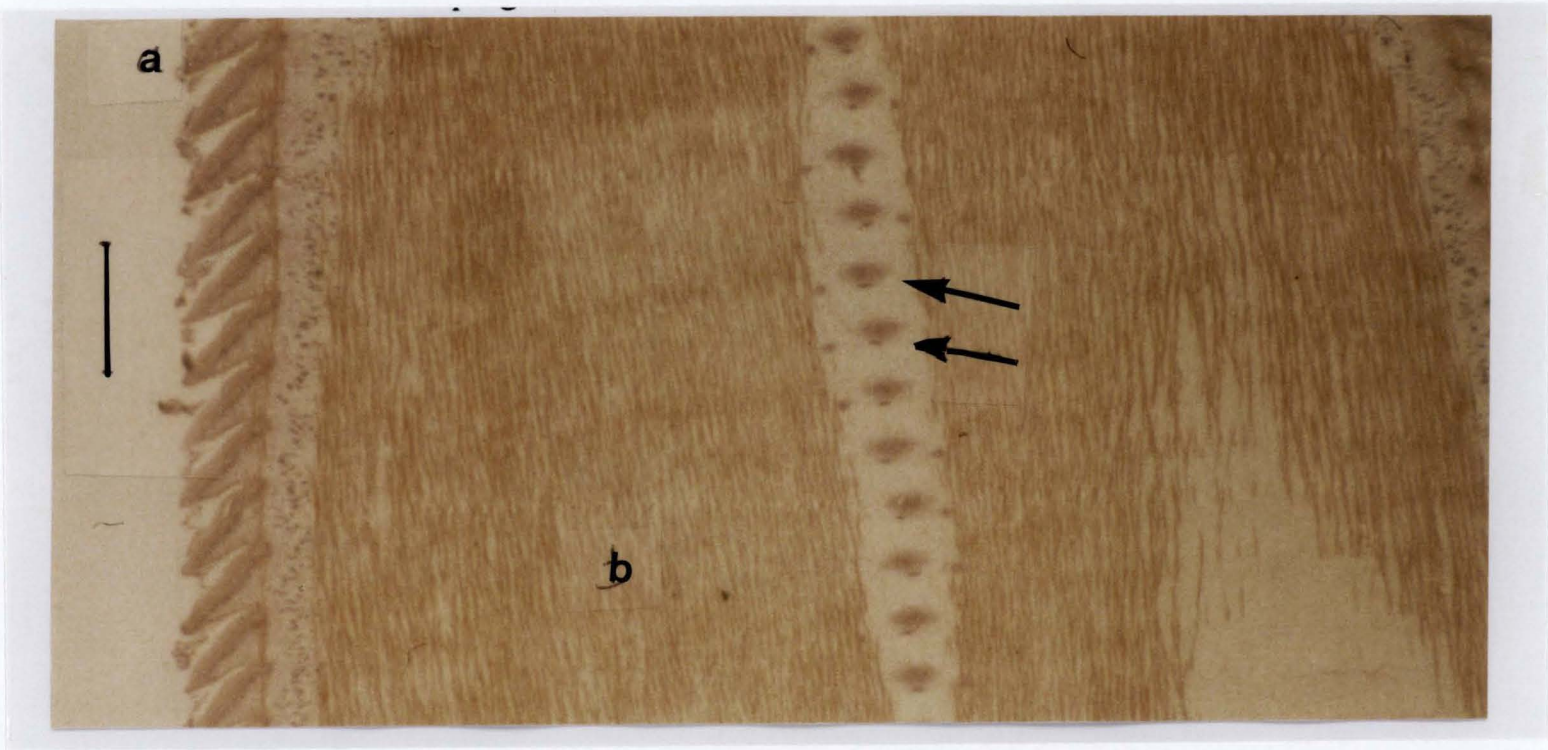


Figure 8.5 Longitudinal section of plerocercoid of *Schistocephalus solidus*, showing well developed genital primordia (arrowed). (a) serrated marginshowing position of individual proglottids. (b) indicates longitudinal muscle fibres. Bar represents 1 mm.

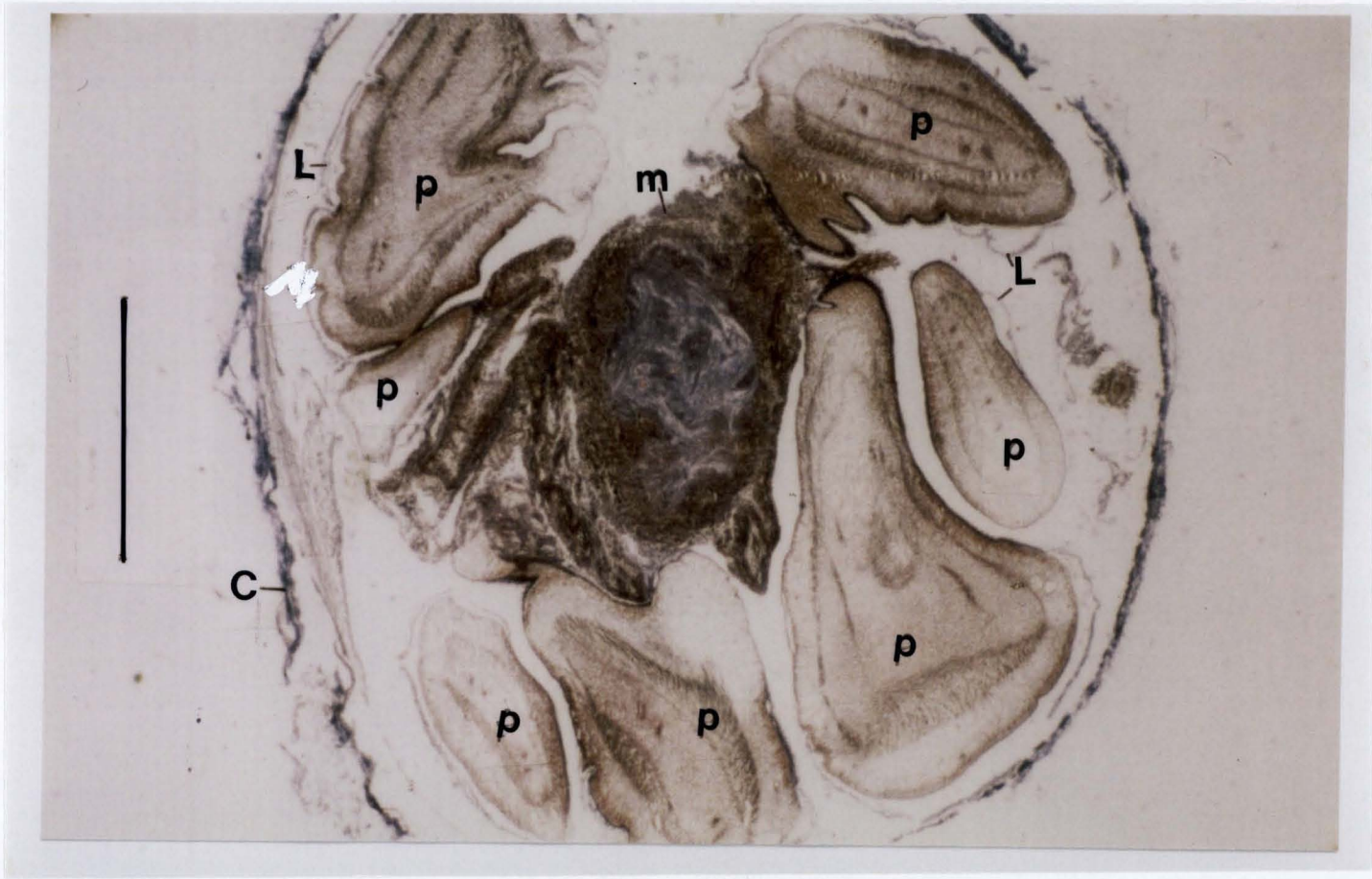


Figure 8.6 Photomicrograph showing section through a *Diphyllobothrium dendriticum* cyst. (p) coiled plerocercoid, (m) non-cellular mass, (c) connective tissue, (L) fine layer of cellular and /or non-cellular material. Bar represents 1 mm.

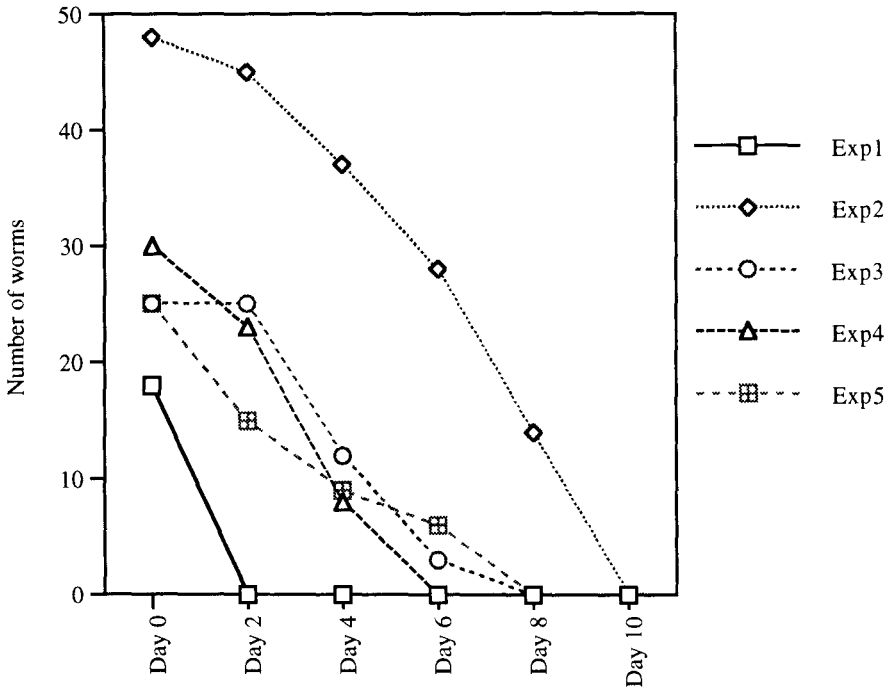


Figure 8.7 Survival of *Diphyllobthrium dendriticum* plerocercoids *in vitro*.

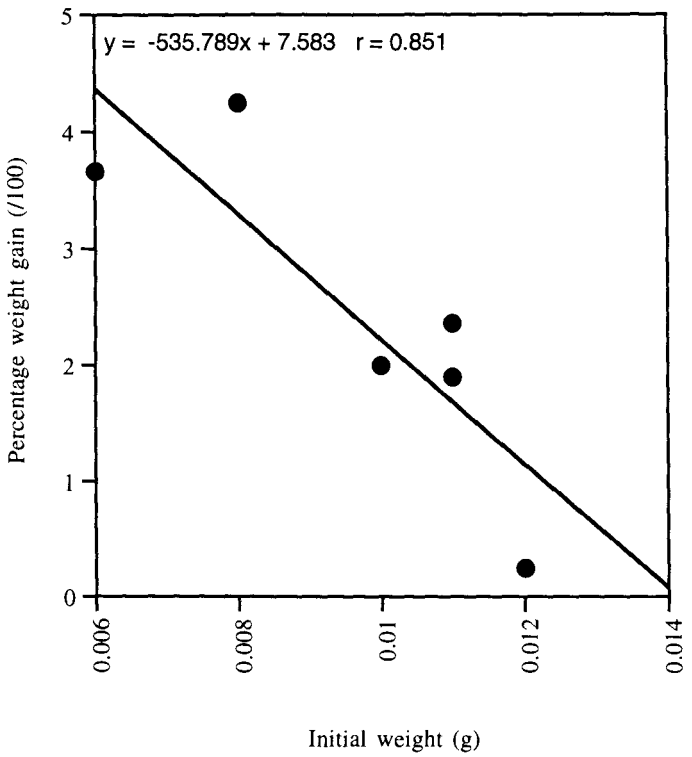


Figure 8.8 Percentage weight gain of *Diphyllobothrium dendriticum* plerocercoids after culture for *in vitro* in relation to initial weight.

Table 8.3 Survival of *Diphyllobothrium dendriticum in vitro.*

Date/Exp no	Tube no	No of worms	Day 2	Day 4	Day 6	Day 8	Day 10
10/5/1995	1	6	all				
Experiment 1	2	6	worms				
	3	6	died				
14/5/1995	1	8	8	8	5	3	3
Experiment 2	2	12	12	11	9	2	1
	3	13	13	10	8	5	3
	4	15	12	8	6	4	3
	1	5	5	4	2	all worms	
14/5/1995	2	5	5	-	-	died	
Experiment 3	3	5	5	2	1		
	4	5	5	2	-		
	5	5	5	4	-		
16/8/1995	1	5	4	-	all worms		
Experiment 4	2	5	3	-	died		
	3	5	5	3			
	4	5	2	-			
	5	5	4	2			
	6	5	5	3			
28/8/1995	1	5	3	2	1	all removed	
Experiment 5	2	5	3	3	2	for	
	3	5	3	1	1	histological study	
	4	5	3	2	2		
	5	5	3	1	-		

Table 8.4 Weight and length of *Diphyllobothrium dendriticum* plerocercoids sampled from *in vitro* culture on successive days after establishment.

	Plerocercoids		2 days *in vitro*		4 days *in vitro*		6 days *in vitro*	
Tube no	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
1	0.011	3.5	0.032	6.0	-	-	-	-
2	0.011	3.2	0.037	10.5	-	-	-	-
3	0.012	3.5	0.015	4.5	0.053	-	-	-
4	0.008	1.5	0.042	6.5	0.012	-	-	-
5	0.010	2.5	0.030	5.0	-	-	-	-
6	0.006	1.5	0.028	3.0	0.009	-	-	-
Average	0.027	2.6	0.030	6.25	0.024	-	-	-

Table 8.5 Weight and length of *Schistocephalus solidus in vitro* culture on successive days after establishment.

	Day 0	Day 1	Day 2	Day 3	Day 4
Tube No.	Weight (g)	Weight (g)	Weight (g)	Weight (g)	Weight (g)
1	0.280	0.324	0.771	------	------
2	0.156	0.118	0.108	0.120	0.129
3	0.022	0.010	0.027	------	------
4	0.013	0.013	0.006	------	------
5	0.172	0.147	------	------	------
6	0.113	0.166	------	------	------
7	0.132	0.117	0.187	------	------
Average	0.143	0.095	0.219	------	------

Figure 8.9 Longitudinal section of *Diphyllobothrium dendriticum* after 6 days *in vitro*. Bar represents 0.5 mm.

Figure 8.10 Scanning electron micrograph of *Diphyllobothrium dendriticum* after 6 days *in vitro*, showing scolex with open bothrium and segmentation like strobila.

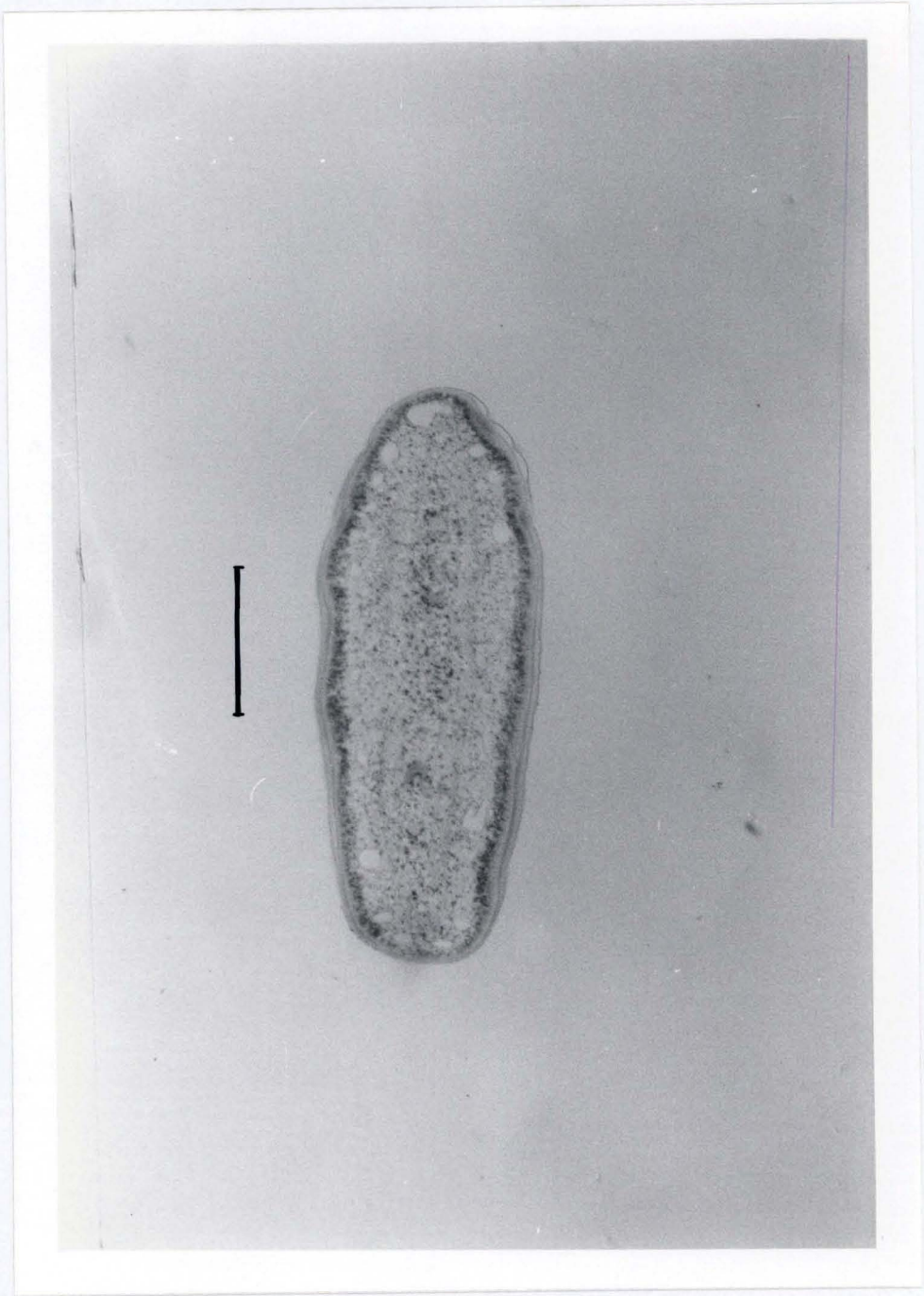


Figure 8.11 Cross section of *Diphyllobothrium dendriticum* after 6 days *in vitro*. Bar represents 0.5 mm.

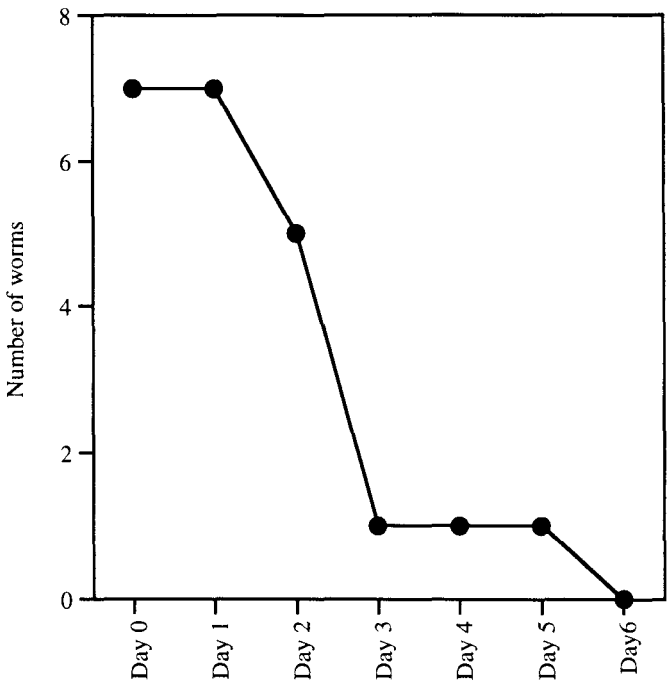


Figure 8.13 Survival of *Schistocephalus solidus in vitro*.

Figure 8.14 Photomicrograph of *Schistocephalus solidus* egg. Bar 70 μm.

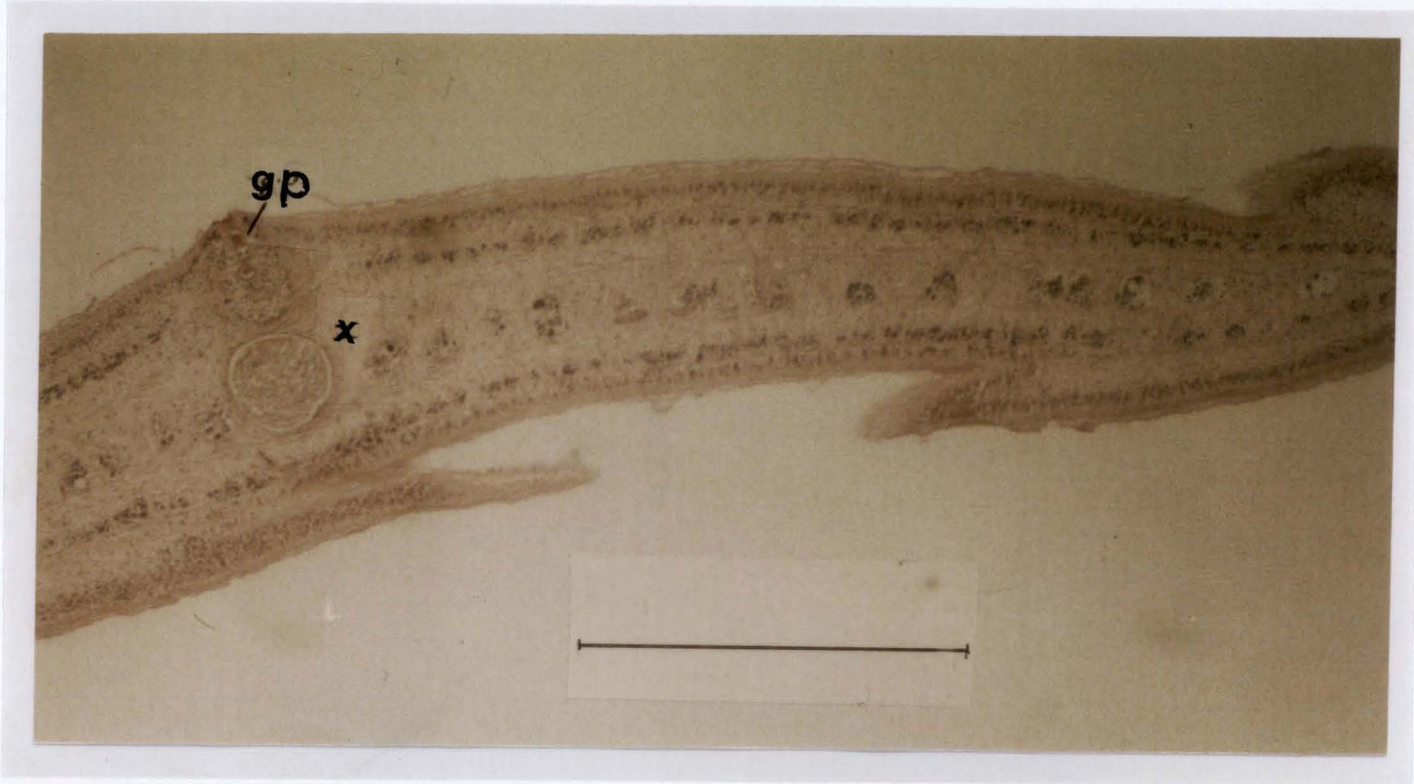


Figure 8.15 Transverse section of *Schistocephalus solidus* worm 5 days *in vitro*. (g.p.) genital pore, (x) reproductive structure. Bar 0.5 mm.

8.5 DISCUSSION

One of the reasons for failure of infection of *Diphyllobothrium dendriticum* establishment to chicken might be small size of plerocercoids. In other words, worms used in this study were not developed enough to adapte to a new environment. In a successful experiment, Sharp *et al.* (1990) fed herring gulls, *Gallus argentatus,* with 30-60 mm long plerocercoids of *Diphyllobothrium dendriticum.* Size-correlated infectivity of plerocercoids has been demonstrated in the past (Hopkins and McCaig, 1963 and Tierney, 1991). Tierney (1991) found that only 5.3 % of *Schistocephalus solidus* plerocercoids up to 50 mg established compared with 55.6 % in the 51-100 mg weight class and in excess of 66.7 % in the heavier classes. An additional reason, in agreement with Archer and Hopkins (1958) is that chicken and duckling may not be suitable definitive hosts for *Diphyllobothrium dendriticum.*

Despite a degree of success in growing *Diphyllobothrium dendriticum in vivo* over the years (Archer and Hopkins, 1958; Bell and Smyth, 1958; Anderson, 1978), little advance has been made in culturing this species to maturity *in vitro.* Smyth (1959), achieved some success when using small fragments cut from the posterior quarter of *D. dendriticum* plerocercoid cultured in concentrated embryo extract. Using this technique, development progressed to stage 5 by day 6, but no primordia of the vitellaria were detected and autolysis set in by day 7 and 8. Little somatic growth was achieved. Smyth suggested that this premature conclusion of growth *in vitro* coincided with a period of intense protein synthesis *in vitro* and that the medium used lacked the necessary nutrients to achieve this.

In the present study, plerocercoids of *Diphyllobothrium dendriticum* have been kept alive and normal in appearence alive for 10 days in EE_{20} until 6 days and in

addition, showed significant growth of plerocercoids. The amino acid, serum, glucose and vitamin supplement was used with the basal medium. This study showed that supplement to medium (duck embryo extract and Tyrode's solution) did not help worms' growth and development rather the reverse as the worms lived the longest in medium with no supplement. Culturing the less differentiated plerocercoids of *Diphyllobothrium* spp. to adults remains a major challenge. Lack of growth and development of *Diphyllobothrium dendriticum* could be due to a number of factors: (1) Possibly, macromolecules specifically produced by the definitive host that are necessary to parasite development did not exist in the media used as predicted by Smyth, 1958; Hopkins and Law, 1978. (2) Bacterial contamination of media and/or parasite is also a possibility. (3) Incorrect osmolarity of pH of the culture media may have inhibited physiological processes. (4) Factors produces by individual worms may have adversely effected other individuals within the same tube. (5) Changes in temperature of media changes may have adversely effected the worms. (6) Damage of worms when the media is changed is also a possibility.

Although eggs of *Schistocephalus solidus* were successfully recovered from one worm in this study, the overall percentage of viable eggs was low. This was undoubtedly due to the restrictive environment in which it was cultured.

8.6 SUMMARY

1. Attempts to grow *Diphyllobothrium dendriticum* plerocercoids *in vivo* in chicks and ducklings were unsuccessful.

2. Plerocercoids of two pseudophyllidean cestode species, *Diphyllobothrium dendriticum* and *Schistocephalus solidus* were cultured *in vitro* in medium containing homogenised duck embryos.

3. *D. dendriticum* showed some development over a six days period but did not develop to a mature stage.

4. Survival was impaired rather than enhanced by supplement with Tyrode's solution.

5. Mean weight of plerocercoids of *Diphyllobothrium dendriticum* increased from 9.6 mg to 27.3 mg by the second day in culture.

6. *S. solidus* gained noticeable weight by second day *in vitro.*

7. *S. solidus* showed full development into sexually mature adults and one worm produce eggs.

Chapter 9 General Discussion

This thesis has set out to describe the results of a series of investigations into the ecology and pathology of helminth infections in Scottish fish populations. With reference to the broad aims identified at the start of the research, the study has produced a number of interesting findings which add to knowledge and understanding of the host-parasite relations.

9.1 PREVALENCE AND INTENSITY OF HELMINTH INFECTIONS IN SCOTTISH TROUT POPULATIONS

Few studies have been carried out on the prevalence and intensity of helminth infections of trout in Scotland. In the present study, the prevalence and intensity of infections (as described by Margolis *et al.*, 1982) in fish populations were investigated. At some sites a very high prevalence and intensity of helminth infections was recorded. For example, 100 % of brown trout and 75 % of rainbow trout in Loch Awe were found to be infected with *Diphyllobothrium* spp. Contrary to Walker *et al.* (1988) who recorded low prevalence (31 %) and intensity (1.2) of *Eubothrium* infection in Arctic charr, in this study, sixty three percent of brown trout and 100 % of rainbow trout from Loch Rannoch were infected with *Eubothrium crassum.* Prevalence of *Echinorhynchus truttae* infection in brown trout from Aurs Burn, Talla Reservoir, Whiteadder Reservoir and Fruid Reservoir ranged from 79-93 %. The highest prevalence of *Neoechinorhynchus rutili* infection in brown trout was recorded in Cochno Loch (100 %) and Loch Maragan (74 %), with individual fish harbouring between 1 and 58 worms. Lassiere (1989) recorded overall 87.6 % infection of *Neoechinorhynchus rutili* in brown trout in Loch Maragan, with individual fish harbouring between 1 and 324

worms. She also observed a seasonal cycle in the prevalence of *N. rutili* infection, with prevalence value 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. She noted that the prevalence of infection of *N. rutili* never fell below 50 % in Loch Maragan. Highest intensities of infection recorded in this study were 339 plerocercoids of *Diphyllobothrium* spp., 118 adult *N. rutili*, 105 adult *E. truttae* and 100 *C. farionis* in individual fish. Bwathondi (1984) found high incidence of *Diphyllobothrium* spp., *Crepidostomum farionis* and *Neoechinorhynchus rutili* infections in Scottish trout, while incidence of *Capillaria* spp., *Estrongylides* sp. and *Proteocephalus* spp. infections were low. Campbell (1974) examined occurrence of *Eustrongylides* sp. in *Salmo trutta* and found that prevalence of infection ranged between 16-40 % during the year.

9.2 HELMINTH COMMUNITY PATTERNS IN SCOTTISH TROUT

In this study, it was found that Scotish trout were infected dominantly by autogenic helminth species. Eight out of 10 species recovered were autogenic and the other two were allogenic. A similar observation was also made by Esch *et al.* (1988) who found that autogenic species were generally the dominant element in trout and responsible for most of the similarity within and between localities. They considered therefore that the restriction of allogenic species of helminth to the certain area and their erratic distribution due to the restricted distribution of birds hosts. They chose findings of Hugghins (1957) as an example which showed that disappearance of resident bird host was responsible for the extinction and subsequent patchy distribution of several species of allogenic parasites of fish. In the present study, *Crepidostomum*

farionis, was the dominant species in the helminth communities in brown trout in 3 of the seven locations and *E. truttae* and *Diphyllobothrium* spp. in two of the locations. Variations of helminth communities between population of the same host species living in a relatively small geographical area was explained by means of the compatibility and encounter filters developed by Combes (1991). In an infrapopulation, there was only the positive association between *C. farionis* and *N. rutili* which are both restricted to the gut. It can be concluded tentatively that possibly one species improves either the establishment or survival of the other and that some effect of the host's immune response may not be involved.

Helminths are macroparasites and each group has a different life-history pattern. The complexity of a particular helminth's life-history influences opportunities for colonisation which in turn plays an important part in structuring helminth communities (Esch *et al.*, 1988). The composition of helminth communities in any specific host in any particular locality may be influenced by factors such as physico-chemical characteristics of a locality (Chubb, 1970), its size (Kennedy, 1978) and the geographical range of the host (Price and Clancy, 1983). Esch *et al.* (1988) suggested that in order to understand patterns in helminth community structure, it is important to distinguish between colonisation potential and colonisation ability. The superior colonisation potential of allogenic species may not always be realised and turned into ability. They concluded from the views of Tinsley and Earle (1983) and Kennedy (1987) that this is because (1) allogenic species may be introduced into a locality by transient hosts which have less time and opportunity to seed the locality with infective stage than resident hosts and (2) the opportunity for successful introduction of a new helminth into a locality may be very limited as a consequence of the very short periods

when transmission of the helminth to its next host or stage is possible that is when transmission windows are very narrow. Helminths introduced by a transient host may miss the window whereas those introduced by residents will have a greater opportunity of encountering it (Esch *et al.*, 1988).

9.3 ENDOPARASITIC HELMINTH INFCETIONS OF SYMPATRIC ARCTIC CHARR POPULATIONS; EFFECTS OF ECOLOGICAL FACTORS ON PATTERNS OF HELMINTH COMMUNITIES

An other aspect of the work carried out in this project was endoparasitic helminth fauna of sympatric morphs of Arctic charr, *Salvelinus alpinus*, from Loch Rannoch, where Arctic charr has been found to be polymorphic (Walker *et al.* 1988; Adams unpublished data). Two morphs of Arctic charr, one benthic and one pelagic were described in Loch Rannoch by Walker *et al.* (1988). More recently, a second benthic morph, was identified by Adams (unpublished data). Overall, five species of endoparasitic helminth were detected. In addition, the morphs were found to have different patterns of helminth communities. A similar study was carried out on two morphs of Arctic charr in Loch Rannoch by Walker *et al.* (1988) and suggested that differences in parasite loading of two morphs was evidence of niche segregation. Several factors may determine the differences in the helminth communities of morphs of Arctic charr in Loch Rannoch, including habitat segregation and qualitative differences between the diets of the morphs. It was observed in this study that the host's ecology appears as an influential factor acting to determine the structure of its helminth ecology. Although, the three morphs are closely related and exist in the same loch (Walker *et al.*, 1988; Adams unpublished data), significant differences in their helminth

communities indicate that the three morphs are different in their ecology and behaviour as well as morphology. One of the most interesting findings in this project was the high prevalence and intensity of *Diphyllobothrium* spp. infections in the pelagic morph and the high prevalence and intensity of *Diplostomum* sp. in the benthic morphs. This may be due to the fact that calm water probably favours high concentrations of swimming cercariae (Davis and Huffman, 1977), therefore benthic morphs are more exposed to *Diplostomum* sp. than are pelagic morphs in Loch Rannoch. In contrast, pelagic morphs are likely to be more exposed to *Diphyllobothrium* spp. than benthic morphs. *Diphyllobothrium* spp. have also been used as a biological indicators to determine migration of salmonid fish and to distinguish the morphs of fish (Dick and Belosevic, 1981; Mackenzie, 1987). Thus, it can be suggested that *Diphyllobothrium* spp. and *Diplostomum* sp. may be used as biological indicators to distinguish the morphs. Similar observations on feeding habitats and differences in parasitism of four Arctic charr morphs were made by Malmaquist *et al.*, (1982, 1986) in Thingvallavatn, Iceland. Relationships between host foraging habits and their subsequent exposure to infection by particular species of parasites have also been studied on many occasions (Kennedy and Burrough, 1978; Kennedy *et al.*, 1992; Curtis, 1995). Differences between the helminth communities of morphs indicated that these three morphs are different ecologically in Loch Rannoch. The clearest difference in parasite fauna in this study was between the pelagic and the two benthic morphs. This is associated with the distinction between the diet of these types (Walker *et al.*, 1988). These observations provide evidence to show how host helminth association may become established and how parasite life-cycles might evolve. It can also be seen that the Arctic charr morphs

are being exposed to different parasite faunas as predicted by Combes's (1991) theoretical encounter filter.

9.4 PREVALENCE OF PSEUDOPHYLLIDEAN CESTODE INFECTIONS IN A FIRST INTERMEDIATE HOST, *CYCLOPS STRENUUS ABYSSORUM*

Infection of *Diphyllobothrium* spp. procercoids in *Cyclops strenuus abyssorum* and population density of zooplankton in Loch Lomond were studied. Overall, 2.6% of *C. str. abyssorum* were found to harbour the procercoid stage of *Diphyllobothrium* spp. The prevalence of infection tended to increase with temperature of water which ranged from 3°C to 16°C during the year. The maximum prevalence was recorded in June at the time of the highest water temperature. The infection was either low or undetectable during winter and early spring. This reflects the negative effects of low water temperature on eggs hatching and also whether existence of definitive hosts, as a consequently of that availability of parasitic eggs. The effects of environmental factors on the life-cycle of cestodes has been demonstrated (Kuhlaw, 1953; Grabiec *et al.*, 1963; Halvorsen, 1966; Sysoev, 1987 and Hanzelova, 1992). The results agreed with those of previous studies carried out by Guttowa (1963), Watson and Lawler (1965), Sergeva and Freze (1981) and Sysoev (1987). It is clear that in colder areas where the definitive hosts migrate, only intermediate hosts would be infected and there would only be a release of eggs from infected definitive host into the ecosystem on a restricted seasonal basis (Henricson, 1978). Observation of no seasonal variation of infection of *C. str. abyssorum* with *Diphyllobothrium dendriticum* encouraged Halvorsen (1966) to suggest that susceptibility to infection varied with season only in some copepod species. Thus, it can be assumed that procercoids could occur in all months of the year in natural

waters, but incidence of the procercoid is likely to be minimal during the cold months and maximal during the warm months.

In accordance with Hanzelova *et al.* (1989), no *C. str. abyssorum* was found to be infected with more than one procercoid of *Diphyllobothrium* spp. This could be explained in that there may be the low numbers of infective coracidia in Loch Lomond's water or there may be only room for one procercoid in haemocoel of each copepod. The seasonal changes of abundance of cyclopoid copepods observed in this study may also be explained by alterations in the ecological and climatic conditions in the lake (Chapman, 1972; Adalsteinsson, 1979; Maitland *et al.*, 1981 and Pomeroy, 1987). It could be concluded that the population dynamics of *Diphyllobothrium* spp. in its first intermediate host, *Cyclops strenuus abyssorum* from Loch Lomond results from processes which are indirectly under the influence of seasons. A seasonal pattern of occurrence of *Diphyllobothrium* infection was observed in both copepod and powan ensuring that successful transmission and reproduction occurred during the year.

9.5 PREVALENCE AND INTENSITY OF *DIPHYLLOBOTHRIUM* SPP. INFECTION IN A SECOND INTERMEDIATE HOST, *COREGONUS LAVARETUS*

The prevalence and intensity of *Diphyllobothrium* spp. infections in relation to host size and sex were studied and effects of infection on powan, *Coregonus lavaretus* were compared with those reported in previously published work. A heavy infection of powan with *Diphyllobothrium* spp. plerocercoids in Loch Lomond was recorded with *Diphyllobothrium dendriticum* being more common than *D. ditremum* although they occurred concurrently. The intensity of infection varied with the size of the fish, being

generally absent or rare in small fish and heavy in bigger fish. In accordance with Anderson *et al.* (1987), plerocercoids found in this study were in irregular shaped cysts of varying size located mainly on the stomach. In the past, plerocercoids of *Diphyllobothrium* spp. were categorised among the most pathogenic of fish parasites and were believed to cause serious losses of salmonid fish (especially trout) in the British Isles (Duguid and Sheppard, 1944; Harris and Hickey, 1947; Fraser, 1960) and in Scandinavia (Henricson, 1977). In addition, it was revealed that migration larvae of *Diphyllobothrium*, particularly in small fish could cause much tissue damage and even mortality (Hoffman and Dunbar, 1961). Another reason to account for the pathology might have been the existence of disorganised encysted or free plerocercoids of *Diphyllobothrium* in the body cavity of fish (e.g. on liver, gonad, surface of swim-bladder, spleen and abdominal musculature) as observed by Fraser (1960). Because the mortality of fish infected with *Diphyllobothrium* spp. was observed in the summer months, Hickey and Harris (1947) suggested that high temperature increases the activity of plerocercoids leading to more damage in the fish. During this study, no evidence was observed to indicate the mortality or even the serious loss of condition of the powan caused by *Diphyllobothrium* spp infections. It can be speculated that either the numbers of plerocercoids of *Diphyllobothrium* were insufficient to produce those results, or water temperature of Loch Lomond does not stay warm enough to induce activity of plerocercoids even during summer months. The finding may reflect the evolution of a localised tolerance or compatibility in this host-parasite system.

9.6 PREVALENCE, INTENSITY AND EFFECTS OF *EUBOTHRIUM CRASSUM* INFECTION ON FARMED SALMON, *SALMO SALAR*

The prevalence and intensity of *Eubothrium crassum* and effects of infection on farmed salmon, *Salmo salar* were investigated. The prevalence and intensity of infection were found to be low when compared with those detected by Kennedy (1978) and Bristow and Berland (1991). This may be due to the lack of availability of infected intermediate hosts. It is unclear where these farmed salmon acquire the infection. Perhaps the fish may be infected when they were stocked at parr stage in freshwater. This seems likely since salmon feed solely on plankton for the first few months of life before switching to more energetically profitable benthic and pelagic macroinvertebrates (Maitland and Campbell, 1992). Negative effects of cestodes on gonad development and hepatosomatic index of sticklebacks have been demonstrated (Arme and Owen, 1967; Wilson, 1971; Sweeting, 1977; Tierney, 1991; Bean and Winfield, 1989, 1992). It was observed in this study that *E. crassum* had negative effects on condition of fish and gonadosomatic index. In spite of these negative effects, it appears that *Eubothrium crassum* is not as harmful to salmon as other pseudophyllidean cestodes are to small fish like stickleback.

9.7 CULTIVATION OF PSEUDOHYLLIDEAN CESTODES

An attempt was made to grow *Diphyllobothrium dendriticum* plerocercoids to maturity *in vitro*. Although there has been a great range of success in maintaining *D. dendriticum in vivo* over the years (Archer and Hopkins, 1958; Bell and Smyth, 1958; Anderson, 1978), little advance has been made in culturing this species *in vitro*. The greatest degree of success was achieved by Smyth (1959) who used small fragments of

D. dendriticum plerocercoids cultured in concentrated embryo extract. In the present study, plerocercoids of *D. dendriticum* were kept alive for 10 days and were normal in appearance up to 6 days. Also the plerocercoids grew significantly. Histological examination showed, however, that there was no clear organogenic development after *in vitro*, but individual proglottids and an increased number of darkly stained nuclei could be detected. The very well defined bothria and segmentation could also be clearly seen from SEM. It is very much open to speculation that lack of development and growth in *D. dendriticum in vitro* may be due to a number of factors: Despite nutrient addition, the media used may not contain all the necessary nutrients for the early stage of organogenesis. Possibly, macromolecules specifically produced by the definitive host are necessary to parasite development as suggested by Hopkins and Law (1978). It is likely that, uptake of nutrients was not successful due to some defect in the tegument, for example damage to the microtiches or loss of transporters in the membrane. Carbohydrate intake needs out stripped availability once internal glycogen stores had been exhausted and energy was no longer available for synthetic processes. Bacterial contamination of media and/or parasites is possible. Worms may be damaged and stressed at the time of media change. Incorrect osmolarity or pH of the culture media may inhibit physiological processes. Changes in temperature at media changes may have adversely effected the worms. Build up of acidic waste products such as succinate and lactate may have effects on the worms. Presence of the light might be disadvantage for parasite growth. Clearly experiments on cultivation of *D. dendriticum* need to be repeated in adequate facilities, using larger numbers of worms and greater resources of medium. *Schistocephalus solidus* has been successfully cultured *in vitro* unlike *D. dendriticum* (Mason, 1965; Sinha, 1967; Smyth, 1946, 1959). Because of success in its

in vitro cultivation, much more is known about the basic physiology and metabolism of *Schistocephalus solidus* (Hopkins and Law, 1978; Korting and Barrett, 1977). In the current study, maturation of *S. solidus in vitro* was achieved. Although eggs were successfully recovered after 5 days *in vitro* from one worm, the overall percentage of viable eggs was very low, perhaps because of the restrictive environment in which it was cultured.

9.8 OVERALL CONCLUSIONS

The results obtained during this programme of research have answered some of the identified questions. However, other questions have not been fully answered and in addition, new issues have arisen as result of the work. There are therefore a number of areas where further work is needed:

9.8.1 The need for experimental infections

In the course of this project it was not possible to develop techniques for experimental infections. *In vitro* culture of pseudophyllidean cestodes needs to be developed in suitable and well designed experimental conditions. Once these are developed, the following questions should be addressed:

(1) What are the effects of environmental parameters upon the establishment, survival and fecundity of helminth populations in controlled laboratory infections of hosts using infected intermediate hosts?

(2) What effect does host sex and age have upon infection dynamics?

(3) What are the effects of helminth infections upon trout health in terms of host survival and fecundity?

(4) Are there any behavioural changes in infected fish with helminths which make them more susceptible to predation by potential definitive hosts?

(5) Do helminths have adverse effects on the fine structure of host tissue?

9.8.2 The factors that influence transmission dynamics.

Additional questions that arise in this topic include:

(1) Do birds have a role to play in the transmission of *Diphyllobothrium* between sites?

(2) To what extent is the observed helminth distribution in Scotland brought about by human activities?

(3) The transmission route of *Eubothrium crassum* to farmed salmon needs to be investigated.

9.8.3 Extension of specific results

(1) Evidence for non-random associations, leads to question about inter and intra specicific competition among parasites in same host. Thus, the question is, what factors determine the dynamics of establishment in terms of spatial arrangement of worms in the body cavity and alimentary tract?

(2) What is the significance of encapsulation of *Diphyllobothrium* in powan, how does it come about and from where does it originate? How significant is this phenomenon in terms of the host health?

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Appendix I. Endoparasitic helminth infections found in *Salmo trutta* (brown trout) and *Oncorhynchus mykiss* (rainbow trout) from different locations in Central Scotland.

Abbrevations: No: Number of fish examined, DH: Definitive host, IH: Intermediate host, IH_2: Second intermediate host, A: Adult, P: Plerocercoid, L: Larva.

Location	Species	No	Helminths	Host Status	Stage
Loch Lomond	Brown trout	8	*Crepidostomum farionis*	DH	A
NS3597 56°08.5' 4°38.9'W W56*			*Diphyllobothrium dendriticum*	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A+P
			Capillaria salvelini	DH	A
Loch Maragan	Brown trout	35	*Neoechinorhynchus rutili*	DH	A
NN4027 56°24.7' 4°35.2'W W51*			*Crepidostomum farionis*	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A+P
			Capillaria salvelini	DH	A
			Raphidascaris acus	DH	A
River Fillan	Brown trout	18	*Neoechinorhynchus rutili*	DH	A
			Echinorhynchus truttae	DH	A
NN3726 56°24.1' 4°38.0'W W50*			*Crepidostomum farionis*	DH	A
			Eubothrium crassum	DH	A+P
Aurs Burn	Brown trout	10	*Neoechinorhynchus rutili*	DH	A
57°03.0' 75°05.0'W W64			*Echinorhynchus truttae*	DH	A
			Crepidostomum farionis	DH	A
			Eubothrium crassum	DH	A+P
			Cystidicola farionis	DH	A
Carbeth Reservoir	Brown trout	20	*Neoechinorhynchus rutili*	DH	A
NS5379 55°59.1' 4°20.9'W W64*			*Crepidostomum farionis*	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A+P
Loch Awe	Brown trout	20	*Crepidostomum farionis*	DH	A
NN0722 56°21.3' 5°07.0'W W50*			*Diphyllobothrium dendriticum*	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A+P
			Capillaria salvelini	DH	A
	Rainbow trout	4	*Diphyllobothrium dendriticum*	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A+P
Dunalister Reservoir	Brown trout	15	*Echinorhynchus truttae*	DH	A
NN7158 56°42.0' 4°07.0W W42*			*Crepidostomum farionis*	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A
			Cyatocephalus truncatus	DH	A
Jaw Reservoir	Brown trout	10	*Neoechinorhynchus rutili*	DH	A
NS4975 55°56.9' 4°42.7'W W64*			*Crepidostomum farionis*	DH	A
			Eubothrium crassum	DH	A
			Cyatocephalus truncatus	DH	A
			Raphidascaris acus	DH	A

Cochno Loch	Brown trout	3	*Neoechinorhynchus rutili*	DH	A
NS4976 55°57.5' 4°24.7'W W64*			*Crepidostomum farionis*	DH	A
			Eubothrium crassum	DH	A
Loch Leven	Brown trout	3	*Diphyllobothrium dendriticum*	IH_2	P
NN0960 56°41.8' 5°06.7'W W41*			*Diphyllobothrium ditremum*	IH_2	P
			Eubothrium crassum	DH	A+P
	Rainbow trout	2	-	-	-
Lochan Creag nan caorann	Brown trout	1	*Neoechinorhynchus rutili*	DH	A
(Secret Loch)			*Cyatocephalus truncatus*	DH	A
56°20.2' 21°08.0'W W22			*Crepidostomum farionis*	DH	A
			Raphidascaris acus	DH	A
	Rainbow trout	1	*Neoechinorhynchus rutili*	DH	A
Hill Loch	Brown trout	7	*Diphyllobothrium dendriticum*	IH_2	P
NS5647 55°42.0' 4°17.0'W W64*			*Diphyllobothrium ditremum*	IH_2	P
	Rainbow trout	1	*Eustrongyloides sp.*	IH	L
Loch Rannoch	Brown trout	19	*Echinorhynchus truttae*	DH	A
NN5957 56°41.3' 4°17.7'W W42*			*Crepidostomum farionis*	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A
			Eustrongyloides sp.	IH	L
	Rainbow trout	7	*Neoechinorhynchus rutili*	DH	A
			Echinorhynchus truttae	DH	A
			Crepidostomum farionis	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A
Burncrooks Reservoir	Brown trout	4	*Neoechinorhynchus rutili*	DH	A
NS4879 55°59.1' 4°25.7'W W64*			*Crepidostomum farionis*	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A
Talla Reservoir	Brown trout	15	*Echinorhynchus truttae*	DH	A
NT1121 55°28.7' 32°24.1'W W72*			*Crepidostomum farionis*	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A
			Cyatocephalus truncatus	DH	A
			Eustrongyloides sp.	IH	L
			Capillaria salvelini	DH	A
Loch Venachar	Brown trout	2	*Crepidostomum farionis*	DH	A
NN5705 56°13.2' 4°17.9'W W57*			*Diphyllobothrium dendriticum*	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A
Loch Rusky	Rainbow trout	13	*Neoechinorhynchus rutili*	DH	A
NN 6103 56°12.2' 4°14.0'W W57*			*Crepidostomum farionis*	DH	A
			Eubothrium crassum	DH	A
			Eustrongyloides sp.	IH	L
Carron Vall Reservoir	Brown trout	22	*Echinorhynchus truttae*	DH	A
NS6983 56°01.6' 4°05.7'W W57*			*Crepidostomum farionis*	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eustrongyloides sp.	IH	L
Whitcadder Reservoir	Brown trout	19	*Neoecinorhynchus rutili*	DH	A

NT6563 55°51.8' 2°33.1'W W67*			*Echinorhynchus truttae*	DH	A
			Crepidostomum farionis	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A
			Eustrongyloides sp.	IH	L
Fruid Reservoir	Brown trout	9	*Echinorhynchus truttae*	DH	A
NT0919 55°27.6' 3°25.9'W W78*			*Crepidostomum farionis*	DH	A
			Diphyllobothrium dendriticum	IH_2	P
			Diphyllobothrium ditremum	IH_2	P
			Eubothrium crassum	DH	A
			Eustrongyloides sp.	IH	L
Lake of Mentieth	Rainbow trout	21	*Diphyllobothrium dendriticum*	IH_2	P
NN5700 56°10.5' 4°17.8'W W57*			*Diphyllobothrium ditremum*	IH_2	P

* Details taken from the *Ordnance Survey Gazetteer of Great Britain*, third edition (1992).

Appendix II Natural hosts of *Diphyllobothrium dendritcum*, *D. ditremum* and *D. latum*.

Parasite species	Hosts	Locality	References
Diphyllobothrium dendriticum	**IH$_1$**		
	Diaptomus gracilis	Hamburg area, Germany	Kuhlow (1953)
		Norway	Halvorsen (1966)
		Lake Karelia, USSR	Sergeeva and Freze (1981)
	Diaptomus gracilioides	Norway	Vik (1963)
	Diaptomus vulgaris	Norway	Vik (1963)
	Cyclops strenuus	Norway	Vik (1963)
		Lake Karelia, USSR	Sergeeva and Freze (1981)
	Mesocyclops leucarti	Lake Karelia, USSR	Sergeeva and Freze (1981)
	IH$_2$		
	Salmo trutta	Reservoirs, South Wales	Duguid and Sheppar (1944)
		Yeo reservoir, England	Fraser (1951)
		Poulaphouca Res., Ireland	Hickey and Harris (1947)
		Anoya Watersystem, Norway	Vik (1957)
		Norway	Vik (1963)
	Onchorhynchus mykiss	England	Fraser (1960)
		North America	Rausch (1954)
		Lake Moreno, Argentina	Revenga (1993)
	Onchorhynchus nerca	North America	Anthony (1964)
	Salmo fario	Norway	Vik (1957)
	Salmo shasta	Norway	Vik (1957)
		Trondelag, Norway	Vik (1957)
	Salvelinus alpinus	Lake Bjellojaure, Sweden	Henricson (1977,1978)
		North America	Freeman and Camieson (1976)
	Salvelinus fontinalis	Lake Moreno, Argentina	Revenga (1993)
	Salvelinus malma	North America	Anthony (1964)
	Salvelinus namaycush	North America	Freeman and Thompson (1969
	Coregonus lavaretus	Lake Pyhajarvi, Finland	Margaretha (1991)
			Ohman-James (1968)
			Bylund (1975)
		Joensuu area, Finland	Wikgren (1964)
	Coregonus albula	Joensuu area, Finland	Wikgren (1964)
	Coregonus migratorius	Lake Baikal,USSR	Chizhova and Gofman-Kadoshinkiva (1960)
	Coregonus zenithicus	North America	Freeman and Thompson (1969
	Coregonus clupeaformis	North America	Dick and Poole (1985)
	Gasterosteus pungitius	Norway	Vik (1953)
	Gasterosteus aculeatus	River Chernaya, USSR	Banina and Isakow (1972)

		Hamburg area, Germany	Kuhlow (1953)
	Pungitius pungitius	River Neva, USSR	Banina and Isakow (1972)
	DH		
	Larus argentatus	Hamburg area, Germany	Kuhlow (1953)
		Norway	Vik (1963)
	Larus canus	Lake Erie, Ohio, USA	Buck et al (1976)
		Lake Bjellojaure, Sweden	Henricson (1978)
	Larus ridibundus	Norv ay	Vik (1963)
	Homo sapines	North America	Rausch and Hillard (1970)
	Dog	North America	Rausch and Hillard (1970)
	Larus californicus	North America	Vermeer (1969)
	Larus deliawarnsis	North America	Vermeer (1969)
	Felis Catus	North America	Threlfall (1969)
Diphyllobothrium ditremum	**IH_1**		
	Diaptomus gracilis	Germany	Luhe (1910)
	Cyclops str. abyssorum	Ireland	Hickey and Harris (1947)
	Cyclops strenuus	Lake Karelia, USSR	Sergeeva and Freze (!981)
	Eudiaptomus Gracilis	Lake Karelia, USSR	Sergeeva and Freze (1981)
	IH_2		
	Salmo trutta	Llyn Tegid, Wales	Aderounmu
		Poulaphouca Res., Ireland	Hickey and Harris (1947)
		Llyn Padarn, Wales	Powell (1966)
		Germany	Luhe (1910)
	Onchorhynchus mykiss	North America	Anthony (1967)
	Salvelinus malma	North America	Anthony (1967)
	Salvelinus alpinus	Llyn Padarn, Wales	Powell (1966)
		Lake Bjellojaure, Sweden	Henricson (1977,1978)
		Quebec lakes, Canada	Berube and Curtis (1986)
	Osmerus eperlangus	Hamburg area, Germany	Kuhlow (1953)
		Finland	Bylund (1975)
	Thymallus thymallus	Llyn Tegid, Wales	Chubb (1961)

	DH		
	Phalacrocorax carbo	Germany	Luhe (1910)
		Sweden	Halvorsen (1970)
		Lyn Padarn	Chubb
		Llyn Tegid	Chubb
		Ireland	Hickey and Harris (1947)
	Phalacrocorax graculus	Norway	Vik (1964)
	Phalacrocorax aristotelis	Alaska	Hillard (1960)
	Ardea cinerea	Sweden	Halvorsen (1970)
		Alaska	Hillard (1960)
	Gavia arctica	USSR	Markowski (1949)
		Sweden	Halvorsen 91970)
		Alaska	Hillard (1960)
	Gavia immer	North America	Hillard (1960)
		Alaska	Hillard (1960)
	Gavia stellata	Alaska	Hillard (1960)
	Mergus merganser	USSR	Markowski (1949)
		Alaska	Hillard (1960)
	Mergus serrator		
Diphyllobothrium latum	**IH_1**		
	Cyclops strenuus	Switzerland	Janicki and Rosen (1917)
		River Svir, USSR	Razumova and Gutkoskaya (1959)
	Diaptomus gracilis	Lake Karpero, Finland	
		Switzerland	Guttowa (1963)
	Termocyclops oithonoides	Lake Karpero, Finland	Guttowa (1963)
	IH_2		
	Esox lucius	Lausanne and Neuchatel	
		River Volga, USSR	Janicki and Rosen (1917)
		Rybinsk Reservoir, USSR	Bogdanova (1958)
		River Kokemaenjoki, Finland	Izyumova (1960)
		Lake Winnipeg, Canada	Wikgren (1963)
		Lake Konche, USSR	Nicholson (1932) Malakov (1961)
	Lota lota	Lake Vortsjarv	
		River Kokemaenjoki, Finland	Tell (1971)
		Lake Konche, USSR	Wikgren (1963)
		Finland	Malakov (1961)
	Perca fluviatilis	Lausanne and Neuchatel	Guttowa (1956,1961)
		Switzerland	Janicki and Rosen (1917)

	Lake Konche	Janicki and Rosen (1917)
	Lake Vortsjarv	Malakov (1961)
	Rybinsk Reservoir, USSR	Tell (1971)
	USSR	Izyumova (1959)
		Tarassov (1934)
Lucioperca lucioperca	River Kokemaenjoki, Finland	
Gymnocephalus cernua	Rybinsk Reservoir, USSR	Wikgren (1963)
Salmo trutta	USSR	Izyumova (1959)
Salmo salar	Norway	Chizhova et al (1962)
Acerina cernua	Germany	Vik (1957,1963)
Percichthys sp.	Lake Moreno, Argentina	Kuhlow (1953)
Salvelinus fontinalis	Lake Moreno, Argentina	Revenga (1993)
		Revenga (1993)
DH		
Several piscivorous mammals		
Homo sapines		Janicki and Rosen (1917)
		Tarassov (1934)
		Kuhlow (1963)
		Guttowa (1956,1961)
		Chizhova et al. (1962)
		Vik (1956,1963)

Abbreviations: IH_1, first intermediate host; IH_2, second intermediate host and DH, definitive host. (After Vik, 1964; Chubb, 1980 and Anderson *et al.*, 1987).

PAGE

NUMBERING

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