Studies on the life history of *Schistocephalus solidus*: field observations and laboratory experiments

By

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A thesis submitted for the degree of

Doctor of Philosophy

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November 1991

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ACKNOWLEDGEMENTS

I would dearly like to thank both my supervisors Felicity Huntingford and David Crompton for their advice, help and particularly for their continued encouragement throughout the course of this study. I would also like to thank the Science and Engineering Research Council for financing the project (GR/E56959) and Profs Stephen Phillips and Graham Coombs for use of the departmental facilities. I would also like to express sincere gratitude to the following people: Peter Wright, who instructed me in the art of successfully keeping sticklebacks and who provided a great many helpful discussions; Roddy McDonald, for his part in the foster care of the sticklebacks and Oscars I, II and III; the Edinburgh Parks Department and the people of Inverleith, for allowing me access to the pond in Inverleith Park; the Meteorological Office, for their permission to consult and use temperature records; Graham Tobasnik, for helping me initiate my protozoan cultures; Bob McNuff (Sciento), for constant advice on the maintenance of copepods and providing me with a regular supply; Fraser Darling, for providing access to the facilities at Yorkhill Hospital; Dennis (Yorkhill), for taking care of the chickens and general technical help during those experiments; Kate Orr, for assisting me in the histological sectioning of stickleback kidneys; Peter Rickus, for setting up the camera used in photographing male sticklebacks; the final year Zoology students (1990-1991), who ranked male colouration.

I would also like to pay thanks to my fishing crew: Gwenda Hughes, Lucy Robertson, Gulshan Parveen, Julie Ewald and Lorna Ballantyne who were there to help, not just when the sun was shining, and James Currall, Neil Metcalfe and Pat Monaghan who gave time and help in the statistical analyses. Many others have contributed in various aspects of the project and I acknowledge their help: Kate Robinson, Nicola Fraser, Isabel Coombs, Herbert Paul, Raymond Stoddart and Liz Denton.

Finally, I would express gratitude to my lab mates (Kate, Fraz, Julie, Lorna, Isabel, Lucy, Gulshan, Gwenda), my additional fun suppliers during these last few years (Claire Ditchburn, Leigh Henderson, Sharon Wilson, Chris Hunter, Roy Armstrong, Dave Donnan, Rob Field), my dancing partner (Wylie Horn) and of course my partner in crime, Gwenda Hughes.

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. All animal procedures were carried out under licence from the Home Office: Project Licence 60/00150; Personal Licence 60/01162.

Jayne Tierney

November 1991

DEDICATION

I dedicate this work to mum, dad and the rest of my expansive family

CONTENTS

SUMMARY

Field and laboratory investigations of the interactions between *Schistocephalus solidus* (Cestoda: Pseudophyllidea) and its hosts were carried out using: the copepod, *Acanthocyclops viridis*; the three-spined stickleback, *Gasterosteus aculeatus* and the chicken, *Gallus gallus*. The first part of the dissertation is concerned with the epidemiology and impact of the plerocercoid stage of *S.solidus* on a natural population of three-spined sticklebacks. Samples of about 60 sticklebacks were collected at roughly monthly intervals between August 1988 and September 1989 from an urban pond in Inverleith, Edinburgh.

A study was made of uninfected fish in the 0+ cohort in order to evaluate the base level biology of the population (Chapter 2). Growth of the sticklebacks was confined to their first autumn, spring and summer of life when temperatures were high and daylight hours long. The normal life span of sticklebacks in this population is 12-18 months. However, some large 0+ fish were lost from the population over winter perhaps as a result of predation by black-headed gulls (*Larus ridibundus*). In previous studies lateral plate counts of 4-5 have been associated with a risk of avian predation. As these counts were observed most frequently in the stickleback population of Inverleith this may also reflect the action of avian predators. There is a single opportunity to reproduce between April and June and those that have bred succumb soon afterwards, but some 1+ sticklebacks (presumably non-breeders) manage to survive until their second winter.

Analysis of stomach contents showed seasonal variation in stomach fullness. Overall, stomachs were less full during the winter than at any other time during the survey. Diet composition in uninfected sticklebacks also varied with season. In Inverleith pond, sticklebacks are largely benthic feeders, relying most heavily on chironomids and to a lesser extent on ostracods and free-living nematodes particularly during the spring and summer. Of the zooplankton consumed cladocerans were the most common and present in stomachs throughout the year. A greater reliance on plant material during winter may have been the result of food scarcity and could partly explain the poor growth at this time.

The composition of the parasite population changed markedly during the life span of the

1988-1989 cohort of sticklebacks (Chapter 3). Logistic regression revealed that time of the year was the single most important determinant of the prevalence of *S.solidus* infection. The intensity of infection was also largely predicted by the time of year but in addition, host size was important, with the largest sticklebacks harbouring the most plerocercoids. By far the greatest proportion of infected fish and the highest infection intensities were recorded in autumn 1988. This increase in both the number of plerocercoids (which was accompanied by an increase in the abundance of small plerocercoids), strongly suggests that a wave of new infections was occurring at this time. Additional features of this wave of infection were: a tendency towards over-dispersion of parasite numbers, large relative weights of parasites and the presence of plerocercoids large enough to be infective to a definitive host.

During winter, many infected fish were lost, notably those harbouring the highest parasite burdens, in terms of plerocercoid numbers, plerocercoid size and the relative weight of the plerocercoids. Such heavily infected sticklebacks may have died because of pathological consequences of their infections. Alternatively, the behaviour of the heavily infected host sticklebacks may have been altered such that they were rendered more susceptible to predation by black-headed gulls, a known definitive host of *S.solidus*. If avian predation is responsible for the loss of infected (and some uninfected) fish, then eggs will be released from the rapidly maturing adults into the pond at this time. The prevalence of infection was found to be fairly stable through the remainder of the survey period, but a further decrease in intensity was observed in fish sampled during summer; again this may have been the result of parasite-induced host mortality.

The growth rate of plerocercoids from single infections parallelled fish growth, being high during autumn, spring and summer. In double infections, however, individual plerocercoid growth was reduced, suggesting that limited nutrients were available for parasite growth and this induced competition between two plerocercoids.

Infection with *S.solidus* plerocercoids was accompanied by changes in the diet, growth, reproduction and longevity of sticklebacks from Inverleith pond (Chapter 4). In both winter and summer, food intake was judged to be lower in infected sticklebacks relative to their uninfected counterparts. There were also disparities in diet composition between uninfected

and infected sticklebacks. Generally, fewer infected sticklebacks consumed many of the common prey types and in addition, they tended to exploit arcellidae (autumn, summer) rotifers (summer) and higher plants (winter) to a greater degree than uninfected sticklebacks. These dietary discrepancies may be the result of differences in habitat choice, in vigilance or in competitive ability of uninfected and infected sticklebacks.

Body condition in infected sticklebacks was observed to be lower than that of uninfected sticklebacks during autumn and winter and was in certain months negatively associated with the parasite index. High rates of plerocercoid growth were apparent at this time; this probably resulted in fewer endogenous resources being available to infected fish, which were therefore unable to maintain their body condition. By contrast, in early autumn, the hepatosomatic indices of infected fish were higher than those of uninfected fish. This may reflect some pathological or physiological response to the acquisition of new infections. Thereafter, the hepatosomatic indices of uninfected and infected sticklebacks were comparable until spring. At this time, there was an large increase in the relative liver weights of uninfected sticklebacks which was completely absent in infected sticklebacks. As no relationship was detected between the hepatosomatic indices and parasite indices, parasite growth did not appear to produce a progressive reduction in liver growth. Instead, the high rate of growth and assimilation of carbohydrate by the plerocercoid probably prevented the accumulation of glycogen in the host liver.

The overall effect of *S.solidus* infection on stickleback reproduction was to reduce their chances of reaching sexual maturity. Most infected males did not mature during the breeding season and their body condition was equivalent to the low levels found in all sticklebacks during winter. Only males that were in good condition became mature whilst sustaining an infection with *S.solidus*. Nuptial colouration, kidney hypertrophy and testes size were unimpaired in these mature infected males, but whether they were capable of successful reproduction is debatable.

Few infected female sticklebacks reached sexual maturity although their body condition was similar to that of uninfected females. However, ripe ovaries were detected only in uninfected females with large livers. A low relative liver weight probably does not prevent the

onset of maturity, but it is envisaged that it prevents or at least reduces the chances of the ovaries of infected female sticklebacks becoming fully gravid.

Initial attempts to maintain the life history of *S.solidus* in the laboratory, based on published techniques, proved unsuccessful. Modified procedures for the infection of the definitive host and for harvesting and embryonating eggs produced in the faeces are explained in Chapter 5. New schemes for maintaining and infecting the copepod and stickleback hosts are described and some were employed during the controlled experimental manipulations detailed in the following chapters.

Aspects of the infectivity of plerocercoid stage and fecundity of the adult stage of *S.solidus* were examined using the chicken, *Gallus gallus* as a definitive host (Chapter 6). To investigate size-related infectivity of the plerocercoid stage to a definitive host, a range of plerocercoids were weighed and each fed to an individual male chicken. Only a very small percentage of plerocercoids, weighing less than 50mg, established compared with in excess of 50% in all other weight classes. In order to examine the factors affecting the quantity and quality of eggs produced by the adult stage, 15 similar-sized plerocercoids (160-218mg) were fed to chickens. The majority of plerocercoids administered established as adults and survived until the experiment was terminated on day 7 post-infection (*p.i.*). Faecal egg counts indicated that all established worms commenced egg production on day 2 *p.i.* with peak output from day 2-3 *p.i.*. By day 7 *p.i.*, egg production in the surviving worms had declined, but was still evident. The final weight of the adult and the average egg output per worm were completely unrelated to the initial weight of the infecting plerocercoid. However, the average egg output was predicted by the final adult dry weight and to a lesser extent by the proportion of weight lost in the transition from plerocercoid to adult, but not by the absolute weight loss. A small-scale study demonstrated a degree of intra- and inter-worm variability in the level of egg development.

The factors influencing the acquisition of *S.solidus* infection by the copepod intermediate host were investigated using *Acanthocyclops viridis* (Chapter 7). Manipulation of the relative density of *S.solidus* coracidia to *A.viridis* copepodites had the most profound impact on infection levels. The prevalence and intensity of infection rose with both increasing concentrations of coracidia and with decreasing host density. However, the total number of

procercoids recovered was only influenced by increasing the density of infective stages. Acquisition of infection by *A.viridis* was largely unaffected by the proximity of coracidia, the length of the exposure period and the level of hunger of the host. There is perhaps an upper limit to the number of coracidia that will successfully establish as procercoids in *A.viridis* and as the procercoids were randomly distributed in most treatment groups this is largely a product of random contact with and infectivity of the coracidia.

The results of this study were discussed in terms of the mechanisms involved in transmission of the parasite at Inverleith pond. In addition, possible coevolutionary outcomes of the interaction between *S.solidus* and its hosts are described.

CHAPTER 1: INTRODUCTION

1.1 HOST-PARASITE RELATIONSHIPS

1.1.1 EPIDEMIOLOGY OF PARASITIC INFECTION

Epidemiology is the study of the dynamics and distribution of disease within a host population. A combination of statistical methods for the assessment of disease parameters and mathematical techniques and theory potentially reveal a great deal about the nature of host-parasite relationships. The overall aim of the study described in this dissertation was to examine aspects of the epidemiology of *Schistocephalus solidus* infection in its intermediate and definitive hosts.

1.1.2 CHARACTERISATION OF INFECTION LEVELS IN A HOST POPULATION

There are a number of descriptive statistics available for quantifying the levels of parasitic infection a population of hosts. Most commonly used are the prevalence and intensity of infection and the frequency distribution of parasite numbers (with the associated pattern of dispersion).

Prevalence

Prevalence is a widely used epidemiological statistic that records the proportion (usually expressed as percentage) of the host population infected with a parasite. This 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.

Intensity of infection

An index of the intensity of infection is ideally based on the total number of parasites per infected host. However, this can rarely be determined except by destructive sampling and is usually only practical in certain helminth and ectoparasitic infections. The total number of parasites cannot be determined when the parasite is a species that multiplies within the host, is very small and numerous or is distributed in many host tissues. In these cases indicators of intensity are employed e.g. the intensity of a malarial infection is described by the blood parasitaemia, which in the case of *Plasmodium* spp., is the number of infected red cells per unit of host blood. The intensity of *Schistocephalus solidus* infection in the three-spined stickleback (*Gasterosteus aculeatus*) is usually defined as the number of plerocercoids per infected host and can only be determined at *post-mortem* examination. However, the weight of an infection with

S.solidus could range from less than 0.001g (personal observation) up to 2.240g (Pennycuick 1971b) and since the weight of the parasite burden appears to be the important determinant of pathology (Pennycuick 1971d) intensity, as described above, may be of limited use in quantifying the debilitating effects of an infection. Another measure of intensity usually used for quantifying the intensity of infection with large parasites such as *S.solidus* and *Ligula intestinalis* infections, relates the weight of the worm burden to the weight of the fish and has been termed the parasite (parasitization) index (Arme & Owen 1967; Pennycuick 1971a-d; Seed 1984)

It is calculated either for *S.solidus* as:

$$\frac{\text{weight of } S.solidus}{\text{weight of stickleback + weight of } S.solidus} \times 100 \qquad (1)$$

or as:

$$\frac{\text{weight of } S.solidus}{\text{weight of stickleback}} \times 100 \qquad (2)$$

Both act equally well as measures of the relative weight of parasite to fish tissue, but the second formula is more easily interpreted e.g if the weight of *S.solidus* was equivalent to the weight of fish tissue, the first formula would give a parasite index of 50% and the second formula would give one of 100%.

Frequency distribution of parasite numbers per host

Prevalence and intensity (when re-defined as the total number of parasites per host) combine to dictate the frequency distribution of parasite numbers per host. There are three basic forms that the distribution of parasite numbers per host can take. An under-dispersed distribution of parasite numbers would be represented by most hosts having similar numbers of parasites. The distribution of parasites can also be randomly distributed throughout the host population. Parasites (particularly helminths) are often aggregated or over-dispersed in their

hosts, so that the bulk of the parasite population is harboured by only a small fraction of the host population (e.g. Pennycuick 1971c; Anderson & May 1982; Bundy, Cooper, Thomson, Didier & Simmons 1987; Robert, Boy & Gabrion 1990). With such a distribution many uninfected or lightly infected individuals and few heavily infected individuals are observed.

These distributions are associated with specific relationships between the variance and the mean: under-dispersed (variance\mean < 1); random (variance\mean =1) and over-dispersed (aggregated, variance\mean > 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 distributions of a range of host-parasite relationships and some have also attempted to understand the factors that generate the various patterns of dispersion. Crofton (1971a) and Bradley (1972) recognised that the death of heavily infected hosts could have a potentially strong regulatory effect on an over-dispersed parasite population and its hosts. By the use of theoretical models of the host-parasite interaction and reference to naturally occurring host-parasite systems, Anderson (1978) was able to propose regulatory and destabilising processes that could influence population growth. He predicted, however, that the impact of these processes would be dependent on the distribution pattern of parasites and hosts. To investigate the dynamic mechanisms that generate the various patterns of dispersion in the distributions of parasites and their hosts, Anderson and Gordon (1982) used Monte Carlo simulation experiments based on models of the growth and decay of parasite populations. These are outlined in Table 1.1:

Table 1.1: The factors that generate dispersion patterns (adapted from Anderson and Gordon 1982)

Under-dispersion	**Over-dispersion**
Parasite mortality	Heterogeneity in host susceptibility to infection
Density-dependent processes	Direct reproduction within the host
Parasite-induced host mortality (Host death rate positively correlated with parasite burden)	Heterogeneity in the ability of hosts to kill parasites

Hence, it would seem that 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 & Gordon 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. Moreover, controlled laboratory experiments have been used recently to explore how dispersion patterns can arise in natural host-parasite associations (Chapter 7).

1.1.3 IMPACT OF PARASITES ON THEIR HOSTS

Epidemiological studies are invaluable in describing host-parasite relationships and in addition, may provide clues to the underlying processes that contribute to the growth and decay of host and parasite populations. However, in order to investigate the specific causes of fluctuations in such populations an examination of the host-parasite relationship at the individual level (i.e. the impact of the parasite on the host and the host on the parasite) is required.

Parasites have been found to have a range of possible detrimental effects on their hosts. 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 & Broom 1980). Some parasites have been found to reduce the amount of nutrients and/or energy available to the host. The intestinal tapeworm, *Diphyllobothrium latum* can absorb vitamin B_{12} and so induce a condition in humans that is similar to pernicious anaemia (von Bonsdorff 1956). Pathological changes in the host associated with parasitic infection may be accompanied by altered host behaviour. For example, infection of dace with *D.spathaceum* results in reduced visual ability and consequently, a lowered feeding efficiency, but there is also an increase in the use of surface water (Crowden & Broom 1980). The latter may render the fish more susceptible to the avian definitive host of the parasite and thus, increase the likelihood of parasite transmission. Such an effect of parasites on the behaviour of intermediate hosts has been reported on a number of occasions, yet there are few instances where predation of infected individuals has

been demonstrated. However, starlings (*Sturnus vulgarus*) have been found to selectively predate infected isopod (*Armadillidium vulgare*) intermediate hosts of the acanthocephalan *Plagiorhynchus cylindraceus* in field and experimental studies and this appears to be a consequence of altered behaviour and habitat selection of the isopod (Moore 1983).

It was intended therefore, to examine interactions in an accessible host-parasite system on which background information is available, namely *Schistocephalus solidus* and its hosts. This involved using both a field survey of the epidemiology and impact of the parasite in the stickleback host and experimental manipulations of the adult and first larval stage of the parasite.

1.2 THE *GASTEROSTEUS ACULEATUS* / *SCHISTOCEPHALUS SOLIDUS* SYSTEM: BIOLOGY OF THE PARASITE

1.2.1 LIFE HISTORY OF *SCHISTOCEPHALUS SOLIDUS*

Schistocephalus solidus is a pseudophyllidean cestode that matures in the gut of numerous bird species. Undifferentiated eggs released from hermaphroditic adults and expelled in the bird faeces, embryonate and hatch in water to release free-swimming coracidia. Upon ingestion by certain species of cyclopoid copepod, a coracidium presumably penetrates the gut wall of the copepod and develops to a procercoid in the haemocoel. If such an infected copepod is consumed by a three-spined stickleback, the procercoid burrows through the gut wall and becomes a plerocercoid in the abdominal cavity. The life history of the parasite is completed when an infected stickleback is eaten by an avian piscivore and the plerocercoid matures and reproduces in the host intestine.

1.2.2 GENERAL BIOLOGY OF THE DEVELOPMENTAL STAGES OF *SCHISTOCEPHALUS SOLIDUS*

1.2.2.1 EGG

The eggs released from the uterus of adult *Schistocephalus solidus* are ovoid with a thick sclerotin (quinone-tanned protein), operculate capsule (Smyth 1956), characteristic of cestodes with well developed vitelleria (Smyth & McManus 1989). At this stage they are unembryonated each ovum being completely obscured by yolk follicles. Development of a fully formed embryo is largely temperature-dependent, taking around 7-12 days at 25-26^{o}C to complete (Hopkins &

Smyth 1951; Mason 1965). The resultant hexacanth (6-hooked) larva occupies nearly the entire volume of the egg (Smyth 1950). Hatching releases the free-swimming, ciliated coracidium via the opercular opening. This process is accelerated by light (Thomas 1947; Mason 1965; Orr & Hopkins 1969), perhaps as a result of a light-activated enzyme attacking the opercular seal, as is believed to occur in the similarly-structured trematode egg (Smyth & Halton 1983).

1.2.2.2 CORACIDIUM

Upon release from the egg, the spherical coracidium makes slow rotating movements (Mason 1965), but is soon found spiralling rapidly in many directions. Disagreement exists as to longevity of the coracidium, with 6-12h being stated by Smyth (1959) and 5-6 days by Thomas (1947). However, Dubinina (1957) and Mason (1965) agreed that they survived for 48h at 21^{o}C and 24h at 26^{o}C.

A great many cyclopoid copepod species and *Diaptomus gracilis* have since been infected with *S.solidus* (Callot & Desportes 1934; Thomas 1947; Clarke 1954; Dubinina 1957; Mason 1965), highlighting the lack of host specificity at this stage. Once a coracidium is ingested by a copepod, the ciliated layer is lost and the resultant oncosphere burrows through the intestine wall (with the aid of hooks and perhaps histolytic secretions of frontal glands, which have been detected in other pseudophyllideans (Kuperman & Davydov 1982)) and eventually resides in the body cavity. The fate of the coracidia, and thus the individual susceptibility of copepods, is likely to be determined by the mechanical, physiological and immunological properties of the copepod intestine and haemocoel.

1.2.2.3 PROCERCOID

Once the procercoid has invaded the copepod haemocoel, it undergoes rapid growth and elongation (Clarke 1954; Mason 1965). Constriction of the posterior end leads to the separation of the hooks into the appendage called the cercomer, which is thought to be the remnants of the oncosphere to be shed (Mackiewicz 1984). Formation of the cercomer is associated with a decrease and eventually a cessation of growth of the procercoid (Clarke 1954; Mason 1965). Calcerous corpuscles (made up of an organic base and inorganic components, primarily calcium) finally appear and become more numerous, marking the last stages in development. Partially-formed microthriches, which amplify the surface for nutritive absorption

in the plerocercoid, are also present (Charles 1971). The time taken to attain this level of development and hence infectivity varies between species, but at 25^{o}C is approximately 8-10 days (Orr & Hopkins 1969). Growth and development of the procercoid appears to be inhibited in multiple infections (Mason 1965).

1.2.2.4 PLEROCERCOID

Three-spined sticklebacks become infected with *S.solidus* plerocercoids by ingesting infected copepods. The procercoid burrows through the gut wall into the body cavity of the stickleback and there, development of the larva takes place.

Host specificity

Although plerocercoids of the genus *Schistocephalus* have been reported from several freshwater species of fish (Dubinina 1959), plerocercoids of *S.solidus* appear to be confined in nature to a single host species, *Gasterosteus aculeatus* (Hopkins & Smyth 1951). Following surgical transfer from *G.aculeatus* to *G.aculeatus*, plerocercoids consistently survived and developed normally, but upon transfer from *G.aculeatus* to *Pungitius pungitius* (the nine-spined stickleback) plerocercoids failed to grow. Transfer to other freshwater fish species resulted in death within 10 days (Bråten 1966). Injection of procercoids into the body cavity gave rise to normal infections in *G.aculeatus*, but not in *P.pungitius* (Orr, Hopkins & Charles 1969). However, the most convincing evidence of *S.solidus* host-specificity comes from the administration of infected copepods via the normal oral route. Both marine and freshwater forms of *G.aculeatus* and *P.pungitius* became infected, but degenerative changes in the cuticle and subsequent death of the plerocercoids were observed in *P.pungitius* (Orr, Hopkins & Charles 1969).

Growth and metabolism

Studies on plerocercoids cultured *in vitro* have indicated that growth is temperature-dependent, being optimal at 23-27^{o}C, and proceeds at a faster rate in smaller plerocercoids (McCaig & Hopkins 1965). *In vivo* studies have further revealed that plerocercoid growth is reduced with increasing infection intensity (Orr & Hopkins 1969; Meakins & Walkey 1973). The increase in size of the plerocercoid is accompanied by accumulation of glycogen stores, perhaps as a consequence of active uptake of glucose through the tegument. By using electron

microscopy studies of the plerocercoid, it was found that uptake of high molecular weight markers appears to occur by a process of tegumental endocytosis (Hopkins, Law & Threadgold 1978; Threadgold & Hopkins 1981). However, Conrad and Peters (1989) could find no evidence of this and in fact deemed one of the markers as toxic. Therefore, how uptake of macromolecules in the plerocercoid is achieved is still debatable.

Glycogen appears to be the major energy source utilised by the plerocercoid, which possesses a very active glycolytic pathway (Körting & Barrett 1977). A full complement of tricarboxylic acid cycle enzymes is also evident and their activities are characteristic of helminths that fix carbon dioxide and adopt a partial reversed cycle (Körting & Barrett 1977). Although no appreciable lipid utilisation is observed *in vitro*, the plerocercoids of *S.solidus* have a complete set of β-oxidation enzymes whose function is unclear (Barrett & Körting 1977).

Development

Upon establishing in the body cavity of the three-spined stickleback, the only marked morphological change from procercoid to plerocercoid is the loss of the cercomer (Clarke 1954). There is rapid growth and an increase in the number of microthriches in these young plerocercoids to levels comparable to those found in large plerocercoids and adults (Charles 1971). The function of these cytoplasmic extensions is presumably to amplify the absorptive area rather like the microvilli of intestinal mucosa.

Internal segmentation precedes any signs of proglottid formation (Clarke 1954; Hopkins & McCaig 1963) and is visible as clusters of densely nucleated cells of genital primordia in the mid-line of 2-3mg plerocercoids (Hopkins & McCaig 1963). The first external segmentation appears in plerocercoids of between 3 and 5mg, around the middle third of the larvae and proceeds anteriorly and posteriorly from this point (Clarke 1954; Mason 1965). Proglottid formation is complete in plerocercoids of 6mg or more, but it is not until worms reach approximately 19mg that the genital primordia (uterus, seminal vesicle, cirrus, testes, ovaries and vitellaria) are formed (Hopkins & McCaig 1963). Thereafter, development of the genitalia is restricted to increases in size of the existing organs and the formation of lumina in the ducts (Hopkins & McCaig 1963). By this stage, the microthriches vary locally in number, size and amplification properties (Threadgold & Robinson 1984).

1.2.2.5 ADULT

The natural definitive host of *S.solidus* is an avian piscivore which acquires infection following ingestion of a stickleback harbouring the plerocercoid stage. The adult then lives and reproduces in the intestine of the bird.

Host specificity

The high degree of host specificity evident in the plerocercoid stage of *S.solidus* (Bråten 1966; Orr, Hopkins & Charles 1969) is absent in the adult stage, which exploits a wide range of host species. Natural infections with adult *S.solidus* have been described in many avian piscivores, especially gulls (Pemberton 1963; Vermeer 1969; Bakke 1985) but also crows (Andrews & Threfall 1975); in addition, some mammals and non-piscivorous birds have been experimentally infected (Hopkins & McCaig 1963; McCaig & Hopkins 1963).

Metabolism

A sharp reduction in the glycogen content of adult *S.solidus* occurs following 24h in a pigeon intestine, suggesting high carbohydrate utilisation (Hopkins 1950). This is supported by the observation that *in vitro* transformation of plerocercoids to adults (by incubating them at 40^{o}C) results in an increased flux of carbohydrate through the glycolytic pathway (Körting & Barrett 1977). As in the plerocercoid, glycogen is the major energy substrate in the presence and in the absence of oxygen. Under anaerobic conditions (as would be experienced in a bird intestine) approximately 70% of the catabolised glygogen is excreted as acetate and propionate, despite the presence of an active lactate dehydrogenase and the ability to produce lactate (Körting & Barrett 1977). It appears then that the energy obtained from glycolysis is produced via carbon dioxide fixation and a partial reversed tricarboxylic acid cycle (Körting & Barrett 1977). The mass action ratios of phosphofructokinase and pyruvate kinase suggest that they are key enzymes in the regulation of the glycolytic pathway (Beis & Barrett 1979).

Maturation and reproduction

Owing to the progenetic (having advanced genital development without maturation) nature of the plerocercoid and its large glycogen reserves, maturation is triggered rapidly as a result of rise in temperature to 40^{o}C (Smyth 1946). However, the rate of gametogenesis and the subsequent production of eggs by adults, is affected by the actual environmental temperature of

the plerocercoids. Thus, temperatures less than 40^{o}C reduce egg production in adults matured in the peritoneum of mice (rectal temp. 36.5^{o}C) and in culture (Hopkins & McCaig 1963; Smyth 1952). Histological studies of adults that developed *in vitro*, have revealed that this effect of temperature may be a consequence of a reduced rate of oogenesis and production of the yolk and shell (Smyth 1952). Furthermore, the capacity for embryonation is low in eggs produced from adults at 33-34^{o}C (Smyth 1952) and this may be due to decreased fertilisation as a result of abnormal spermatogenesis (Smyth 1952). Even when complete development of the male and female genitalia takes place, the production of fertile eggs relies on physical conditions that compress the worm and encourage sperm transfer from the cirrus to the seminal receptaculum (Smyth 1954). This occurs naturally in the intestine of the definitive host and can be achieved *in vitro* with the aid of dialysis tubing (Smyth 1954).

There is evidence from culturing individual worms that self-fertilisation (cirrus inserted into the vagina of proglottids from the same worm) may occur in adult *S.solidus* (Smyth 1954). In addition, isoenzyme analysis of naturally occurring plerocercoids did not reveal any polymorphic variants in the 4 enzymes being studied (McManus 1985); this lack of variation would be expected if self-fertilisation were the normal mode of insemination.

1.3 THE *GASTEROSTEUS ACULEATUS* / *SCHISTOCEPHALUS SOLIDUS* SYSTEM: BIOLOGY OF THE HOST

The three-spined stickleback, *Gasterosteus aculeatus*, is found in freshwater, brackish and marine habitats in the world's temperate regions. The freshwater populations are non-migratory, whilst the marine populations are anadromous and breed in fresh or brackish water (see Wootton 1976, 1984 for a review of general biology and morphology).

A number features of stickleback biology make its interaction with *S.solidus* an attractive system to study and there is a large literature available on the subject (detailed in relevant chapters). Numerous authors have examined the natural diet of the stickleback and found it to be very varied and related to factors such as availability, season etc. Moreover, experimental investigations have found that prey selection relies primarily on visual cues, but will be influenced by the state of hunger and the competitive ability of the stickleback, in addition to the perceived risk of predation. Therefore, infection with *S.solidus* could potentially influence

the diet via many channels. Sticklebacks also have well developed anti-predator behaviour, the extent of which depends on both inherent factors and experience. Again, there is scope for *S.solidus* infection to interfere with development of the behavioural repertoire or indeed manipulate it. Finally, being a small fish with a short life span and a costly reproductive period, there are many possibilities for such a large parasite to alter the normal scheme of growth and development.

1.4 AIMS

The aims of this study are:

Field survey

a) To characterise the effect of season on the prevalence, intensity and distribution of *S.solidus* plerocercoids in a population of sticklebacks (Chapter 3).

b) To characterise the effect of sex and size of stickleback on the prevalence and intensity *S.solidus* infection (Chapter 3).

c) To examine the patterns of plerocercoid growth in single and multiple infections of sticklebacks (Chapter 3).

d) To investigate the impact of *S.solidus* infection on the diet, body condition and reproductive capacity of a population of sticklebacks (Chapter 4) using a base level study of uninfected fish in the same site (Chapter 2) for comparison.

Experimental studies

e) To revise the methods for maintaining the life history of *S.solidus* in the laboratory and to develop techniques for obtaining controlled infections of hosts (Chapter 5).

f) To look at the infectivity of plerocercoids and the fecundity and egg quality of adults of *S.solidus* in relation to worm size (Chapter 6).

g) To explore acquisition of *S.solidus* infection by a first intermediate host, *Acanthocyclops viridis* in relation to the relative densities of host and parasite, hunger and the length of the exposure period (Chapter 7).

CHAPTER 2: THE BIOLOGY OF THE THREE-SPINED STICKLEBACK (*GASTEROSTEUS ACULEATUS*) OF INVERLEITH POND

2.1 INTRODUCTION

In order to examine the host-parasite interactions of a naturally occurring *Gasterosteus aculeatus/Schistocephalus solidus* system, a thorough knowledge of the base level biology of sticklebacks free from *S.solidus* infection was first required. Aspects of the stickleback's biology that will be of concern are morphology, growth, development, diet and behaviour.

2.1.1 GENERAL BIOLOGY AND MORPHOLOGY OF *GASTEROSTEUS ACULEATUS*

The stickleback is a small, laterally compressed fish which usually swims by means of a large pair of pectoral fins, with strong muscular bases (see Wootton 1976, 1984 for a review of general biology). Contrastingly, each pelvic fin is reduced to a spine and a single soft fin ray. The dorsal and anal fins are situated posteriorly on the body and just anterior to the dorsal fin are the three spines from which the fish derives its name. An important property of stickleback spines is that they can be locked in an erect position. Owing to the special characteristics of the locking mechanism, downward pressure on the spines (like that exerted by the jaws of a predator) will not cause them to collapse. Spine deficient morphs do occur and it has been suggested that they have evolved in response to an environmental calcium deficiency (Giles 1983a), but other evidence strongly indicates that a shift from vertebrate to insect predation is responsible (Bell 1988).

Unlike most teleost fish, the three-spined stickleback lacks scales and is instead, armoured with bony plates (or scutes). A row of plates exists along the back and is fairly constant in number (around 6). Along the flanks are lateral plates which show intra- and inter-population variation in frequency. The number and arrangement of the lateral plates, allows sticklebacks to be categorised into three recognised plate morphs: trachurus, leirus and semi-armatus.

The trachurus form has completely plated sides (30-35 plates), beginning just forward of the pectoral fins, terminating in a caudal keel on each side. The leirus morph has few lateral plates (0-9) in the anterior region of the body and lacks a caudal keel. The semi-armatus stickleback has characteristics which are intermediate between those of the leirus and trachurus forms. Some anterior plates exist, as does a caudal keel, but they are separated by a plateless region. In the past the terms trachurus and leirus have been used to classify freshwater and

marine populations of sticklebacks (Hagen 1967), but are being used here only to describe morphological variants, as advised by Bakker and Sevenster (1988).

2.1.2 GROWTH AND LIFE HISTORY OF *GASTEROSTEUS ACULEATUS*

Growth is a change in the size of an individual, usually measured in units of weight, length or energy. It has been found to be influenced by both exogenous factors (e.g.food supply, temperature) and endogenous factors (e.g. genetic control, body size) each interacting to produce a spectrum of individual growth patterns (Wootton 1990).

A true picture of growth in a population of fishes can be obtained only by following length or weight of the same individuals over a period of time. When this is not possible, repeated destructive sampling may give a good approximation, provided that age classes can be discriminated. However, an event such as breeding, which results in increased numbers of small fish in a sample, will also influence the apparent mean length or weight for that sample. Therefore, careful interpretation of growth patterns as calculated by the latter method is essential.

Once sticklebacks have hatched, most immediately begin to grow and to develop the morphological traits described above. Primarily growth is sustained by the yolk sac and thereafter, the young fish begins to take food from its environment. A high initial rate of growth is common amongst natural populations of sticklebacks (Jones & Hynes 1950; Allen & Wootton 1982), probably as a result of relatively warm, autumn water temperatures and a plentiful food supply. During the first winter of life there is often a lag in growth (Mann 1971; Allen & Wootton 1982), which may be accounted for by low water temperatures, poor food availability or limited daylight for foraging. With the approach of spring/summer and increased temperatures, growth tends to resume (Mann 1971; Allen & Wootton. 1982). In annual populations, breeding will then occur and death usually follows in the autumn and winter (Mann 1971; Ukegbu 1986).

In populations where the maximum life span exceeds 12-18 months, breeding rarely takes place in the first year. Instead, in successive years, growth continues at a lower level, until the fish have matured and bred (Jones & Hynes 1950; Pennycuick 1971d). It is important, therefore, to analyse the age structure of a group of sticklebacks before attempting to assess

growth rates. This may be achieved either by looking at the pattern of rings on the otoliths (Jones & Hynes 1950; Allen & Wootton 1982) or by using the frequency distributions of their lengths. Prerequisites of the latter method are a population with a short breeding season and a fairly high rate of growth. Under these conditions, new fry are recruited in a series of annual waves, which form distinct peaks on a length frequency histogram.

2.1.3 DIET OF *GASTEROSTEUS ACULEATUS*

The diet of the three-spined stickleback has been investigated extensively (e.g. Hynes 1950; Walkey 1967; Allen & Wootton 1984 and in Scottish populations: Ukegbu & Huntingford 1988; Ibrahim & Huntingford 1989). These studies indicate that sticklebacks are predominantly carnivorous fish, feeding on a range of invertebrate prey types. Overall, copepods and cladocera were the common zooplanktonic prey and insect larvae/nymphs and ostracods were the most frequently consumed benthic prey. However, individual populations often vary in the relative proportions of the prey types selected. For example, Ibrahim (1988a) studying two Scottish populations found that in their natural habitats, one population fed on benthos whilst the other fed on plankton. Laboratory experiments on the diet choice of the two populations revealed that both preferred plankton (Ibrahim 1988b), leading him to conclude that the natural diet was a reflection of food availability in the respective habitats. Furthermore, the naturally planktonic population had significantly smaller gapes and finer gill raker spacing than the naturally benthic population, which probably allowed them to feed more efficiently on plankton. This would suggest that these populations exhibit morphological adaptations linked to prey availability. Intra-population differences in diet have also been detected in sticklebacks, in relation to variables such as season and fish age and sex.

Seasonal variation in the diet was described by Allen & Wootton (1984) and Ukegbu & Huntingford (1988). Both noticed a summer increase in the consumption of copepods and Allen & Wootton (1984) found that chironomid larvae and ostracods were taken throughout the year. Plant material is a rare component of the diet, but was consumed in a seasonal pattern. The sticklebacks in the studies of Walkey (1967) and Allen & Wootton (1984) consumed algae and higher plant material during the winter. It is possible that seasonality in diet structure in these studies also reflect, changes in the availability of food, rather than a shift

in diet choice. This can be illustrated by cannibalism of stickleback eggs which was a feature of all the above studies and occurs only during the breeding season when eggs are present.

Feeding habits can also vary with fish gender and this is usually most apparent during the breeding season (Manzer 1976). Mature males devote themselves to defending a territory, nest construction, attracting mates and looking after fry. This will tend to limit the time and space that males have available for foraging. Instead mature females exist in loose schools and do not enter the territories of males until they are gravid and ready to spawn. Females are therefore likely, to have more time for foraging and probably cover a greater area of the habitat. Fish size is an obvious determinant of diet (Hynes 1950; Ukegbu & Huntingford 1988) since the size of fish will limit the size range of prey that can be handled and consumed.

2.1.4 PREDATORS AND ANTI-PREDATOR DEFENCE OF *GASTEROSTEUS ACULEATUS*

Three-spined sticklebacks are subject to predation by a range of animal groups including birds, fish, mammals, reptiles and invertebrates. Sticklebacks themselves contribute as predators by cannibalising the eggs and fry of conspecifics (Hynes 1950; Ukegbu & Huntingford 1988; Foster 1988). Habitat diversity and the associated variation in the occurrence and intensity of predation lead to differences in the morphology and behaviour of these fishes.

The importance of predation at a particular locality has been investigated in a number of ways. Assessing which predators exist at a site and whether sticklebacks are likely to form part of their diet was a qualitative method employed by Gross (1977, 1978) and Giles & Huntingford (1984). Predation intensity has also been estimated by directly examining predator stomachs for the presence of sticklebacks at different times of year (Moodie 1972; Hagen & Gilbertson 1973).

Predation appears to be linked to morphological characteristics of the stickleback. A size comparison between sticklebacks in the stomachs of predatory fish, with those in the population as whole revealed a tendency for small fish to be predated. Having short dorsal spines and eight lateral plates also seemed to predispose this population to predation (Moodie 1972). Many authors have reported that sticklebacks with long dorsal spines and a modal lateral

plate number of seven occur most frequently at sites that experience high fish predation (Hagen & Gilbertson 1973; Moodie & Reimchen 1976) or a combination of high fish and bird predation (Hagen & Gilbertson 1972; Gross 1978). However, a mode of five was found in response to garter snake predators (Bell & Haglund 1978); modes of three or four when the main predators were birds and modes close to zero when invertebrates were the main predators (Reimchen 1980).

Whilst there are clear indications from the above studies that morphological variability is closely related to predation, there are few functional explanations for this variation. Reimchen (1991) suggests that such variation reflects adaptations to piscivores with differing means of pursuing, manipulating and consuming prey. For example, having long dorsal and pelvic spines would confer an advantage to a stickleback encountering a trout, because it is a gape-limited predator. By contrast, they would prove useless during in an encounter with a river otter, which would break off the spines before attempting ingestion (Reimchen 1991).

Although much attention has been given to the influence of predation on morphological variation, the effects on intra-specific behavioural differences have also been studied (Huntingford 1982; Giles 1984; Giles & Huntingford 1984; Tulley & Huntingford 1987; Foster 1988; Foster, Garcia & Town 1988). Each stage in the life span of a stickleback appears to have associated behavioural adaptations to the type and intensity of predation experienced.

The response to predators develops with size irrespective of exposure to predators (Giles 1984), suggesting the anti-predator behaviour has some inherited component. However, it seems that greater exposure to predators results in an increased anti-predator response, such that fish from high risk sites react most strongly to a predator or predatory stimulus (Giles & Huntingford 1984; Tulley & Huntingford 1987).

It is not only the anti-predator response of sticklebacks that is influenced by predation risk, but the behavioural interactions with conspecifics. Huntingford (1982) observed that intra-specific aggression was lowest in populations where the risk from predation was high and Foster (1988) found nesting males displaying diversionary behaviour to protect their young against cannibalistic foraging groups.

2.1.5 AIMS

The major aim of this chapter is to describe the general biology of a population of sticklebacks that were exposed to *S.solidus*. By concentrating on those sticklebacks that were free from infection, the effects of *S.solidus* on the biology of the stickleback could be more clearly assessed at a later stage (Chapter 4).

2.2 MATERIALS AND METHODS

2.2.1 STICKLEBACK POPULATION

The host stickleback population is located in a small, shallow urban pond situated in a public park in Edinburgh (74N 27E O.S. Second Series Sheet 66). Two quite separate parts of the pond exist, providing slightly different habitats for the sticklebacks and probably accounts for the differences detected in their parasite fauna. To avoid confusion, sampling was confined to the large pond, holding the bulk of the population.

This population was selected for the survey for a number of reasons. Primarily, it is a natural source of *S.solidus* infection, which can be reliably sampled throughout the year. Also, literature is available on the breeding, paternal and anti-predator behaviour of the sticklebacks from this site (Huntingford 1982; Tulley & Huntingford 1987). Finally, it is also known that there are no other fish species present and the main avian inhabitants are black-headed gulls (*Larus ridibundus*), which nest nearby.

2.2.2 DETERMINATION OF SAMPLE SIZE

It was necessary to decide on the minimum sample size, that would provide an adequate representation of the parameters of interest. Therefore, the mean and variance of the length and weight of sticklebacks and the prevalence and intensity of *S.solidus* infection were plotted for different sample sizes of fish (caught in August 1988). These were found to stabilise at a sample size of around 60 and so this was the figure that was aimed for when sampling.

2.2.3. COLLECTION AND PRESERVATION OF SAMPLES

Collections were taken at approximately monthly intervals between August 1988 and September 1989. To ensure that fish were sampled from other than just the pond margins, a small trawl was employed. This incorporated a net 90cm wide and 65cm deep, the mouth of which was held open by weights. It was supported by a wooden beam and pulled by a 25m

polypropylene rope. A sample of at least 60 fish was taken on all but the first occasion, because at this time the desired sample size had yet to be calculated. They were either killed in the field or back at the laboratory by exposure to an excess of benzocaine anaesthesia and then frozen at -20^{O}C until they could be dissected.

2.2.4 DATA COLLECTION

Stickleback body measurements

For all sticklebacks sampled, the following measurements were recorded:

Number of lateral plates - the number of plates down each side of the body counted separately. Any counts greater that 15 were combined.

Standard length - the length from the tip of the snout to the caudal peduncle to the nearest mm.

Total weight - fish were blotted and weighed to the nearest mg.

Liver weight - the liver was separated from the intestine, blotted and weighed to the nearest mg.

Carcass weight - once all the visceral organs had been removed (liver, heart, intestine, kidneys, gonads) the inside and outside of the fish was blotted and it was weighed to the nearest mg.

Sex and maturity

Prior to dissection, males collected during the breeding season and exhibiting nuptial colouration, were first thawed and then photographed using a 35mm camera with fixed aperture size, shutter speed, light source and distance from the subject (excepting a small focus distance). Additionally, the wet weight of testes and kidneys were noted and the trunk portions of the kidneys preserved in 10% buffered formalin. In adult females and immature fish, gender and the extent of maturity could only be determined by dissection and inspection of the gonads. They were categorised as follows:

Undifferentiated- gonads absent or indistinguishable as male or female or fish below 30mm.

Immature female - gonads with small white undeveloped oocytes.

Mature (inter-spawning) female - gonads with developing oocytes (yellow, granular) and mature ova (yellow translucent).

Mature (gravid) female - gonads with mature ova only (yellow, translucent).

Immature male - no nuptial colouration and gonads covered with melanophores (black pigment

cells) giving a grey/black speckled appearance.

Mature - red nuptial colouration and gonads covered with melanophores

Quantifying stomach contents

A number of methods are available for analysing the diet of fishes (Hynes 1950; Hyslop 1980), two of which were employed in this study. The first was the occurrence method, which involves recording the number of fish with stomachs containing one or more individuals of each food category. This is then expressed as a percentage of all fish stomachs examined, to illustrate what percentage of each sample has consumed each food type. This method is quick and simple to apply, but gives no indication of the quantity of each food item in stomachs.

To overcome this, an essentially volumetric points method was employed based on that of Hynes (1950). Each stomach was first given points for fullness and then food categories were allocated a proportion of these points, relating to their estimated contribution to the stomach fullness. The following points were awarded for fullness: 0 = empty; 6 = quarter full; 12 = half full; 18 = three-quarters full; 24 = full; 30 = distended, and then the food types were assigned to their major taxonomic groups (Ward & Whipple 1959). The volume that was occupied by each food type, relative to the total food volume (stomach fullness), was then estimated and in view of the approximation of the method, the points for these food items were restricted to a combination of 1, 2, 3, 6, 12, 18, 24, 30. The method provides an estimate of the bulk of food items, but it has the disadvantage of assigning more points to a few large items, compared with many small items.

The number of stomachs in each fullness category was then expressed as a percentage of the total stomachs being examined. Secondly, the points awarded to a food type was expressed as percentage of the points awarded for stomach fullness i.e. the volume occupied by a food type as a percentage of the total volume of food in a stomach.

Parasite status

The body cavity, intestine, viscera, urinary bladder and eyes were all checked for the presence of helminth parasites. *S.solidus* plerocercoids were counted and weighed individually and the quantity and stages of other helminth parasites were recorded.

2.2.5 DISCRIMINATION BETWEEN AGE CLASSES

To enable the above parameters to be followed for a one year period in a single age class of sticklebacks, total monthly samples had to be separated into age classes. Length frequency histograms were plotted for each sample collected (Figure 2.1) and from these cumulative percentage frequencies were derived and plotted on arithmetic probability paper.

A normal frequency distribution is reflected by a linear plot, but if two overlapping normal distributions exist the result will be 2 straight lines, separated by a change in the direction of the graph. The point at which the graph changes direction (the point of inflexion), indicates where the two distributions divide. Occasionally, the point of inflexion was unclear, but by examining the shape of the appropriate length frequency histogram the situation was always resolved. All the samples were thus separated into adults and young of the year. Furthermore, individuals judged to be infected with *Schistocephalus solidus* were removed from these initial analyses.

2.2.6 ENVIRONMENTAL CONDITIONS

Neither water nor air temperatures were recorded during the course of the survey, despite temperature being important in the growth and development of most animals. However, air temperatures at the Royal Botanic Gardens recording point were obtained from the Meteorological Office, Edinburgh for the period of the survey. The Royal Botanic Gardens are adjacent to Inverleith Park and so these temperatures are assumed to reflect conditions at Inverleith pond, during the period of the survey.

2.2.7 DATA ANALYSES

Growth

The growth of sticklebacks was expressed as an instantaneous percentage growth rate per unit of time, using both weight and length as the indicators of changes in size:

$$\text{Specific growth rate} = \frac{(\ln W_2 - \ln W_1)}{t_2 - t_1} \times 100$$

where:

W_1 = the mean weight of sample 1 (mg)

W_2 = the mean weight of sample 2 (mg)

t_1 = the date of sample 1 (days)

t_2 = the date of sample 2 (days)

To determine the specific growth rate in terms of length the same formula was used but mean lengths (mm) were used instead of mean weights. Both provide measures of growth that relate to the sampling time scale.

Sex and maturity

As sample sizes were not sufficient to compare the monthly occurrence of fish at different stages of maturity, it was necessary to combine samples and instead analyse frequencies, with respect to season:

December - February	= winter
March - May	= spring
June - August	= summer
September - November	= autumn

A Chi-Square test of independence was utilised. However, the data were still plotted by month to illustrate subtle changes.

Diet

Again, samples had to be categorised according to season to provide sufficient numbers for the statistical analysis of diet. Both the raw frequencies from the occurrence method and stomach fullness were compared for seasonal variation using the Chi-Square test of independence. The percentage bulk contribution of dietary items was compared by season using a Kruskal Wallis one-way ANOVA. All the dietary data were plotted by month again to provide greater detail.

2.3 RESULTS

2.3.1 GROWTH AND LIFE HISTORY OF UNINFECTED STICKLEBACKS

Life history

An examination of the length frequency distributions of complete samples, that had been separated into age classes, provided information about the life-history of Inverleith sticklebacks (Figure 2.1(a-m)). In August (Figure 2.1(a)) there were two length modes present, one at 21-25mm and another at 46-50mm. These represent the two age classes that were present. The small fish are actually fry produced during the 1988 breeding season (age = 0+) by adults that had bred during their first year of life (age = 1+). Few 1+ adults remained in August, suggesting that many had died post-breeding. This is not surprising, as dead adults were frequently observed, when sampling took place late towards the end of the breeding season.

The numbers of 1+ fish were observed to decline steadily throughout autumn and winter, but some persisted until early June (Figure 2.1(e-k)). Subsequently, a new mode of small fish appeared, indicative of 1989 fry (0+). The two modes were less well separated than in the previous year, but two age classes could still be discriminated. Thus, sticklebacks from Inverleith pond have a life span of 12-18 months, they breed in their first year and die shortly afterwards. The remainder of results described in this chapter will be confined to 0+ sticklebacks that were not infected with *Schistocephalus solidus*.

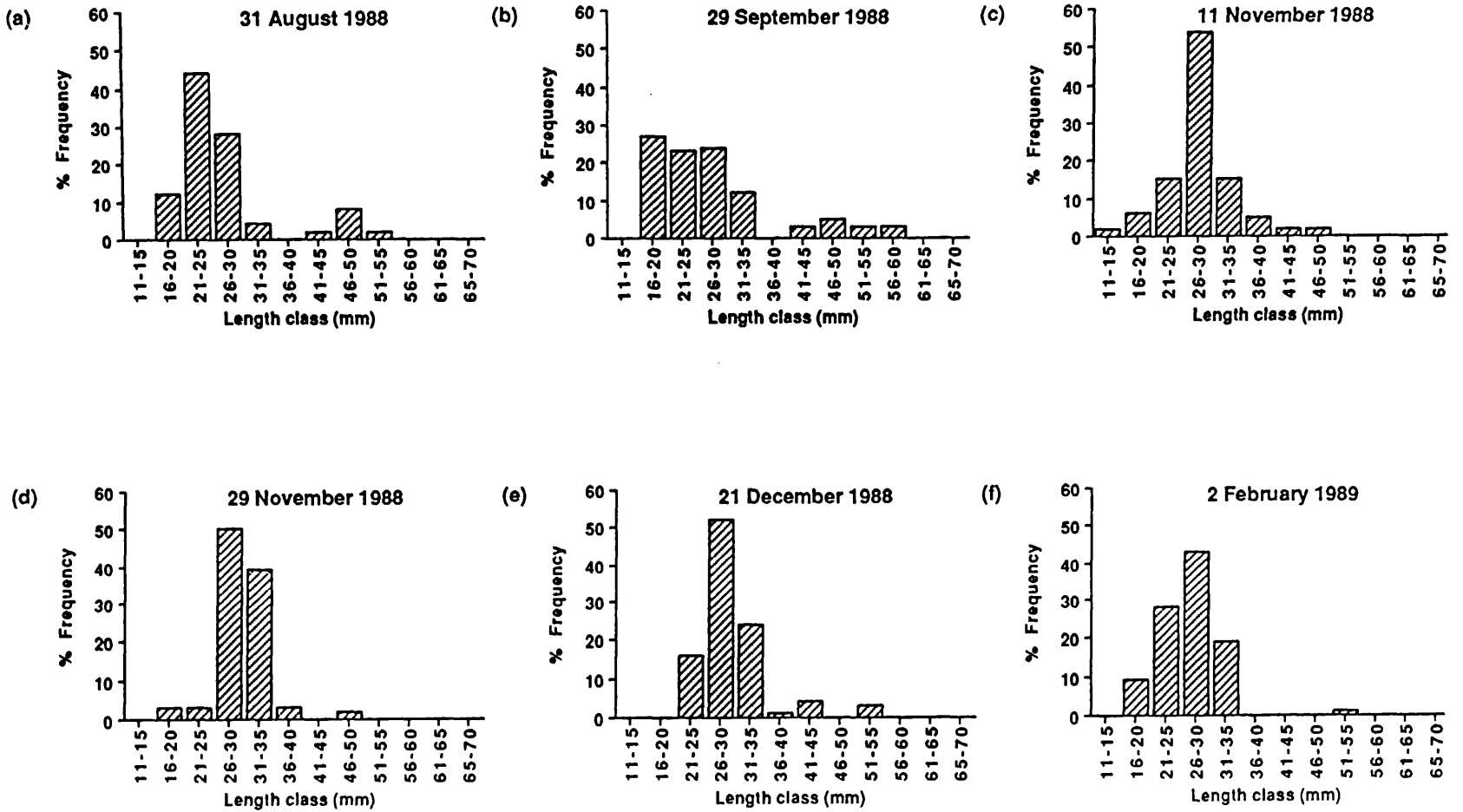
Environmental temperature

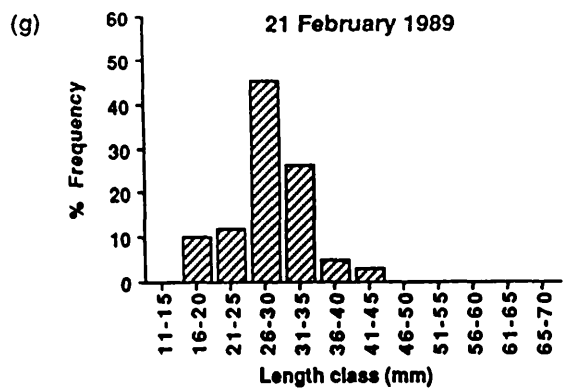
Monthly fluctuations in mean air temperature from August 1988 until September 1989 are given in Figure 2.2. There was a rapid drop in monthly temperature from 15^{O}C in August to 6^{O}C in November and it rarely exceeded 7^{O}C for the rest of the winter. Only in April did the air temperature begin to rise and did so dramatically from 6^{O}C to a high of 17^{O}C in July. From then on the mean monthly air temperature decreased.

Growth

Growth of uninfected, young-of-the-year was followed from August 1988 until September 1989 by examining the rate of change in mean weight and length per day. After an initial period of weight loss, there were high autumnal rates of growth in terms of weight (Figure 2.3). During winter the negative values that were found for the specific growth rate,

Figure 2.1: Length frequency distributions of complete monthly samples of sticklebacks.


(a)
31 August 1988
(b)
29 September 1988
(c)
11 November 1988
(d)
29 November 1988
(e)
21 December 1988
(f)
2 February 1989
% Frequency
Length class (mm)
0
10
20
30
40
50
60
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
65-70


(g)
21 February 1989
% Frequency
0
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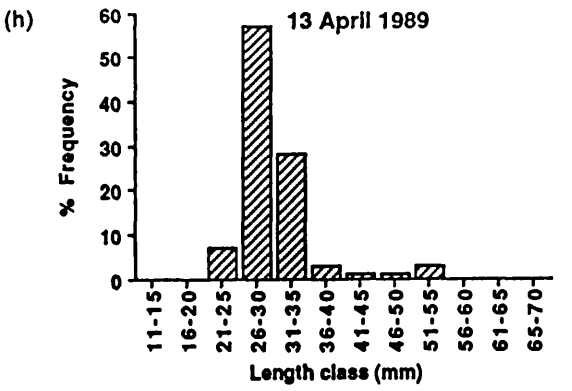
20
30
40
50
60
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
65-70
Length class (mm)


(h)
13 April 1989
% Frequency
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20
30
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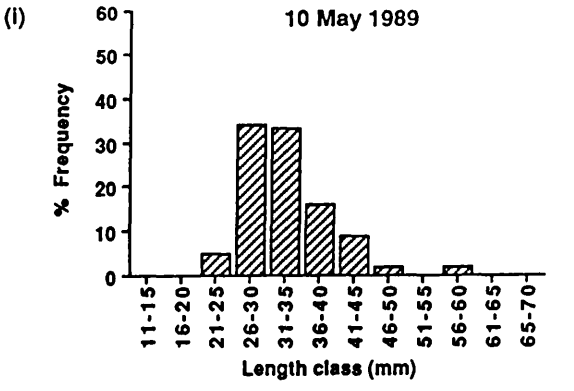
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
65-70
Length class (mm)


(i)
10 May 1989
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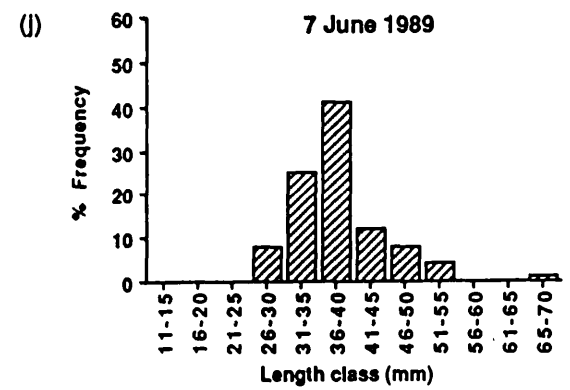
36-40
41-45
46-50
51-55
56-60
61-65
65-70
Length class (mm)


(j)
7 June 1989
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11-15
16-20
21-25
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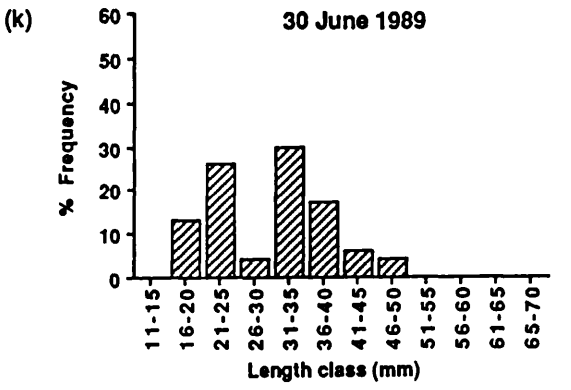
41-45
46-50
51-55
56-60
61-65
65-70
Length class (mm)


(k)
30 June 1989
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11-15
16-20
21-25
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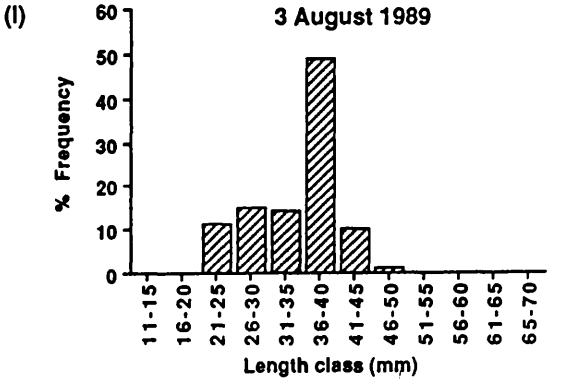
36-40
41-45
46-50
51-55
56-60
61-65
65-70
Length class (mm)


(l)
3 August 1989
% Frequency
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40
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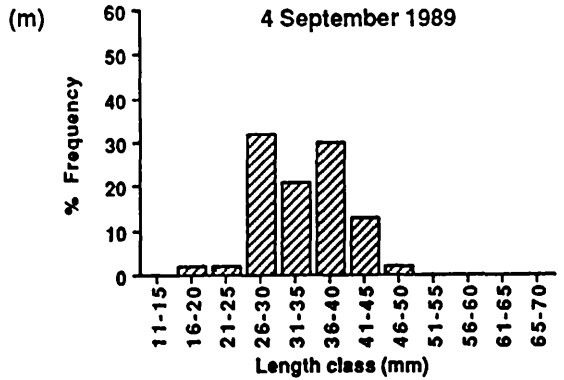
60
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
65-70
Length class (mm)


(m)
4 September 1989
% Frequency
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40
30
20
10
0
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
65-70
Length class (mm)

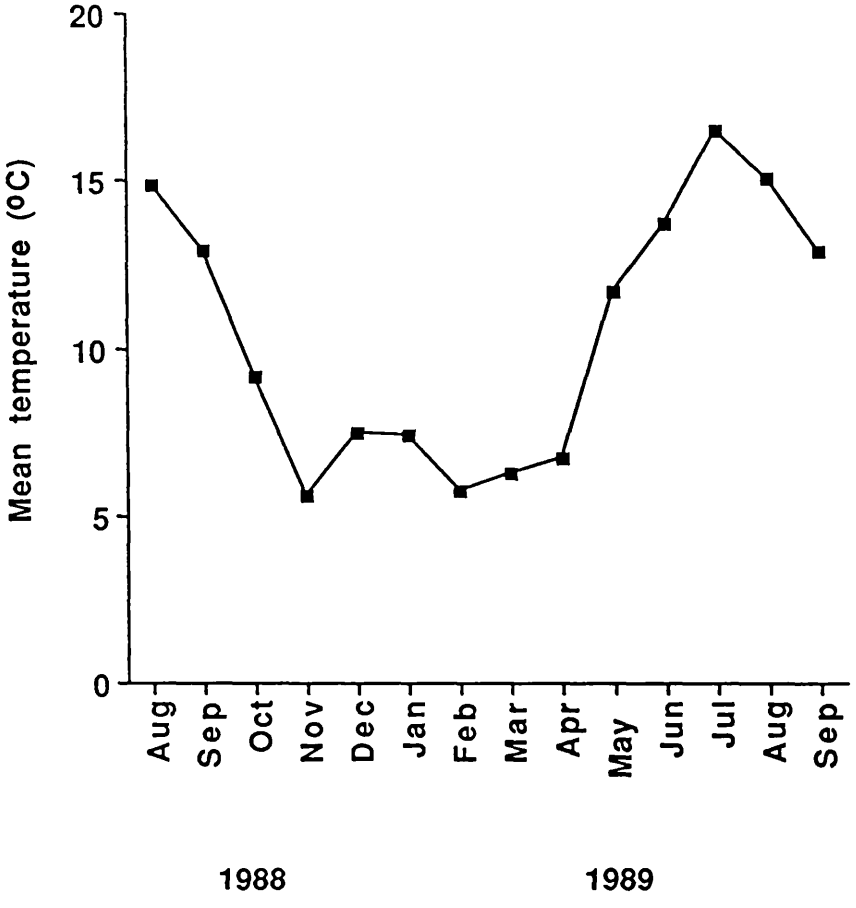


Figure 2.2: Monthly changes in the mean air temperature at the Royal Botanic Gardens, Edinburgh.

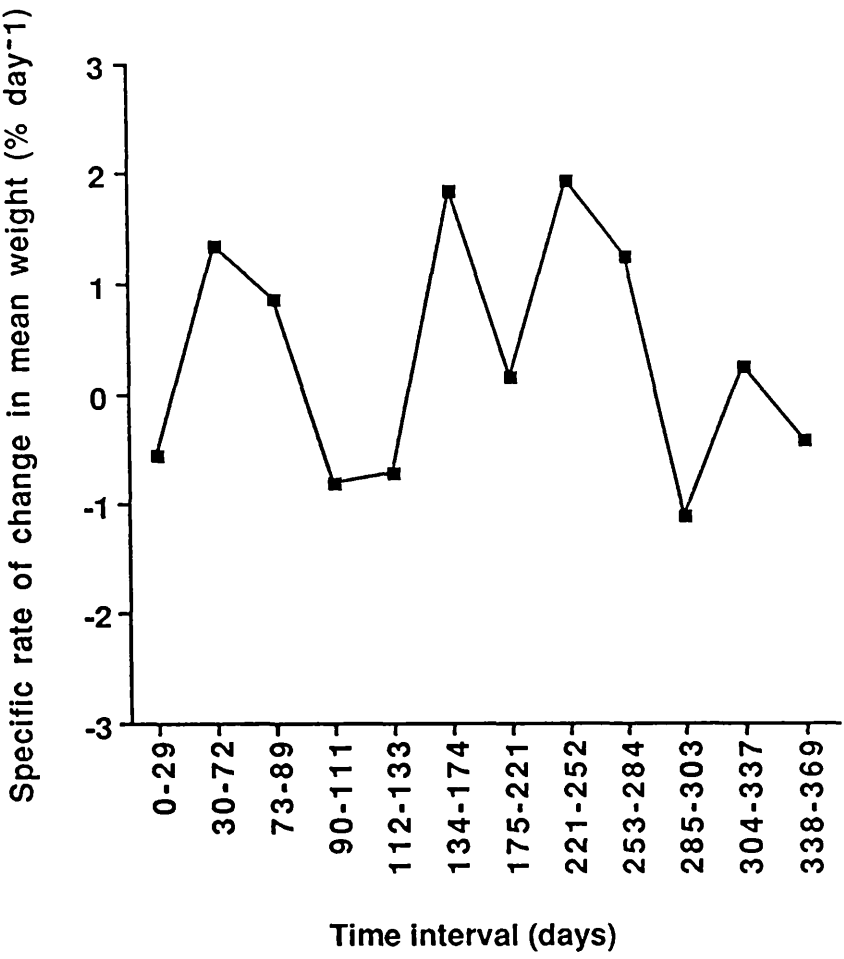


Figure 2.3: Rate of change in the mean weight of monthly samples of uninfected, 0+ sticklebacks.

reflect of a large drop in the mean weight of samples, suggesting very poor growth. It resumes in late winter, but slows somewhat before peaking in late spring and summer 1989. For the remainder of the survey the rate of growth declined and again negative values were sometimes obtained.

A similar picture of growth is presented by the rate of change in length (Figure 2.4). There was a drop in overall length between the first two sampling points which can only be explained by a loss of the largest fish from the population, because skeletal size is not expected to shrink. There followed a period of increased growth in length, but during early winter fish losses recurred (negative values). A higher rate growth in length occurred throughout spring, but again there appears to be fish losses in summer and autumn and also poor growth.

A comparison of changes in the mean weight and length of samples reveals other subtle clues about the growth of sticklebacks from Inverleith pond. In the time between third and fourth sample growth by length was faster than that by weight and this is repeated later in the survey between samples 9 and 10. Differences in the rates of length and weight increases may have consequences for the condition of the fish and this will be discussed further in Chapter 4.

Breeding cycle

The frequency of sticklebacks at different stages of maturity varied with season (Chi-Square, $X^2=308.356$, d.f.=20, $P<0.001$). More than 90% of the summer 1988 sample of uninfected, young of the year sticklebacks were classed as undifferentiated with respect to sex (Figure 2.5). These new fry began to grow (see above) and develop such that, by autumn 1988 65% of fish could be sexually differentiated and some even had mature gonads. A reduction in the relative proportion of sexually developed sticklebacks took place in early winter but rose again in late winter.

Mature fish of both sexes, that were capable of breeding, were not observed until May, which denotes the beginning of the breeding season. Recruitment of mature sticklebacks took place throughout most of the summer and breeding probably continued until mature males and females were depleted. A large contingent of sticklebacks had not attained full sexual maturity by the end of the survey and some definitely would not have had any opportunity to breed.

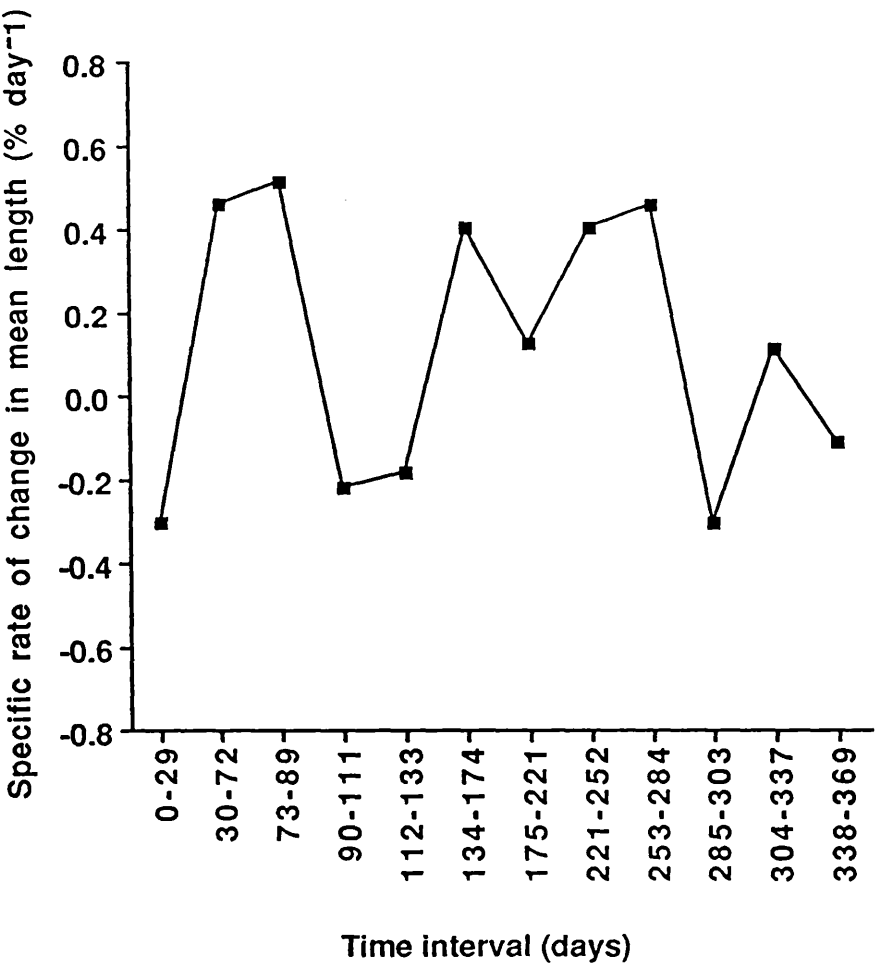
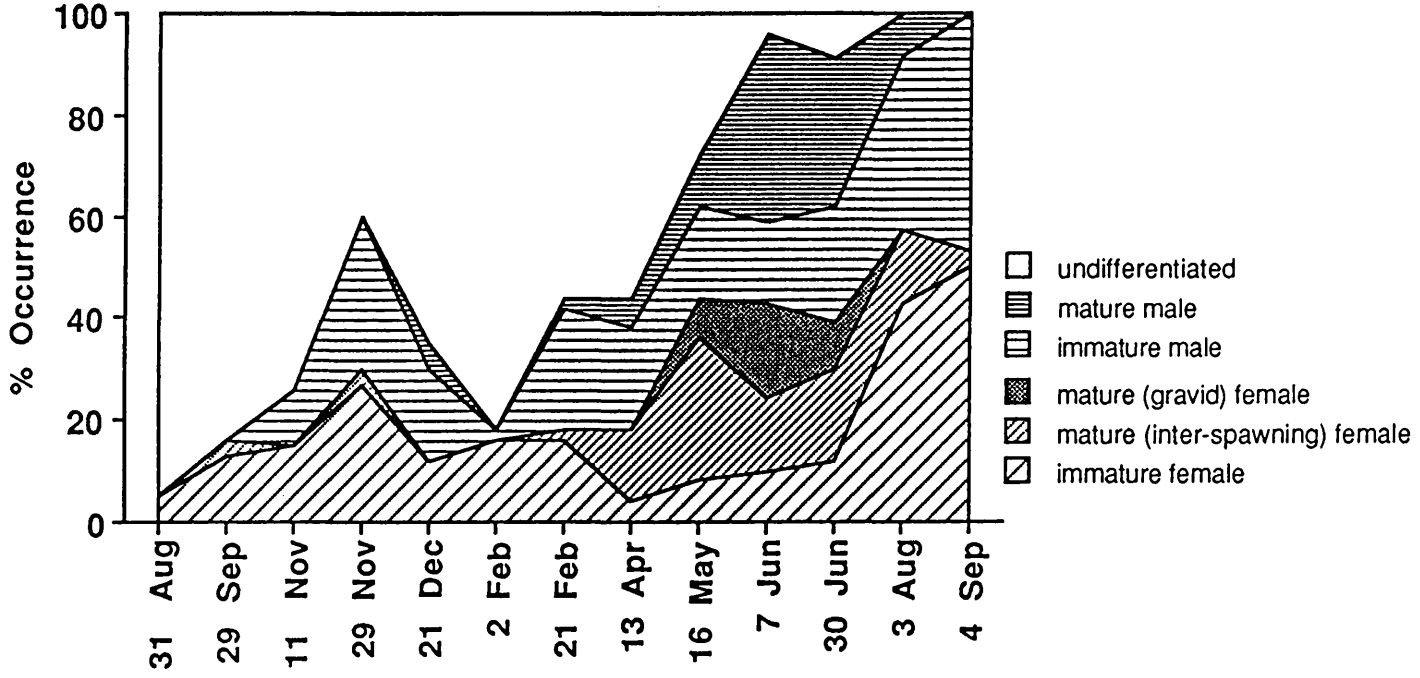


Figure 2.4: Rate of change in the mean length of monthly samples of uninfected, 0+ sticklebacks.

Figure 2.5: Occurrence of uninfected, 0+ sticklebacks at different stages of maturity, in monthly samples from 31 August 1988 to 4 September 1989.


% Occurrence
100
80
60
40
20
0
31 Aug
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
13 Apr
16 May
7 Jun
30 Jun
3 Aug
4 Sep
undifferentiated
mature male
immature male
mature (gravid) female
mature (inter-spawning) female
immature female

2.3.2 LATERAL PLATE MORPHOLOGY OF UNINFECTED STICKLEBACKS

The major aspect of morphology studied was the plate morphology and was followed in the uninfected 0+ cohort of sticklebacks.

Plate morphs

Although no quantitative record of the occurrence of plate morphs was made and the maximum number of lateral plates counted was 16 per side, the three plate morphs were represented in Inverleith sticklebacks. By far the highest proportion had a leirus plate distribution. Semi-armatus forms occurred rarely and trachurus forms were only observed on a few occasions.

Lateral plate distributions

There was generally good agreement between the number of lateral plates on each side of the body. Asymmetrical plate counts were found in only 2% of fish. As asymmetry was very infrequent, the distribution of plate numbers for each sample was based on left side counts only. The distribution of these counts per sample is given in Figures 2.6(a-m). It appears that there were at least two modes to the lateral plate distribution, the size of which varied according to the time of year.

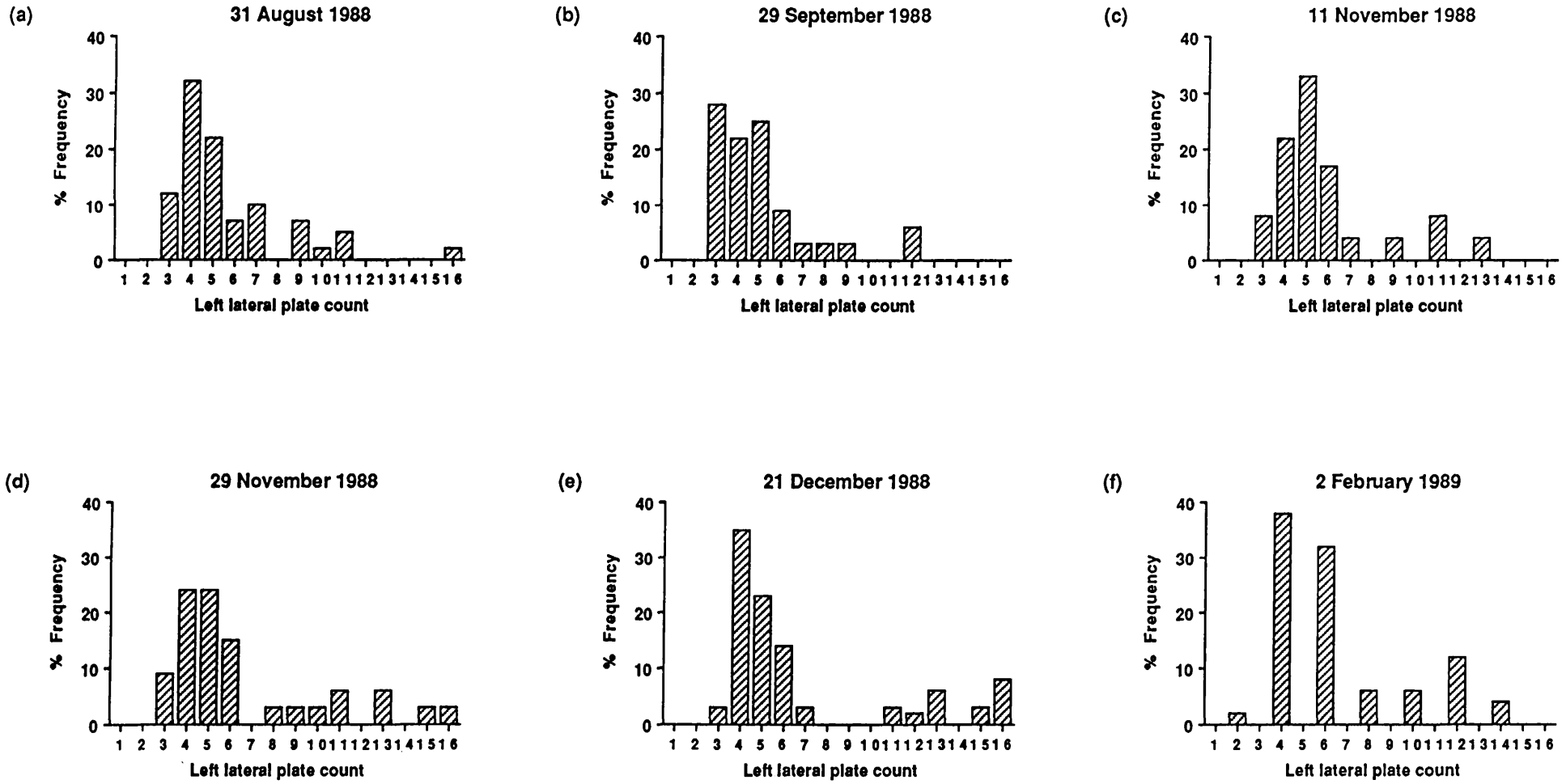
The largest and most consistent mode was around 4-5 lateral plates (range 2-7). Between 62 and 87% of samples of uninfected young-of-the-year sticklebacks had lateral plate counts in this range. As there were so few with plate counts above 7 it was difficult to distinguish clearly, other patterns. In some months, plate counts of 9, 10 and 11 were common (Figure 2.6(a) and (d)) and in others, counts of 13, 14 and 15 were the most common (Figure 2.6(j) and (l)). The transient nature of these modes may simply reflect an unrepresentative sample size and it is therefore, probably unwise to draw any conclusions about the distributions of plate counts exceeding 7.

2.3.3 DIET OF UNINFECTED STICKLEBACKS

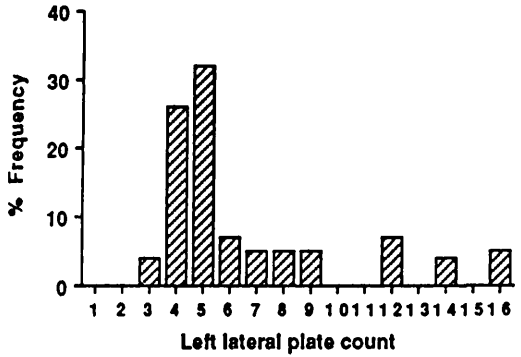
Stomach fullness

Seasonal changes were found in the numbers of uninfected, young of the year sticklebacks with various categories of stomach fullness (Chi-Square, $X^2=61.978$, d.f.$=16$, $P<0.001$). Few fish (0-7%) with empty stomachs were found during the early part of the survey

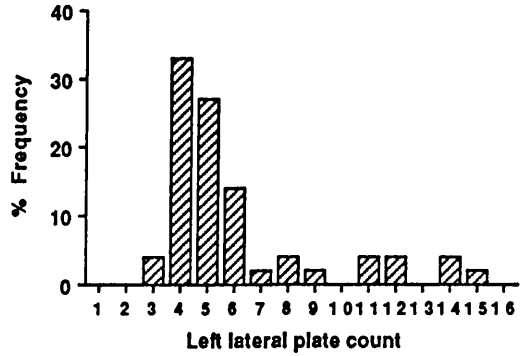
Figure 2.6: Left lateral plate frequency distributions in monthly samples of uninfected, 0+ sticklebacks.


(a)
31 August 1988
(b)
29 September 1988
(c)
11 November 1988
(d)
29 November 1988
(e)
21 December 1988
(f)
2 February 1989
% Frequency
Left lateral plate count
0
10
20
30
40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

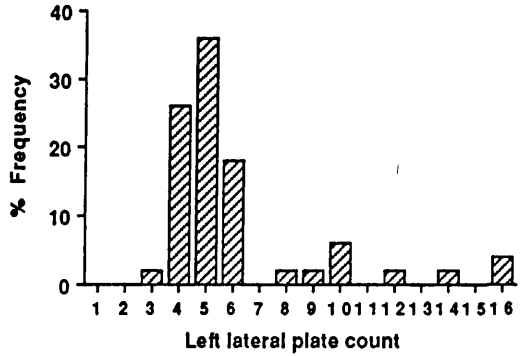
(g) 21 February 1989



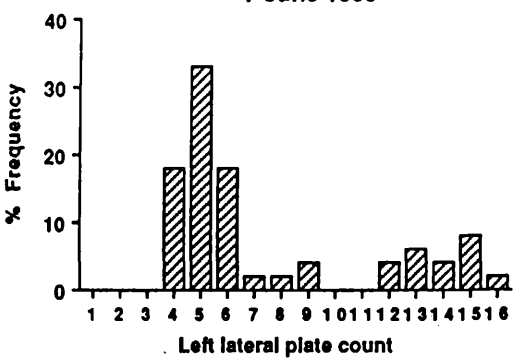
(h) 13 April 1989



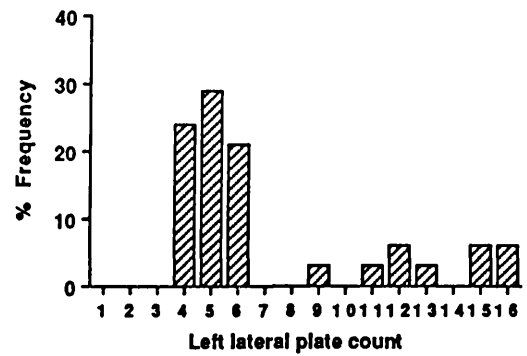
(i) 16 May 1989



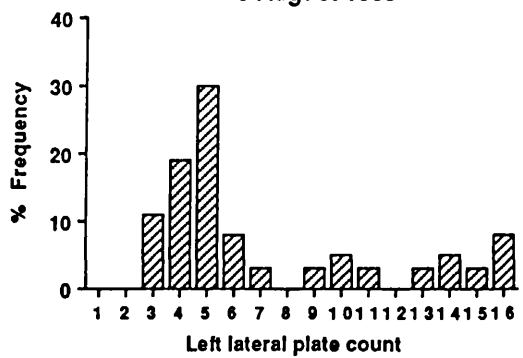
(j) 7 June 1989



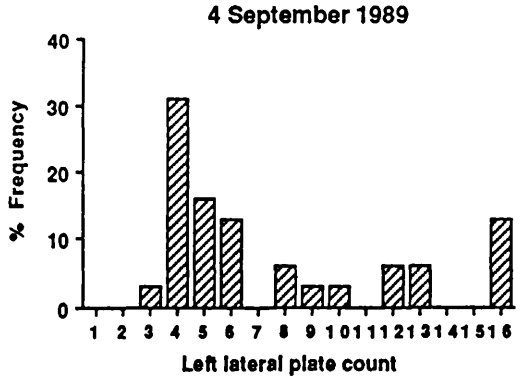
(k) 30 June 1989



(l) 3 August 1989



(m)


4 September 1989
40
30
20
10
0
% Frequency
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Left lateral plate count

(Figure 2.7), but in autumn 1989 the figure was much higher (19%). Sticklebacks with 1/4 full stomachs were also fairly uncommon (7-18%). Most stomachs assigned to this category were from fish caught in their first autumn and winter of life (15-18%), but later in the survey their frequency diminished (7-9%).

The quantity of fish having 1/2 full stomachs increased steadily in autumn and winter (27-45%) and did not decrease dramatically (to 9%) until the autumn of 1989. By contrast, the frequency of fish with 3/4 full stomachs was static during the autumn and winter of 1988 (33%), but then rose throughout the following spring and summer to a maximum in autumn 1989 (69%). Fish with full and distended stomachs were quite rare (0-17%), but peak numbers were found in the autumn 1988 and spring and summer 1989 samples.

Occurrence of dietary items in stickleback stomachs

The percentage of stickleback stomachs containing each of the major food types described is given in Figures 2.8(a-k). Cyclopoid copepods, cladocerans and rotifers were the few planktonic prey consumed by Inverleith sticklebacks and each had its own seasonal pattern of occurrence in stomachs. There was significant variation with season in the number of stickleback stomachs containing cyclopoid copepods (Chi-Square, X^2=46.058, d.f=4, P<0.001). During autumn and winter 1988 the percentage of fish consuming cyclopoids was rather low (11-34%) compared with the spring and summer 1989 when the figure had often doubled (Figure 2.8(a)).

Cladocerans were preyed upon consistently throughout the year and therefore by all size ranges of fish (Chi-Square, X^2=6.738, d.f.=4, P>0.05, N.S). They always occurred in more than 40% of stomachs per sample (Figure 2.8(b)). This was not the case for rotifers which seemed to be a common component of the diet of young fish only. Up to 93% of fish had eaten rotifers in autumn, but by the following summer almost no fish had consumed them (Figure 2.8(c)).

Larvae and pupae of the family chironomidae, ostracods, free-living nematodes and arcellidae (testate amoebae) were the main benthic prey. The presence of chironomids in stomachs altered with season (Chi-Square, X^2=163.007, d.f.=4, P<0.001), tending to increase as the survey progressed (Figure 6.8(d)). By the summer of 1989, almost all fish sampled had

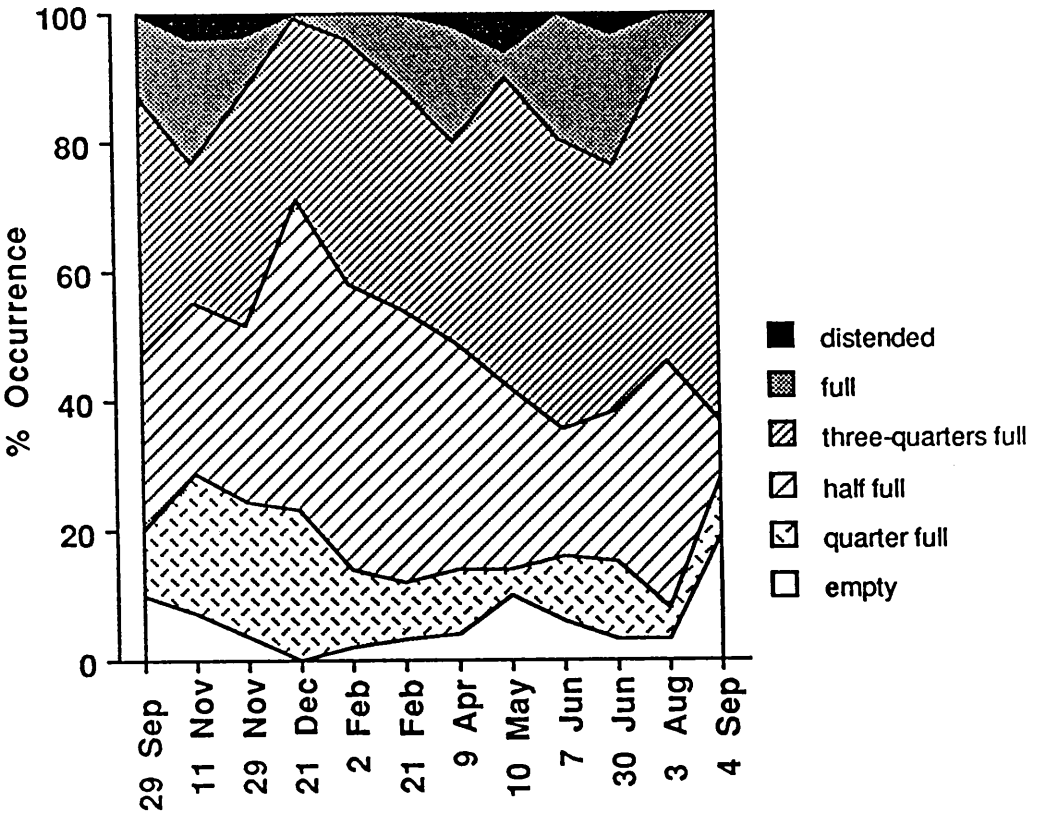
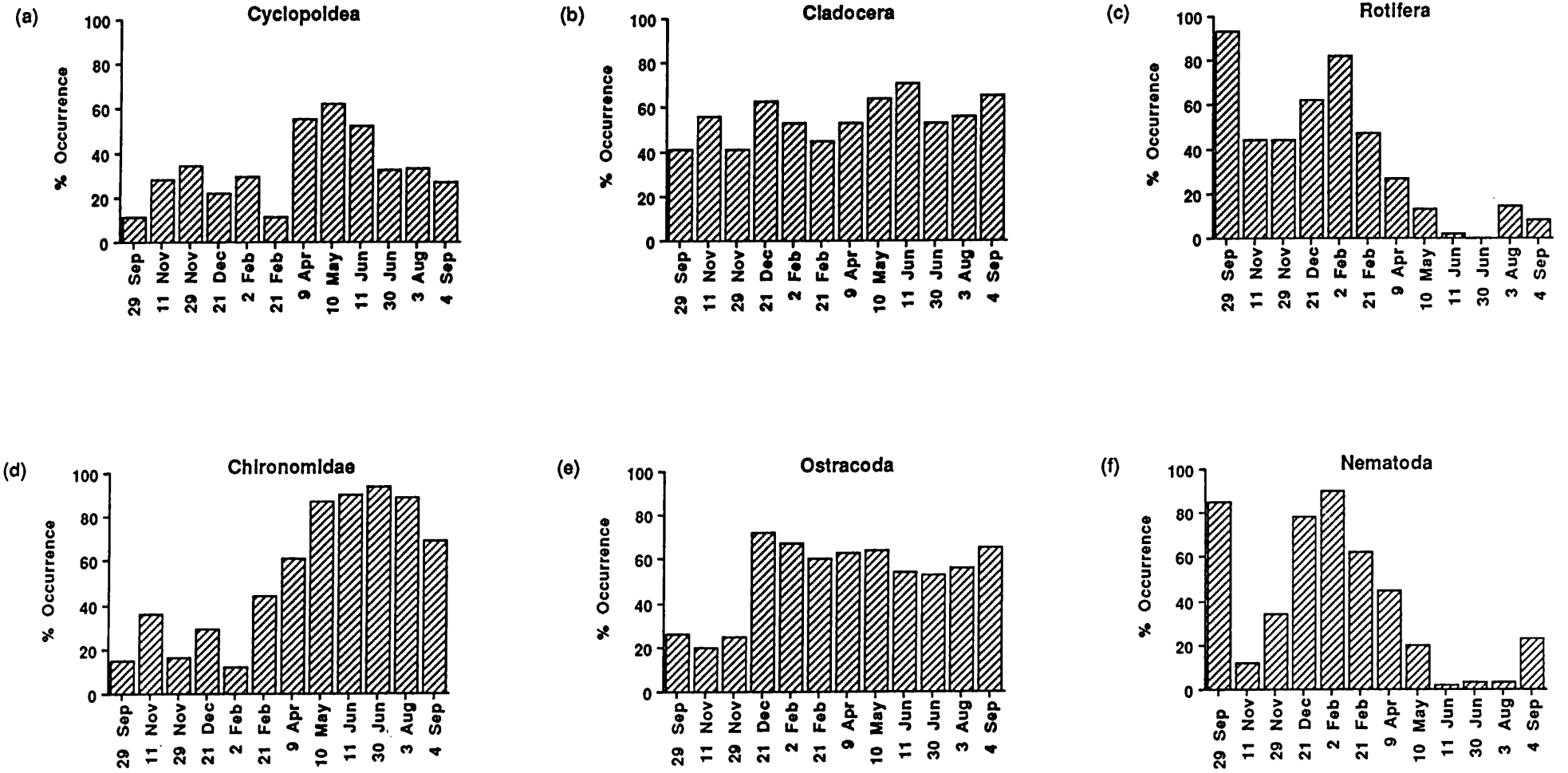
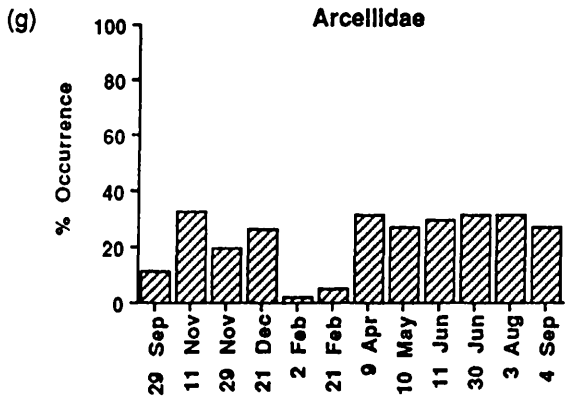
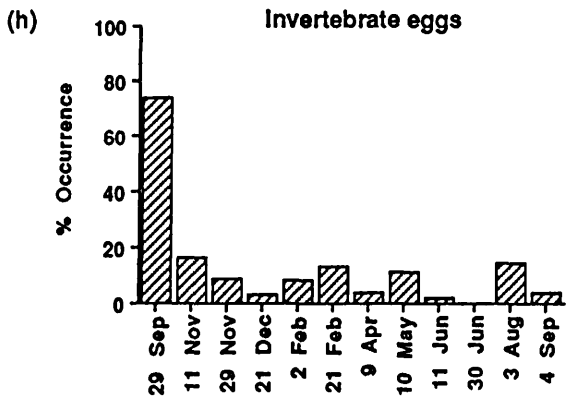


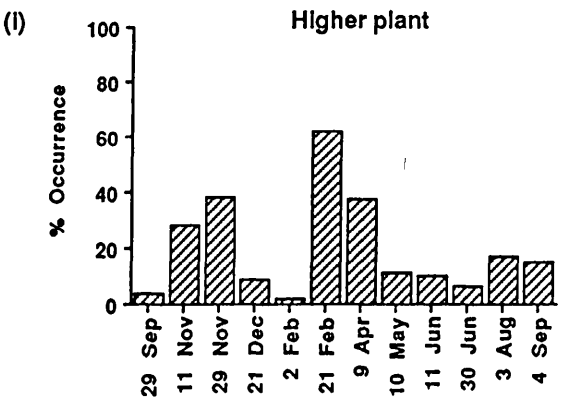
Figure 2.7: Occurrence of uninfected, 0+ sticklebacks with each category of stomach fullness, in monthly samples from 31 August 1988 to 4 September 1989.

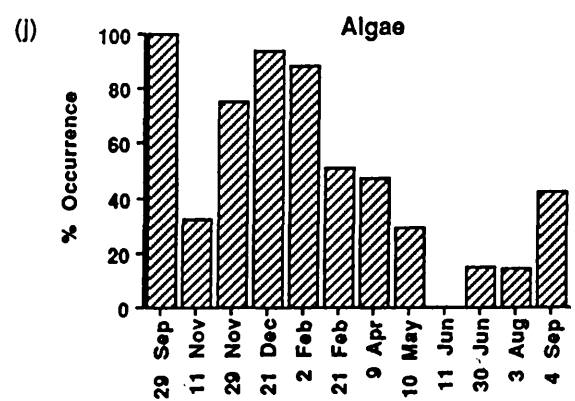
Figure 2.8: Occurrence of each of the major food items in the stomachs of uninfected, 0+ sticklebacks, in monthly samples.

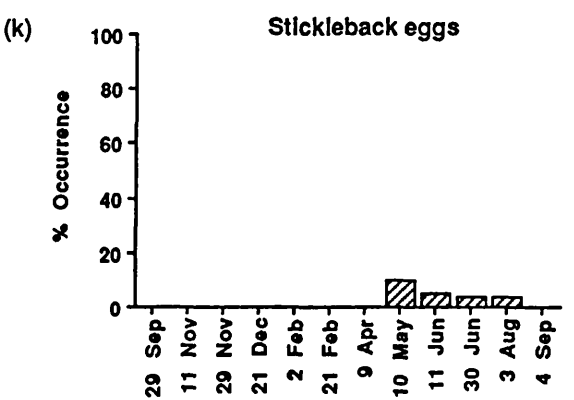

(a)
Cyclopoidea
(b)
Cladocera
(c)
Rotifera
(d)
Chironomidae
(e)
Ostracoda
(f)
Nematoda
% Occurrence
0
20
40
60
80
100
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(g)
Arcellidae
% Occurrence
100
80
60
40
20
0
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(h)
Invertebrate eggs
% Occurrence
100
80
60
40
20
0
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(I)
Higher plant
% Occurrence
100
80
60
40
20
0
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(J)
Algae
% Occurrence
100
80
60
40
20
0
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(k)
Stickleback eggs
% Occurrence
100
80
60
40
20
0
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep

consumed chironomids (89-94%), suggesting that they are a favoured food of adult sticklebacks.

The only seasonality in the occurrence of ostracods in stomachs was between autumn 1988 and the rest of survey (Chi-Square, X^2=49.948, d.f.=4, P<0.001). During autumn, ostracods were found in 20-25% of stickleback stomachs, but thereafter they were continually observed in excess of 50% of stomachs (Figure 2.8(e)). Nematodes however, were preyed upon with different seasonal pattern (Chi-Square, X^2=163.527, d.f.=4, P<0.001)). Following an 85% occurrence in stomachs in September 1988, there was a drop to 12% (Figure 2.8(f). Winter was a time of peak utilisation of nematodes, but by spring and summer 1989 few fish were found to have eaten them.

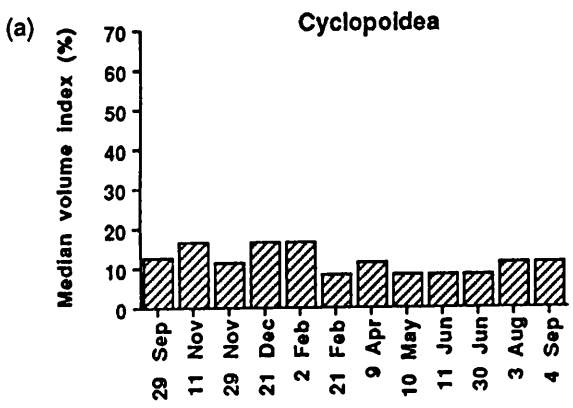
Arcellidae were taken by a small number of fish (2-31%) throughout the year but some seasonal variation did exist (Chi-Square, X^2=16.373, d.f.=4, P<0.01) evident as a lack of arcellidae in stomachs during winter (Figure 2.8(g)). Again invertebrate eggs were consumed by few of the sampled fish, but they were taken on a seasonal basis (Chi-Square, X^2=46.859, d.f.=4, P<0.001). In the sample from September 1988 it was common to find them in stickleback stomachs (74%), but rare (0-16%) for the rest of the survey (Figure 2.8(h)).

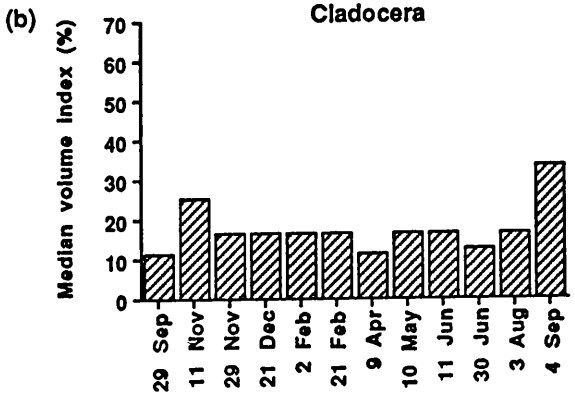
In addition to prey, plant material was also ingested (Figure 2.8(i) and (j)). Season had some effect on the frequency of stomachs containing higher plant material (Chi-Square, X^2=9.642, d.f.=4, P<0.05), but a greater effect on the numbers containing algae (Chi-Square, X^2=153.297, d.f.=4, P<0.001). There were two peaks in the percentage of stomachs containing higher plants in late November (38%) and late February (62%), but overall algae were encountered in many more stickleback stomachs. They were observed in all fish stomachs in the September 1988 sample and then in 32-94% of stomachs for the remainder of autumn, winter and spring. Algae were less important for the rest of the survey. Stickleback eggs were only taken by a very small proportion of the sampled fish during the breeding season (Figure 2.8(k))

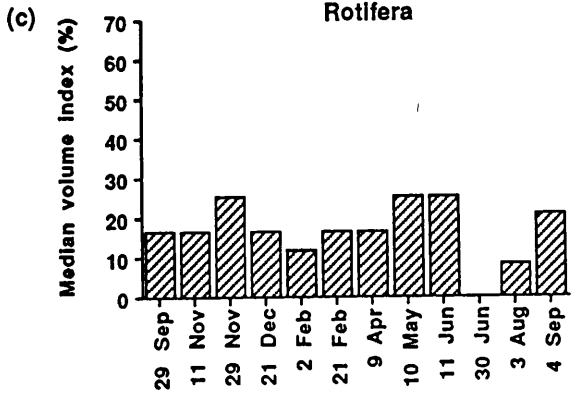
The contribution of dietary items to food volume

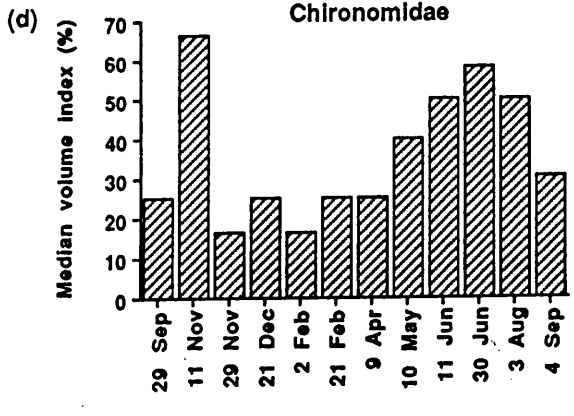
The points allocated to each of the major food items as a percentage of the total bulk food (the volume index), is shown in Figure 2.9(a-k). Cyclopoid copepods constituted a small proportion of the total volume of food in stomachs the whole year round. Their median contribution was never more than 16% per sample (Figure 2.9(a)), but in the spring and

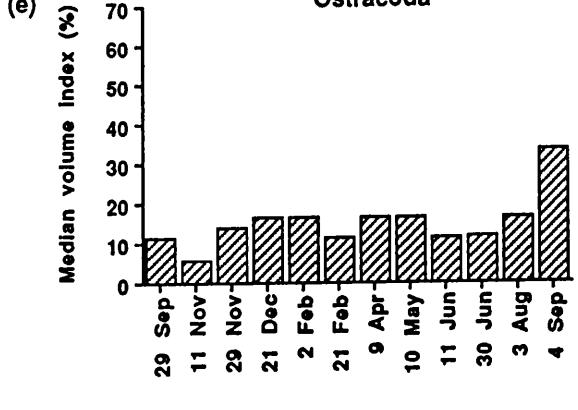
Figure 2.9: Median volume index of food items in the stomachs of uninfected, 0+ sticklebacks, in monthly samples.

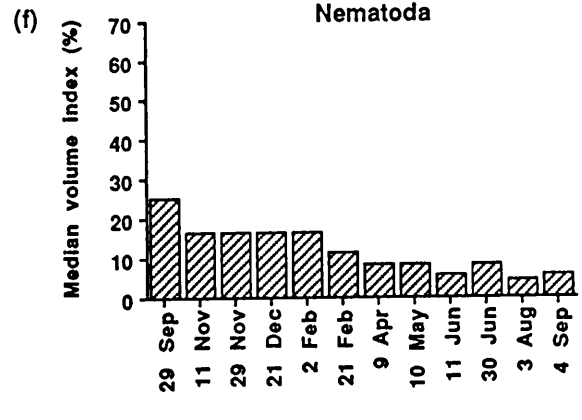

(a)
Cyclopoidea
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep

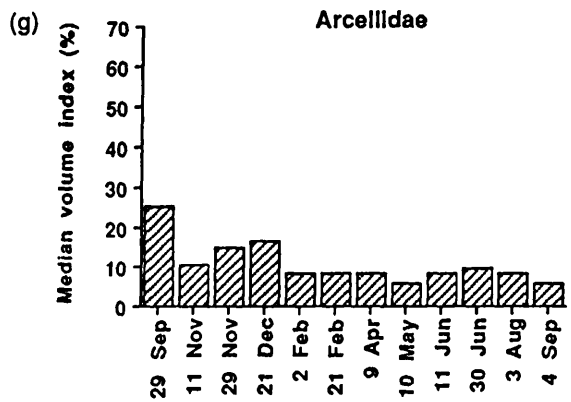

(b)
Cladocera
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep

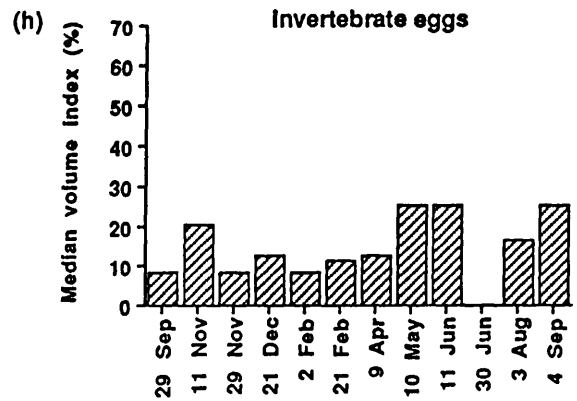

(c)
Rotifera
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep

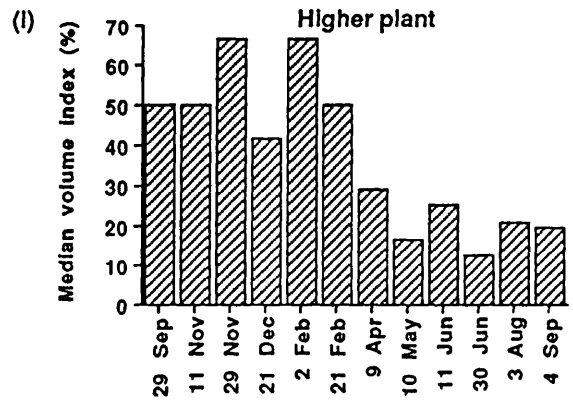

(d)
Chironomidae
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep

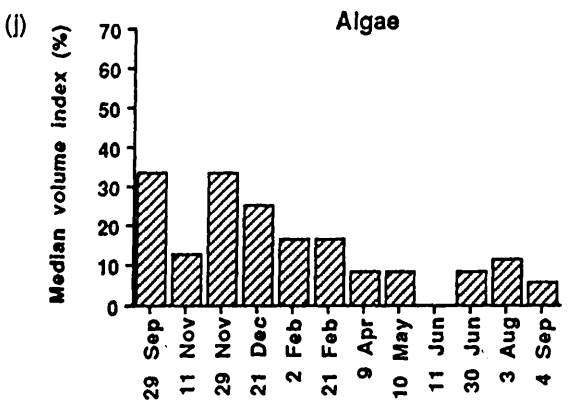

(e)
Ostracoda
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep

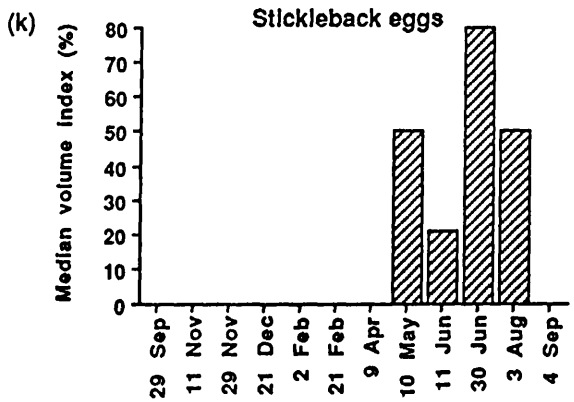

(f)
Nematoda
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(g)
Arcellidae
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(h)
Invertebrate eggs
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(i)
Higher plant
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(j)
Algae
Median volume index (%)
0
10
20
30
40
50
60
70
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep


(k)
Stickleback eggs
Median volume index (%)
0
10
20
30
40
50
60
70
80
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
9 Apr
10 May
11 Jun
30 Jun
3 Aug
4 Sep

summer samples it was somewhat less (8-11%) (Kruskall Wallis ANOVA, $H=12.92$, d.f.$=4$, $P<0.05$). Similarly, cladocerans varied seasonally with respect to the volume of food that they accounted for (Kruskal Wallis ANOVA, $H=12.67$, d.f.$=4$, $P<0.05$), this being greater in autumn 1989 than at any other time of the year (Figure 2.9(b). They also constituted a greater proportion of the stomachs they occupied (medians of 11-33%). No significant seasonal differences were found (Kruskall Wallis ANOVA, $H=6.24$, d.f.$=4$, $P>0.05$, N.S.), in the volume of stomachs that were taken up by rotifers (Figure 6.9(c). Overall, the bulk of these planktonic prey rarely exceeded 33%.

Chironomids formed a steadily increasing percentage of the food volume of Inverleith sticklebacks (Figure 2.9(d)). Despite being an important bulk prey item throughout the year (medians of 17-67%), they formed the largest part during the summer of 1989 (Kruskal Wallis ANOVA, $H=25.04$, d.f.$=4$, $P<0.001$). Ostracods accounted for a smaller and more consistent proportion of the food in stomachs (Figure 2.9(e)), but did show seasonal change, being particularly common in autumn 1989 compared with rest of the year (Kruskal Wallis ANOVA, $H=18.00$, d.f.$=4$, $P<0.01$).

The main intake of nematodes was early in the life of the sticklebacks (Figure 2.9(f)) when as much as 25% of the food volume was composed by them. A marked seasonal reduction was evident thereafter (Kruskal Wallis ANOVA, $H=44.23$, d.f.$=4$, $P<0.001$). An almost identical trend was observed for arcellidae (Figure 2.9(g)), with them constituting a lesser part of food in stickleback stomachs as the seasons passed (Kruskal Wallis ANOVA, $H=14.20$, d.f.$=4$, $P<0.01$). Invertebrate eggs, by contrast, were consumed in small volumes (Figure 2.9(h)), with no statistically apparent seasonal trend (Kruskal Wallis ANOVA, $H=5.27$, d.f.$=4$, $P>0.05$, N.S.).

When higher plant matter was detected in stomachs its volume tended to be quite large (Figure 2.9(i)), but this was more apparent in autumn and winter of 1988 (medians of 40-67%), with a decline in 1989 (Kruskal Wallis ANOVA, $H=28.40$, d.f.$=4$, $P<0.001$). Similarly the volume of stomach occupied by algae was fairly high (Figure 2.9(j)), but more so in autumn and winter compared with the remainder of the year (Kruskal Wallis ANOVA, $H=53.86$, d.f.$=4$, $P<0.001$). Cannibalism of stickleback eggs was a major source of dietary bulk (Figure 2.9(k)),

but obviously only when eggs were available. Within the breeding period the bulk contribution of stickleback eggs did not differ significantly across the seasons (Kruskal Wallis ANOVA, $H=0.09$, d.f.$=1$, $P>0.05$, N.S.). Other dietary items were found, but even when pooled they constituted a minimal part of the total volume of food in stomachs.

Overview of the diet of Inverleith sticklebacks

The occurrence method and the points method enabled the diet of the Inverleith pond sticklebacks to be viewed from two perspectives. A combination of the two illustrates which dietary items are important to which fish and at what times of the year. In summary, zooplankton is a relatively small feature of the diet except perhaps in young fish. Of the three types of zooplankton, cladocerans were the most important as they were consumed by many fish and in quite substantial volumes throughout the year. The sticklebacks of Inverleith pond are largely benthic feeders, relying very heavily on chironomids and to a lesser extent on ostracods and nematodes. This is particularly apparent in spring and summer. During winter, there is a greater dependence on plant matter and during the breeding season at least, for a few fish cannibalism can be a major source of dietary bulk.

2.3.4 PREDATORS AND ANTI-PREDATOR DEFENCE OF UNINFECTED STICKLEBACKS

No quantitative analysis of predation was made during the survey period, but the presence of any potential predators was noted. As no other fish species inhabit the pond the probable risks of predation are from invertebrates, birds and conspecifics.

During sampling, it was usual to pick up aquatic invertebrates but no potential invertebrate predators were ever detected. However, a colony of black-headed gulls (*Larus ridibundus*) did frequent the pond and the adjacent playing fields. Black-headed gulls feed mainly on insects and earthworms which they could obtain from the playing fields and pond area, but they are also known to feed on fish from shallow water, mainly during winter. Indeed sticklebacks have been recorded in their diet (See Cramp (Ed.) 1983 for a description of *L.ridibundus*). Based on this evidence it is expected that avian predation is fairly low at Inverleith, except perhaps during winter, and this would explain the poor anti-predator behaviour shown by this population of sticklebacks (Huntingford 1982; Wright & Huntingford

pers. comm.).

2.4 DISCUSSION

The purpose of the work described in this chapter was to establish the characteristics of growth, development and diet, of a population of sticklebacks. By examining fish that were without *S.solidus* infection in a single age class, the study was not confounded by the effects of age of the host and the presence of this helminth.

Sticklebacks from Inverleith pond have a growth pattern which seems to be partially related to environmental conditions. Relatively high autumn temperatures and possibly greater food availability coincided with a spurt of growth in recently hatched sticklebacks. During early winter, the negative values for the specific growth rate can only be attributed to a loss of many of the largest 0+ fish. This is further supported by the corresponding reduction in the occurrence of fish in the 31-35 and 36-40mm length classes (Figure 2.1(d-f)). In addition there was a winter decline in the numbers of sexually differentiated fish, which are likely to have been the largest fish in the population. There were no stickleback carcasses to indicate death by disease and/or poor body condition, yet there is just such evidence of large scale death following the breeding season. Despite the limited use of sticklebacks in their diet, circumstantial evidence points to predation by black-headed gulls as a cause of fish loss because 1) these gulls are more commonly piscivorous during winter and indeed, they were often seen on the pond at this time of year and 2) the most frequently occurring plate count of 4-5 has previously been shown to be linked to avian predation (Reimchen 1980).

Later in winter, poor growth was observed and low winter temperatures may have been responsible. Elliot (1975a,b) working on brown trout (*Salmo trutta* L.) found that at low temperatures food consumption was reduced and this was probably the result of slow evacuation rates of food from the trout stomachs (Elliot 1972). Evacuation rates of the stomach of the three-spined stickleback are also affected by temperature (Beukema 1968) and so it is likely that consumption was similarly affected. Additionally, less hours of daylight may have restricted the time available for foraging. No more fish had empty stomachs at this time of the year as any other, but diel sampling of fish may have revealed whether the number of meals per day had declined.

Perhaps another important consideration in explaining the winter pattern of growth, is that the sticklebacks' diet depended more heavily on plant matter during winter. Studies on other omnivorous fish have shown that a plant based diet results in a poorer growth rate and efficiency (Payne 1979; Hofer, Krewedl & Koch 1985). Thus, the quality of food in the diet of the sticklebacks from Inverleith (in terms of its metabolisable energy) may also have contributed to the slow growth rates observed.

A new wave of increased growth occurred in spring and summer and development to sexual maturity was maximal at this time. Many factors could have been involved: temperature and daylight hours were rising and a higher proportion of stomachs were 3/4 full, full or distended, suggesting more voracious foraging activity and since the diet was almost exclusively carnivorous, more energy may have been available for growth.

Again, in the later months of the survey there were indications of declining fish growth and loss of large fish. Initially, the loss would represent the death of large post-breeding adults but eventually death of non-breeders would also have been a factor. Almost certainly the breeding adults died because of poor body condition and associated disease, as they were frequently observed dead or dying at the pond margins, in late summer. Non-breeders however, may have succumbed to predation by gulls in their second winter of life, as was proposed in the case of young fry.

2.5 CONCLUSIONS

Sticklebacks in Inverleith pond survive essentially as an annual population, whose growth is restricted to their first autumn, spring and summer of life. In addition to the effects of temperature and daylight on food intake, seasonal changes in diet may also have an impact on their growth. They have a single opportunity to breed in their first summer of life, after which they die. The morphology of the population and the ecology of the bird life suggests that winter predation may affect both the young of the year and adults that have failed to breed.

CHAPTER 3: THE EPIDEMIOLOGY AND GROWTH OF *SCHISTOCEPHALUS SOLIDUS* IN THE STICKLEBACK POPULATION OF INVERLEITH POND, EDINBURGH

3.1 INTRODUCTION

3.1.1 PREVALENCE OF *SCHISTOCEPHALUS SOLIDUS* INFECTION IN *GASTEROSTEUS ACULEATUS*

Seed (1984) found that sticklebacks collected in June from a reservoir in North Wales had a high prevalence of *S.solidus* infection, whereas a much lower prevalence was observed in those sampled during October/November. Therefore, even with only two sampling points, there was some disparity in the prevalence of infection with season. Chappell (1969a,b) and Arme and Owen (1967) undertook studies that extended for periods greater than a year. Both found some seasonal changes in prevalence, notably that there were many infected sticklebacks during the summer and fewer in winter. However, Chappell (1969a) detected a low autumn prevalence whilst Arme & Owen (1967) found the prevalence to be very high at this time. Other authors (Hopkins & Smyth 1951; Clarke 1954; Pennycuick 1971a) did not observe much seasonal variation in the percentage of infected fish, but the latter two investigations did reveal differences between years.

Two studies related the size of hosts to the prevalence of *S.solidus* infection, both with the same conclusions (Chappell 1969b; Pennycuick 1971b); each observed an increase in prevalence with increasing host length. The same two studies looked for possible sex effects on prevalence, but neither could detect significant differences between males and females.

Only Pennycuick (1971b) compared the prevalence of *S.solidus* infection between age classes of sticklebacks. She found it to be lower in the 0+ class of fish than in any of the other age classes present in samples (1+, 2+ and 3+ years).

3.1.2 INTENSITY OF *SCHISTOCEPHALUS SOLIDUS* INFECTION IN *GASTEROSTEUS ACULEATUS*

In two stickleback populations, seasonal changes in the intensity of infection (in terms of worms numbers) were apparent. Rising mean intensities were observed in spring and summer by Arme & Owen (1967) and in summer and autumn by Pennycuick (1971a). Neither found an appreciable drop in intensity during winter. Chappell (1969a) could detect no seasonal variation in intensity.

Using the ratio of plerocercoid weight to fish weight as a measure of intensity (the

parasite index described earlier), a rather different seasonal pattern was observed. The mean parasite index in the populations studied by Clarke (formula 2, 1954) and Pennycuick (formula 1, 1971a) rose in the summer in a similar fashion as the mean intensity, yet Arme & Owen (formula 1, 1967) found a steady lowering of the parasite index throughout the year.

Pennycuick (1971b) noticed that intensity increased with length of stickleback, up to approximately 60mm, but decreased in fish above this size. A similar, but less pronounced increase in intensity with fish size was described by Chappell (1969b), yet in this case only up to a length of 50mm. Neither author looked for size-linked variability in the parasite index. The intensity of *S.solidus* infection and the parasite index could not be statistically distinguished between males and females (Chappell 1969b; Pennycuick 1971b), but Pennycuick (1971b) discovered that the intensity of infection was much higher in 1+, 2+ and 3+ fish, compared with 0+ fish. However, 1+ and 2+ fish had only marginally larger parasite indices than the young-of-the-year sticklebacks.

3.1.3 FREQUENCY DISTRIBUTION OF *SCHISTOCEPHALUS SOLIDUS* PLEROCERCOID NUMBERS IN *GASTEROSTEUS ACULEATUS*

Pennycuick (1971c) carried out a detailed study of the frequency distribution of plerocercoid numbers in sticklebacks. The distribution for each sample varied, but was always over-dispersed (indicated by the ratio of the variance to the mean). She suggested that the over-dispersion was due to infection being acquired in a series of non-random waves. A lack of randomness in exposure could result from spatial aggregation of infected copepods, an aggregated distribution of procercoids in the copepod population, heterogeneity in the diet of sticklebacks or heterogeneity in the genetics of the host population. In addition, successive waves of infection could cause temporal segregation in the exposure of hosts. All of these examples are possible mechanisms for generating differential susceptibility of hosts, put forward by Anderson and Gordon (1982) as a generator of over-dispersion.

As the distribution patterns of *S.solidus* were similar between fish of different sexes and ages, Pennycuick (1971c) concluded that the observed over-dispersion, could not simply be a reflection of a heterogeneous host population structure. It was also proposed that parasite-induced host mortality had caused a reduction in over-dispersion in some samples. However,

for this to be the case mortality must increase with increasing intensity of infection (Pennycuick 1971c), yet it appears to be the weight of the *S.solidus* burden that induces pathology in sticklebacks, rather than the number of plerocercoids (Pennycuick 1971d).

3.1.4 GROWTH OF *SCHISTOCEPHALUS SOLIDUS* PLEROCERCOIDS *IN VIVO* AND *IN VITRO*

The growth of *S.solidus* has been examined both *in vitro* and *in vivo*. *In vitro* growth has been found to increase between temperatures of $4^{o}C$ and $23^{o}C$ and seems to be optimal between $23^{o}C$ and $27^{o}C$ (Sinha & Hopkins 1967). Also, large plerocercoids have a much lesser specific growth rate (% increase in weight per 8 days) than small plerocercoids, such that a plerocercoid double the weight of another, has approximately half the specific growth rate (McCaig and Hopkins 1965). Laboratory studies of *in vivo* growth have also been carried out (Orr & Hopkins 1969; Meakins & Walkey 1973). Both studies give indications that the growth rate of plerocercoids is depressed in higher intensity infections.

Changes in mean plerocercoid weight from successive samples of sticklebacks have been used as an indicator of growth of the parasite in natural populations, in much the same way as size changes are used to study fish growth and with similar assumptions (Chapter 2). Clarke (1954) observed that the mean plerocercoid weight rose in the summer and fell in the winter suggesting that either plerocercoids were losing weight, there was a loss of large plerocercoids or there was an increase in the number of small plerocercoids. These fluctuations followed closely the observed changes in water temperature and to a lesser extent the changes in mean fish weight. Temperature could have influenced the growth of the parasite, but Clarke (1954) postulated that the winter reduction in fish weight was due to an efflux of the largest fish from the sampling area (the margins) into deeper water. If those fish carried the largest parasites (as is suggested by the studies above), then this may have been the important determinant of the changes in worm weights. Pennycuick (1971a) also found higher mean plerocercoid weights in her summer sample compared with the previous and following winter samples. In addition, Hopkins and Smyth (1951) and Pennycuick (1971a) suggested an abundance of small worms detected in autumn and winter were indicators of new waves of infection, restricted to these times of year. By contrast, Clarke (1954) found small plerocercoids throughout the entire year

which implied that, at his study site, sticklebacks could acquire *S.solidus* infections at any time.

3.1.5 PROBLEMS WITH INTERPRETATION OF PREVIOUS STUDIES

Although the life history of *S.solidus* has been elucidated by the work of the above authors, some of the results are conflicting. Prevalence, intensity and the parasite index varied seasonally at some localities and at others, between years. In some cases, these variables were affected by size or age and in other instances they were not. Also, some authors found evidence for continuously occurring new infections, whilst others suggested that new infections occurred in waves at specific times of year. There are many possible explanations for the discrepancies between the results of the studies. Simply, the disparities could reflect habitats and stickleback populations that were differentially supportive of the parasite. However, as limited information was given on the habitats and more importantly, of the biology of the host populations, the plausibility of this explanation cannot be assessed. Therefore, the present study, has at the outset, provided the general biology of the host sticklebacks (Chapter 2,) such that aspects of the epidemiology of *S.solidus* might be interpreted against a broader background of information.

Other features of the previous studies may have detracted from their contribution to an understanding of the life history of *S.solidus*. Almost all workers fished using hand nets from marginal areas of water bodies, which may have resulted in non-random sampling of fish and thus of parasites. Also, there may have been selective catching of infected fish if their behaviour had been altered by *S.solidus* infection or they were spatially segregated from uninfected fish (see Section 3.4). To alleviate these difficulties, the present study incorporated a small trawl which was pulled at speed through many areas and covering the full depth of the pond. It was intended that a more representative sample of fish would be obtained if a large area was covered and that the sample would not be biased towards infected fish. Also, with a good trawl speed, fish with all levels of anti-predator behaviour should be caught.

Many of the analyses in other investigations treated factors which may affect prevalence, intensity and growth individually as though they were independent, without allowing for the possibility that they may be confounded. For example, the weight of *S.solidus* increased with length of fish and sometimes with season. It is not surprising that the weight of the parasite

changes seasonally because stickleback length often varies with season (Chapter 2) and the parasite weight varies with fish length (this Chapter). This study examines the factors which may affect *S.solidus* infection and also addresses whether these factors might interact.

Furthermore, the age of sticklebacks was only considered by Pennycuick (1971b) as a possible influence on the infection parameters, yet successive age classes of fish are likely to have different patterns of foraging and social behaviour, growth and mortality. Consequently, infection levels and the growth and mortality of *S.solidus* are also bound to vary between cohorts of fish. Thus this survey concentrates on *S.solidus* infection in young-of-the-year sticklebacks only.

Finally, studying the growth of *S.solidus* plerocercoids in a natural stickleback population will always be problematic if the timing of the input of new infections is unknown. If this can be assessed, then an examination of changes in weights of plerocercoids will provide an estimate of growth. However, it is also important to be aware that plerocercoid growth may be reduced in multiple infections and the combination of data from various intensity infections may provide an inaccurate estimate of growth of *S.solidus*.

3.1.6 AIMS

The aims of the study described in this chapter are to investigate whether the prevalence and intensity of *S.solidus* infection alter with season, and size and sex of sticklebacks within a single cohort of fish. Also, the distribution of parasite numbers per host, the parasite indices and the infectivity of the plerocercoids will be examined for clues to the dynamics of *S.solidus* infection. Finally, the pattern of plerocercoid growth will be investigated in single and multiple infections and in the light of these data, predictions about the life history of the parasite will be made.

3.2 MATERIALS AND METHODS

3.2.1 DATA COLLECTION

Schistocephalus solidus plerocercoids were collected, counted and weighed as described in Chapter 2.2. The same chapter explains how stickleback lengths and weights were derived. The common epidemiological statistics were used to evaluate the status of *S.solidus* within the stickleback population and are defined as follows:

$$\text{Prevalence} = \frac{\text{number of infected hosts}}{\text{number of hosts sampled}} \times 100$$

Intensity = number of parasites per infected host

$$\text{Parasite index} = \frac{\text{weight of } \textit{S.solidus}}{\text{weight of fish}} \times 100$$

The above form of the parasite index was used, because it gives a more easily interpreted description of the relationship between the weight of the *S.solidus* burden and the fish weight. Therefore, a parasite index of 100% would occur when the weight of plerocercoids was equivalent to the weight of the stickleback. Using the data obtained in Chapter 6, the infected sticklebacks were categorised according to whether the plerocercoids they harboured were likely to be uninfective (<=50mg) or infective (>50mg) to the definitive host or a combination of both types of plerocercoids.

3.2.2 DATA ANALYSES

Prevalence

In order to examine the independent effects of the month of survey and the size and sex of fish on the prevalence of *S.solidus* infection in young-of-the-year sticklebacks, a stepwise logistic regression analysis was used. This logistic model has a binary dependent (response) variable and is capable of describing the effects of a number of covariates (predictors) on the probability of this binary variable e.g.

$$\text{probability} = \frac{\exp(b_0 + b_1x_1 + b_2x_2)}{1 + \exp(b_0 + b_1x_1 + b_2x_2)}$$

where ($b_0 + b_1x_1 + b_2x_2$) is a simple linear function of the 2 covariates x_1, x_2 with coefficients (b_1, b_2) and intercept b_0 (see McCullagh & Nelder 1989 for complete description). Logistic regression was particularly appropriate for studying the effects of month of survey, length of stickleback and sex of stickleback on the probability of being infected. The analysis was carried out using the BMDP statistical package, which bases entry of covariates into the regression on the log-likelihood ratio statistic for the logistic model, which can be approximated by the Chi-Square distribution. BMDP also provides Chi-Square tests that describe the improvement in the prediction capability of the model as each covariate is incorporated, whether the predicted values fit the data (goodness-of-fit) and whether the logistic model is appropriate to the data ('C.C. Brown' goodness-of-fit)

Intensity

It was also necessary to determine the independent effects of month of survey, length of stickleback and sex of stickleback on the intensity of *S.solidus* infection. Classical stepwise linear regression could not be used, as it relies on the dependent variable having a normal error distribution, which is influenced by both the mean and the variance. The distribution of *S.solidus* numbers was clearly not normal and to determine whether it was random or over-dispersed the variance-to-mean ratio was ascertained. This calculation requires a zero class and so was carried out on the intensities minus 1. The value of 2.5 obtained for the variance-to-mean ratio suggests only slight over-dispersion. Therefore, a stepwise Poisson regression was used to determine the important predictors of intensity, because the difference between it and a binomial regression is negligible for such modest amounts of over-dispersion (McCullagh & Nelder 1989). Poisson regression is based on a log-linear model of the form:

$$\log(y) = (b_0 + b_1x_1 + b_2x_2 \ldots)$$

where b_0 is the intercept and b_1 and b_2 are coefficients of the covariates x_1 and x_2 (see McCullagh & Nelder 1989 for a complete description).

The analysis was carried out on the intensity minus 1 data (and back-transformed later) using the GENSTAT statistical package. The dispersion parameter (variance-to-mean ratio)

was not fixed at 1 as is usual for the Poisson model, to allow for the slight over-dispersion. The programme reports the deviance of the data from the Poisson model i.e. the log-likelihood ratio statistic, which is approximated by the F distribution. An analysis of deviance allows the fit of a model to be interpreted (in a similar way to the analysis of variance in linear regression). When a good model is obtained, GENSTAT can use it to provide a set of predicted y-values from a range of x-values to illustrate combined effects of different covariates.

Frequency distributions of parasite numbers per host

As the frequency distributions of parasite numbers per host are thought to shed light on the dynamic interactions of populations, survey samples were treated individually, so that each could be regarded as a 'snapshot' of the dynamics of both the host and parasite populations at the time of collection. The variance-to-mean ratios were calculated for each distribution and used as indices of dispersion. They were further converted to Chi-Square values by multiplying by the degrees of freedom (n-1) and to test whether the variance was significantly different from the mean, z scores were calculated according to the following formula (see Fowler & Cohen, B.T.O. Guide 22):

$$z = \sqrt{(2X^2)} - \sqrt{(2(n-1)-1)}$$

Where $z<1.96$ there is no significant difference between the variance and the mean ($P>0.05$) and the distribution is regarded as random, but if $d>1.96$ then the variance is significantly greater than mean ($P<0.05$) and the distribution is considered to be over-dispersed. To determine whether differences existed between distributions, the number of parasites were classed as 0, 1, 2, 3+ plerocercoids per stickleback, grouped into seasons and analysed as a contingency table by the Chi-Square test of independence.

Parasite index

The proportional nature of the parasite index and its non-normal distribution, invalidated use of parametric statistical tests. Usually, this can be rectified by arc sine transformation of the data, but as the parasite index of fish with very small plerocercoids was often 0% and that of fish with large plerocercoids >100%, this was impossible. Consequently,

comparisons of median parasite indices were made between months and length classes using the non-parametric Kruskal Wallis one-way analysis of variance.

To illustrate the monthly distribution of parasite indices, sticklebacks were classified into groups having parasite indices between 0 and 20%, 21-40%, 41-60%, 61-80%, 81-100% and greater than 100%. The percentage frequency distributions of fish falling into each of these ranges were plotted. However, the major trends were ascertained by comparing the raw frequencies of fish in the categories 0-20%, 21-40%, 41-60% and 61+% between seasons.

Infectivity of plerocercoids

The percentage distribution of sticklebacks in each plerocercoid infectivity category was determined for each sample. To compare the proportions of fish falling into each category, the data were combined into seasons and the resultant frequencies compared using the Chi-square test of independence.

Growth

The specific growth rate of plerocercoids was determined in a similar fashion to that of uninfected fish in Chapter 2:

$$\text{Specific growth rate} = \frac{(\ln W_2 - \ln W_1)}{t_2 - t_1} \times 100$$

where:

W_1 = the mean weight of plerocercoids from sample 1 (mg)

W_2 = the mean weight of plerocercoids from sample 2 (mg)

t_1 = the date of sample 1 (days)

t_2 = the date of sample 2 (days)

Comparisons of mean plerocercoid weights, whether between months or length classes, were made using parametric one-way analyses of variance. The mean weights of plerocercoids from single and double infections were compared with the student's t-test.

3.3 RESULTS AND INTERPRETATION

3.3.1 PREVALENCE OF *SCHISTOCEPHALUS SOLIDUS* INFECTION IN STICKLEBACKS

The prevalence of *S.solidus* infection was found to vary considerably over the months of the survey (Figure 3.1), ranging from 6.8-58.5%. To determine what factors may influence the probability of being infected, stepwise logistic regression was used. Length of stickleback (mm) was entered as a continuous variable and month of sampling (1-13) as a categorical variable. Frequently, the sex of the stickleback could not be determined, especially in young fish in the early months of the survey. Nevertheless, by including undifferentiated as one of the categories, 'sex' could be included as a categorical variable (1=female, 2=male, 3=undifferentiated; see Chapter 2 for definitions). However, the inclusion of these sex categories reduced the appropriateness of the logistic model (C.C. Brown Goodness of fit X^2, P reduced from 1.000 to 0.141), probably as a result of the disproportionate representation of these categories across months, leading to a very high number of cells with expected values less than 5. As there appeared to be no overall difference in prevalence between males and females (Chi-Square, $X^2=0.152$, d.f=1, $P>0.05$, N.S.), the analysis was repeated using only month of survey and length of fish as dependent variables.

The second regression revealed that month of survey was the single best predictor of infection status and its inclusion in the model led to a significant improvement on the constant (Chi-Square, $X^2=78.449$, d.f=12, $P<0.001$). Length did not improve the model sufficiently to merit its inclusion (Chi-Square, $X^2=1.92$, d.f=1, $P=0.1662$), suggesting that length had no independent effect on prevalence that could not be explained by month. It is probable that the difference in prevalence detected between length classes (Chi-Square, $X^2=16.390$, d.f=3, $P<0.001$) is actually a function of the strong relationship between stickleback length and month (Spearman rank correlation, $r_s=0.678$, N=785, $P<0.001$). As month was the single covariate in the model, the probability of being infected by *S.solidus* was the actual observed prevalence for each month. The regression equation for the probability of being infected is:

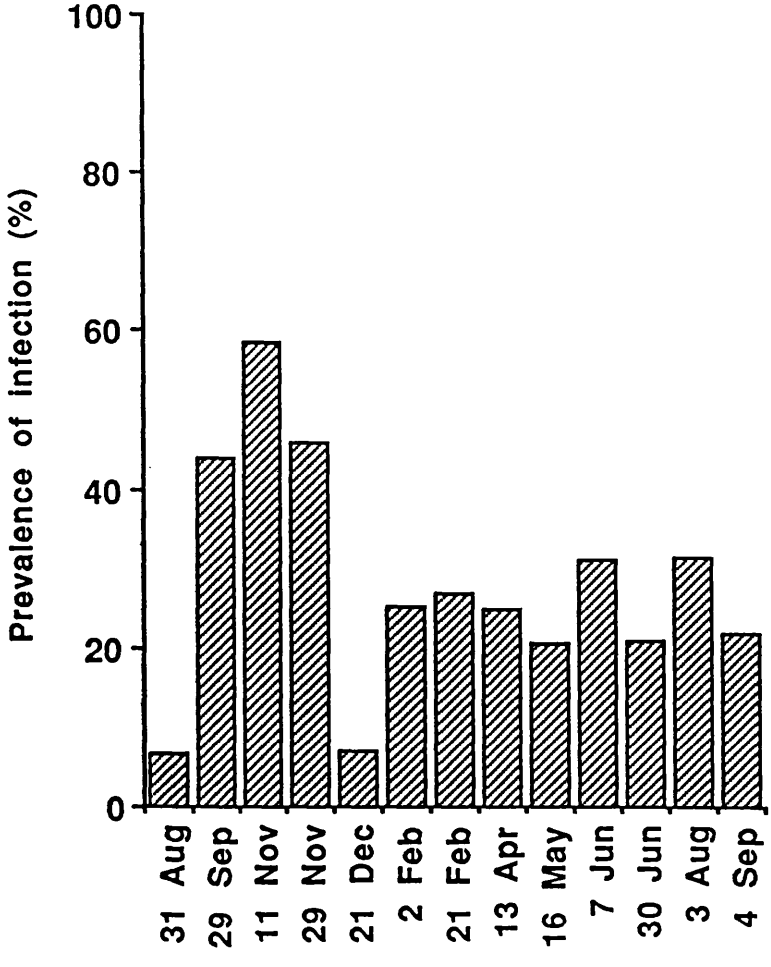


Figure 3.1: Monthly prevalence of *S.solidus* infection from 31 August 1988 to 4 September 1989 = monthly changes in the probability of being infected with *S.solidus* in 0+ sticklebacks, as predicted by stepwise logistic regression.

$$\text{Probability of being infected} = \frac{\exp(1.0726 + \text{month coefficient})}{1 + \exp(1.0726 + \text{month coefficient})}$$

The lowest prevalence and thus, the lowest probability of being infected with *S.solidus* was in August 1988 (Figure 3.1). Sticklebacks were most likely to be infected in autumn 1988 (September-November) with a peak of prevalence in November (45.9-58.5%). Thereafter, a drop in prevalence was detected and the probability of being infected was stable for the remaining months of the survey (approx 20-30%).

From these prevalence data, it would appear that new infections are acquired mostly, if not exclusively, by 0+ sticklebacks during their first autumn of life, irrespective of fish size. The drop and subsequent stability of the infection prevalence suggests that there was either a large winter loss of infected sticklebacks that were subsequently not replaced or a large winter loss of infected sticklebacks followed by an equilibrium of loss and gain of infected fish.

3.3.2 INTENSITY OF *SCHISTOCEPHALUS SOLIDUS* INFECTION IN STICKLEBACKS

The median intensity of infection was consistently 1 across the months of the survey, excepting an increase to 1.5 in early November (Figure 3.2). Month of survey, length of stickleback and 'sex' (reproductive status) were used as covariates in the stepwise Poisson regression (in the same form as in the logistic regression above), to investigate which were the important determinants of intensity. Month of survey was entered as the first covariate in the model, because its inclusion brought about the biggest reduction in the residual mean deviance. Indeed, month of sampling accounted for a significant degree of variation in intensity (Analysis of Deviance, mean deviance ratio=2.75, d.f=12,221, $P<0.01$ (approximated from F tables)). However, having accounted for the monthly variation in the intensity, length was also found to account for a highly significant quantity of the variation in intensity (Analysis of Deviance, mean deviance ratio=21.01, d.f=1,210, $P<0.01$). The inclusion of length as a covariate was responsible for a drop in the residual deviance and this led to an improvement in the amount of variation that was explained by month (Analysis of Deviance, mean deviance ratio=3.01, d.f=12,210, $P<0.01$).

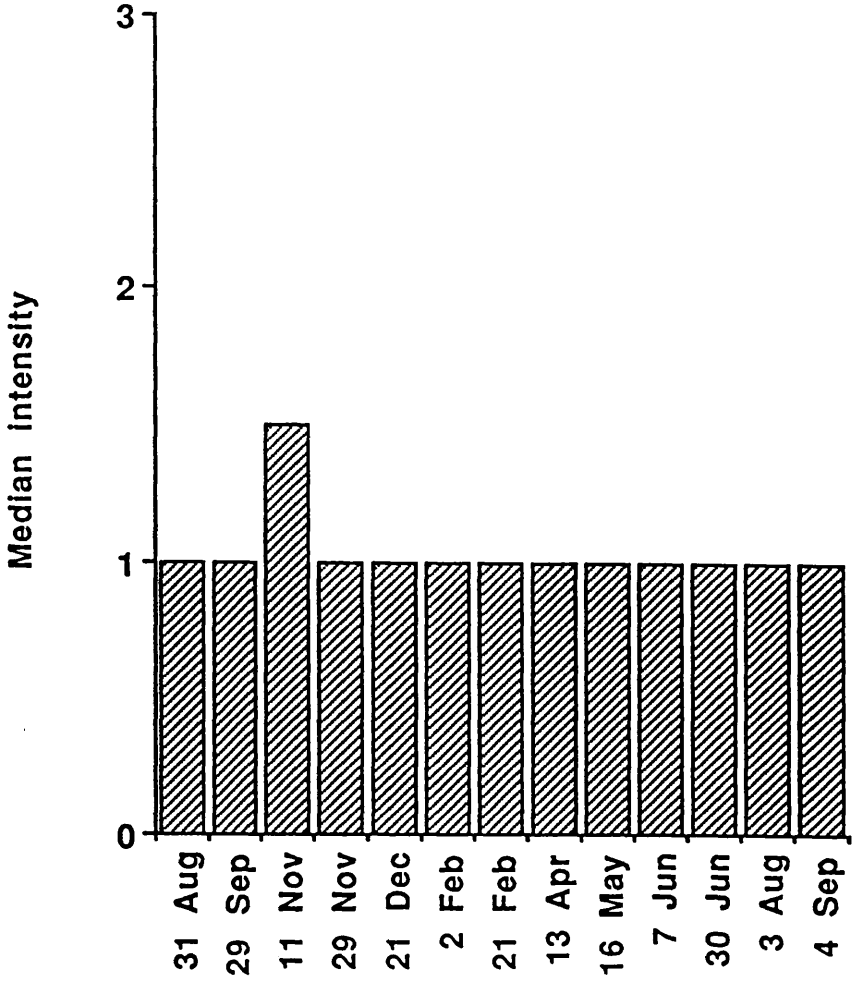


Figure 3.2: Monthly median intensity of *S.solidus* infection from 31 August 1988 to 4 September 1989 in infected 0+ sticklebacks.

Neither the length-month interaction nor the reproductive status of stickleback appeared to significantly affect intensity (Analysis of Deviance, mean deviance ratio (length-month)=1.74, d.f=12,196, P>0.05, N.S; mean deviance ratio (sex)= 2.53, d.f.=2,196, P>0.05, N.S). Thus, the final model included only the constant, month of survey and the length of stickleback and the relevant coefficients. Hence:

Intensity = exp(-7.98 + month constant + 0.0873 (length))

The results of this analysis suggest that intensity is primarily affected by the time of year but, once this has been accounted for, the intensity increases with length of stickleback. The intensities predicted by the model for each month of the survey and for the commonly occurring lengths of stickleback, are shown in Figure 3.3. It is apparent that only at certain times of the year do fish harbour high intensity infections, particularly in autumn. The model also predicts that the largest sticklebacks are infected by a greater number of plerocercoids than smaller sticklebacks within a given month. This trend is strongest in autumn 1988 and it is possible that size at this time is merely an indicator of age, and thus exposure time.

These epidemiological statistics of prevalence and intensity have indicated that *S.solidus* infection in sticklebacks from Inverleith pond occurs in an autumnal wave. The drop in prevalence in winter is also evidence for infected fish losses. Worm losses and gains were investigated further by reference to the distribution of numbers of parasites per host, with a view to identifying the contributing dynamic processes.

3.3.3 FREQUENCY DISTRIBUTIONS OF NUMBERS OF *SCHISTOCEPHALUS SOLIDUS* PLEROCERCOIDS IN STICKLEBACKS

The distribution of parasite numbers per host, for each month of the survey and the corresponding variance-to-mean ratios are shown in Figure 3.4(a-m). Significant seasonal differences were apparent (Chi-Square, X^2=84.168, d.f=12, P<0.001). As described earlier, the prevalence was low at the end of the summer in 1988, but rose sharply during autumn. Associated with the low summer prevalence is a variance-to-mean ratio of just less than unity, which suggests a close to random distribution of plerocercoid numbers in the sample of fish (Figure 3.4(a)). The sharp autumn increase in prevalence coincided with increasing over-dispersion and with greater than expected numbers of high intensity infections (Figure 3.4(b-d).

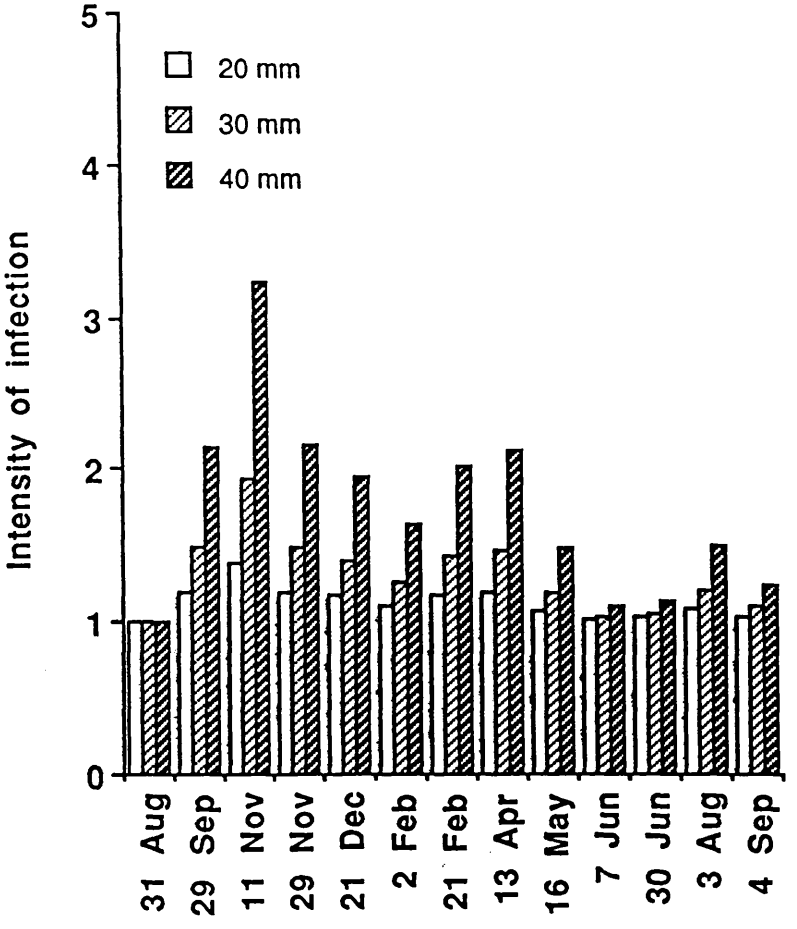
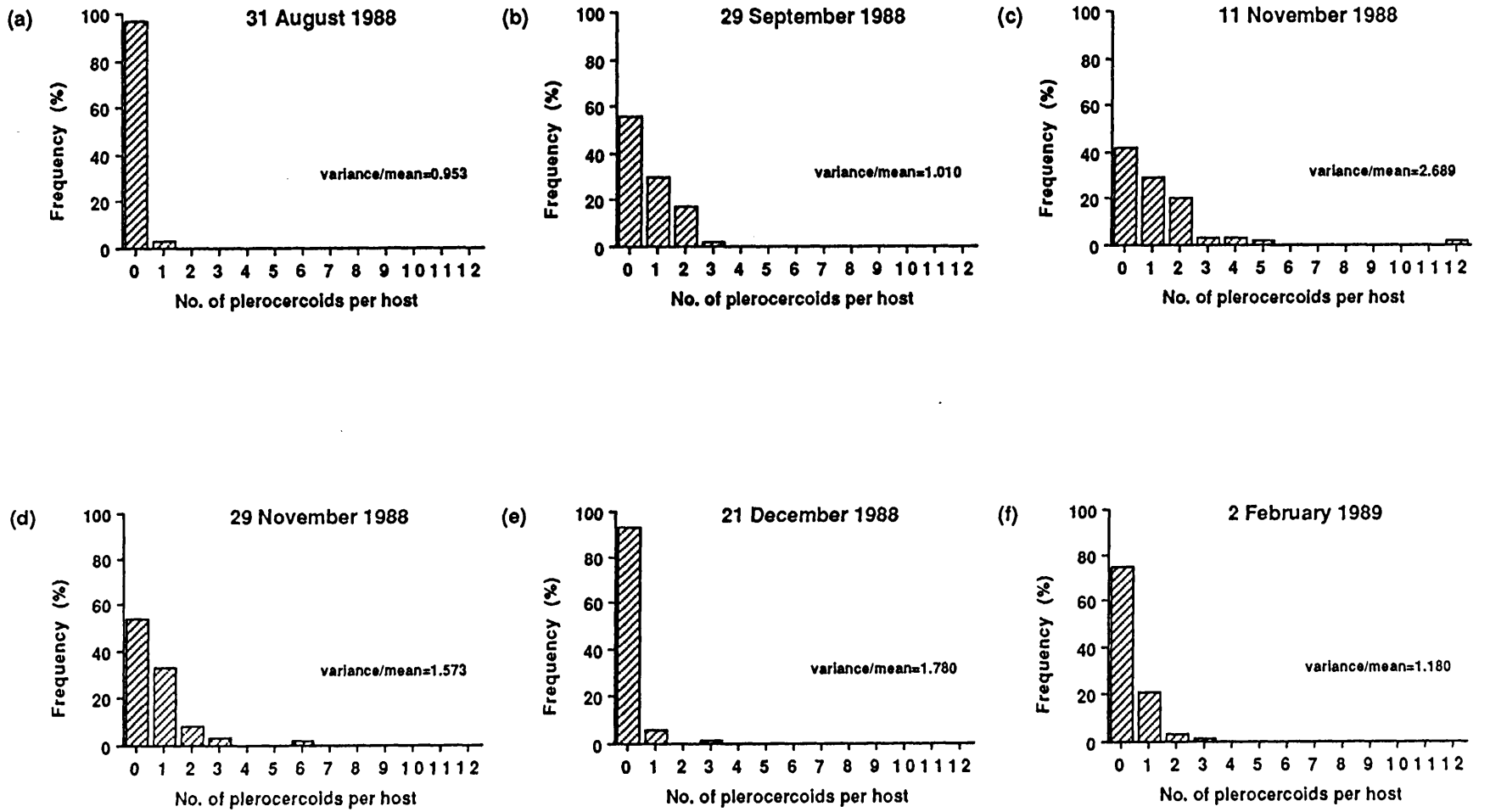
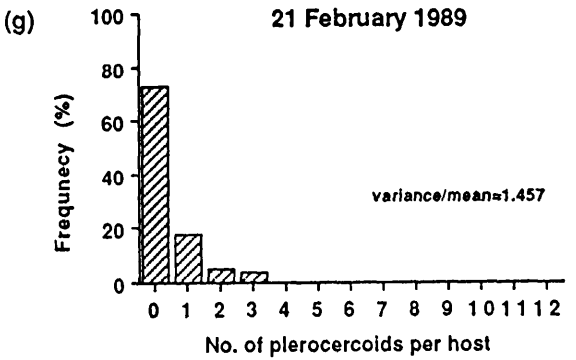
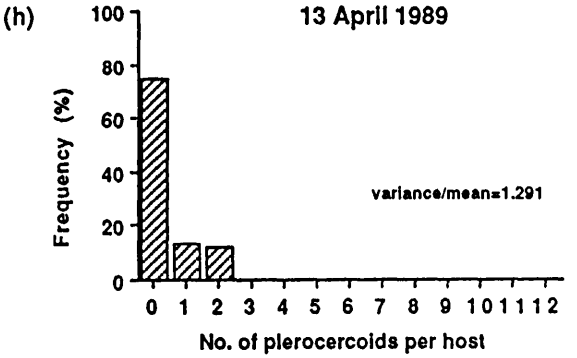


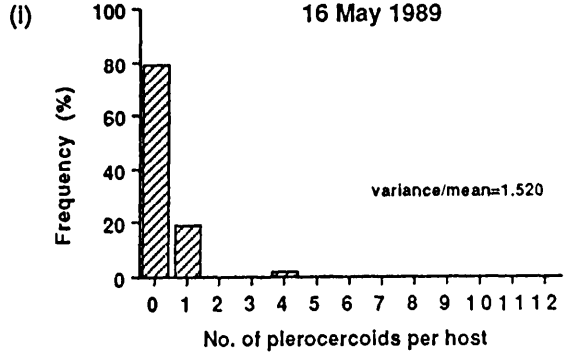
Figure 3.3: Monthly changes in the intensity of *S.solidus* infection from 31 August 1988 to 4 September 1989 in 0+ sticklebacks of 20mm, 30mm and 40mm standard length, as predicted by stepwise Poisson regression.

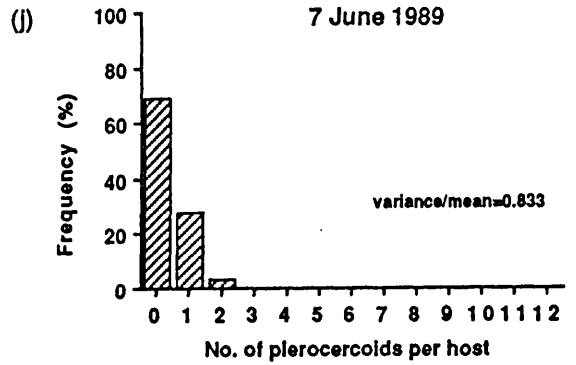
Figure 3.4 Frequency distribution of *S.solidus* plerocercoid numbers per host and corresponding variance-to-mean ratios, for monthly samples of 0+ sticklebacks.

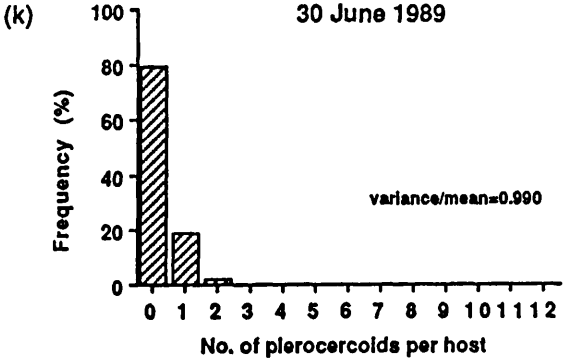

(a)
31 August 1988
variance/mean=0.953
(b)
29 September 1988
variance/mean=1.010
(c)
11 November 1988
variance/mean=2.689
(d)
29 November 1988
variance/mean=1.573
(e)
21 December 1988
variance/mean=1.780
(f)
2 February 1989
variance/mean=1.180
Frequency (%)
No. of plerocercoids per host
0 1 2 3 4 5 6 7 8 9 10 11 12
0 20 40 60 80 100

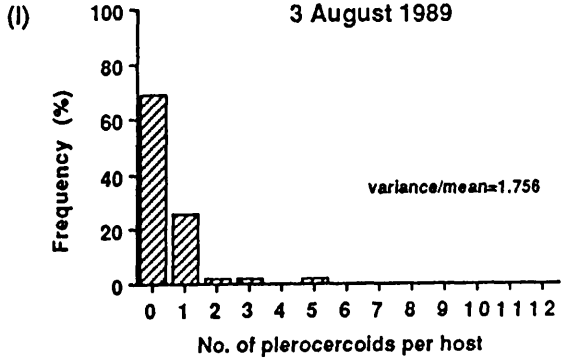

(g)
21 February 1989
Frequnecy (%)
variance/mean=1.457
No. of plerocercoids per host

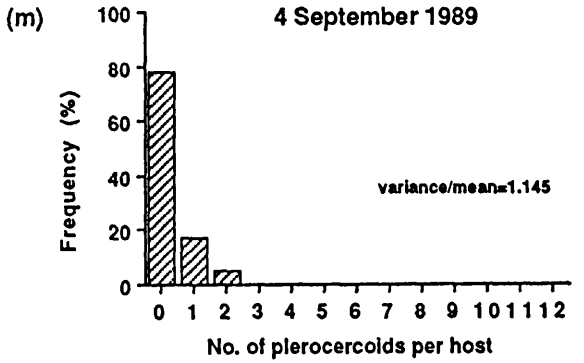

(h)
13 April 1989
Frequency (%)
variance/mean=1.291
No. of plerocercoids per host


(i)
16 May 1989
Frequency (%)
variance/mean=1.520
No. of plerocercoids per host


(j)
7 June 1989
Frequency (%)
variance/mean=0.833
No. of plerocercoids per host


(k)
30 June 1989
Frequency (%)
variance/mean=0.990
No. of plerocercoids per host


(l)
3 August 1989
Frequency (%)
variance/mean=1.756
No. of plerocercoids per host


(m)
4 September 1989
Frequency (%)
100
80
60
40
20
0
variance/mean=1.145
0 1 2 3 4 5 6 7 8 9 10 11 12
No. of plerocercoids per host

Although this indicates that a greater proportion of the *S.solidus* population were being harboured by a smaller proportion of the stickleback population (in higher intensity infections), the distribution was still not highly aggregated.

Despite the observed drop in prevalence during winter, there was still some aggregation of the parasite population in a small number of hosts. However, the highest intensity infections were no longer apparent (Figure 3.4(e-g)). Over-dispersion continued to be a small feature of the distribution of *S.solidus* in sticklebacks during the months of spring (Figure 3.4(h-i)). However, in the early summer samples (Figure 3.4(j-k)) most infected fish harboured a single plerocercoid and this led to a somewhat random/under-dispersed distribution pattern. In the remainder of the survey samples *S.solidus* was found to be minimally over-dispersed in sticklebacks (Figure 3.4(l-m)).

Variability in the frequency distributions was therefore evident within the survey period. Particularly, a low prevalence coincided with a reduced occurrence of high intensity infections. When compared with the much higher variance-to-mean ratios (3.93-17.35) observed by Pennycuick (1971c), the overall impression was that *S.solidus* was much less over-dispersed in sticklebacks from Inverleith pond. Nevertheless, the overall distribution did not differ significantly from a negative binomial distribution (Chi-Square, X^2=4.360, d.f=4, P>0.05, N.S.), but did differ from a Poisson distribution (Chi-Square, X^2=77.643, d.f=1, P<0.001).

3.3.4 PARASITE INDEX IN STICKLEBACKS

Although the frequency of occurrence of high intensity infections was found to fall at certain times of the year, perhaps resulting from parasite induced host mortality or predation, it was postulated by Pennycuick (1971d) that the weight of the *S.solidus* burden may be a more important determinant of the pathogenicity of the parasite.

Monthly changes in the parasite index

Monthly median parasite indices were compared to examine whether the relative weight of parasites changed with time and found to be significantly different (Kruskal Wallis ANOVA, F=38.28, d.f=12, P<0.001). There was a low relative weight of parasite to fish in August, probably because the plerocercoids were small and recently acquired (Figure 3.5). By autumn the weight of plerocercoids was equivalent to almost half the fishes' weight, which would be

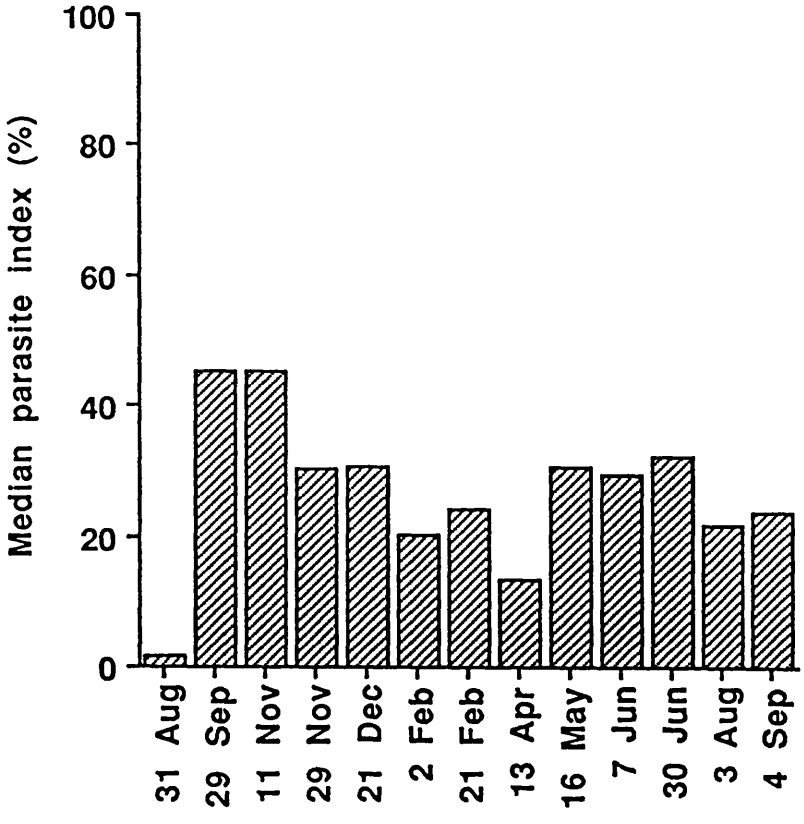


Figure 3.5: Monthly changes in the median parasite index from 31 August 1989 to 4 September 1989 in *S.solidus*-infected, 0+ sticklebacks.

expected if the parasites were growing at a relatively faster rate than the fish. The subsequent reduction in the median parasite index could have resulted from either a low rate of parasite growth or a loss of fish with large parasite burdens. The further increase in the median parasite indices in the summer samples may reflect high plerocercoid growth; the final drop in the parasite indices strongly suggests more losses of fish harbouring big burdens.

The prevalence and intensity data have already pointed to worm losses over winter. If the sticklebacks with high weights of *S.solidus* were those that had succumbed, this would explain the low median parasite indices observed at this time. Similar events may have been the cause of the reduction in median parasite index observed later in the survey. In order to determine more precisely whether heavily infected fish were being lost via death or predation, it was necessary to examine the distribution of parasite indices.

Monthly frequency distribution of parasite indices

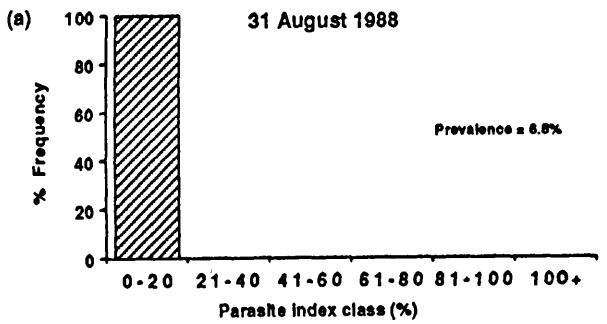
Parasite index distributions for each month are shown in Figure 3.6(a-m) and considerable seasonal changes were evident (Chi-Square, X^2=33.869, d.f.=9, P<0.001). Many fish in the autumn samples had high parasite indices (Figure 3.6(b-d)), which is not surprising since the median parasite indices at this time were also high. In September, the distribution was bimodal at 0-20% and 41-60%, probably as a result of repeated infections. There are single modes in the early and late November samples at 41-60% and 21-40% respectively. As suspected, there were dramatic changes in the distribution pattern of parasite indices in the winter samples. Far fewer large parasite indices were detected and the mode in each of the three months was around 0-20%. The reduction in the prevalence provides evidence for an overall loss of infected fish, but the alteration of the distribution of indices points to selective loss of heavily infected sticklebacks.

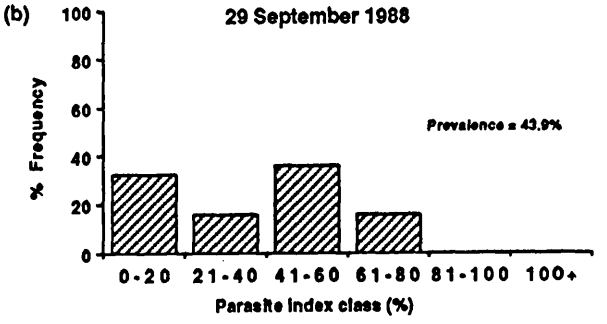
In the spring and summer samples, a shift of the distribution of parasite indices was detected such that most fish harboured parasite burdens that were greater than 20% of their body weight. However, there was not an abundance of parasite indices greater than 40%, as was observed in autumn and this accounts for the lower median parasite indices at this time.

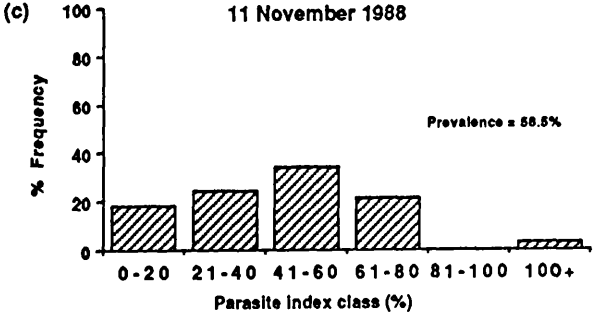
Size of sticklebacks and the parasite index

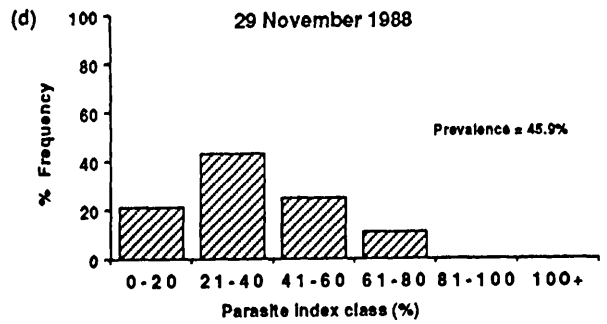
Having ascertained that the median parasite index changed during the survey, it was

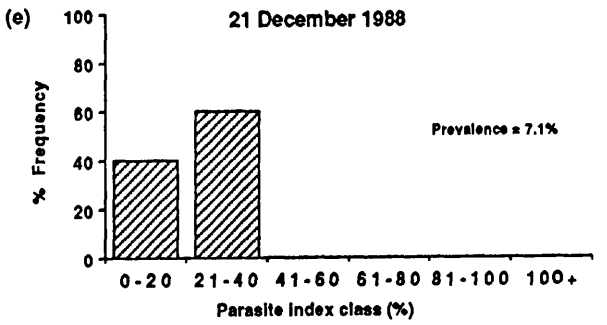
Figure 3.6: Frequency distributions of parasite indices and corresponding prevalences, for monthly samples of *S.solidus*-infected, 0+ sticklebacks.

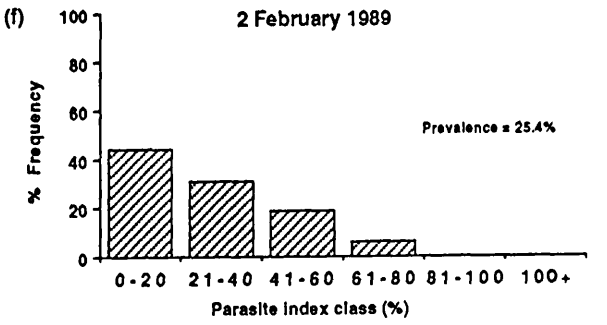

(a)
31 August 1988
Prevalence = 6.8%
% Frequency
0-20 21-40 41-60 61-80 81-100 100+
Parasite index class (%)

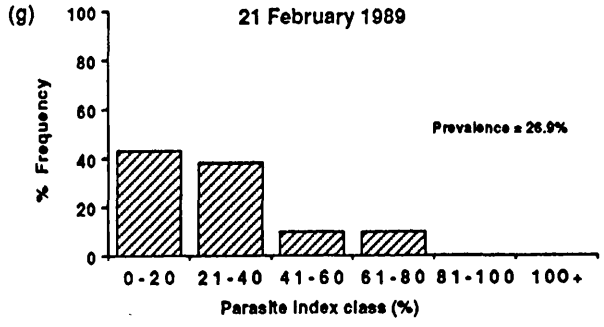

(b)
29 September 1988
Prevalence = 43.9%
% Frequency
0-20 21-40 41-60 61-80 81-100 100+
Parasite index class (%)

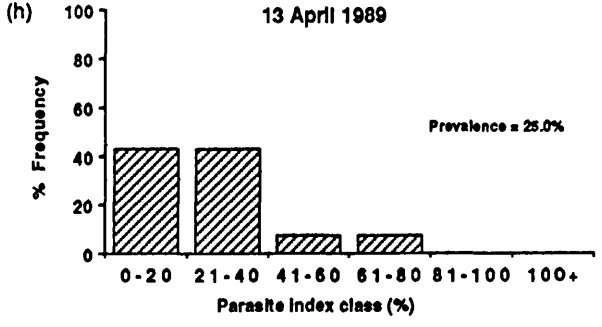

(c)
11 November 1988
Prevalence = 58.5%
% Frequency
0-20 21-40 41-60 61-80 81-100 100+
Parasite index class (%)

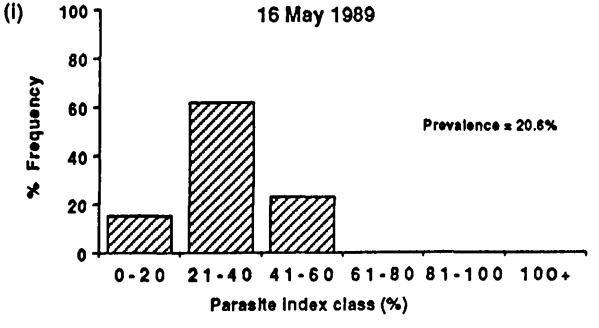

(d)
29 November 1988
Prevalence = 45.9%
% Frequency
0-20 21-40 41-60 61-80 81-100 100+
Parasite index class (%)

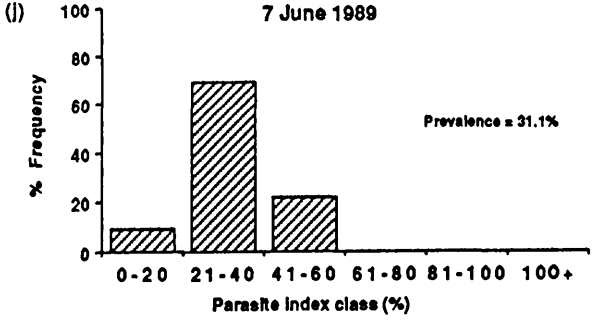

(e)
21 December 1988
Prevalence = 7.1%
% Frequency
0-20 21-40 41-60 61-80 81-100 100+
Parasite index class (%)

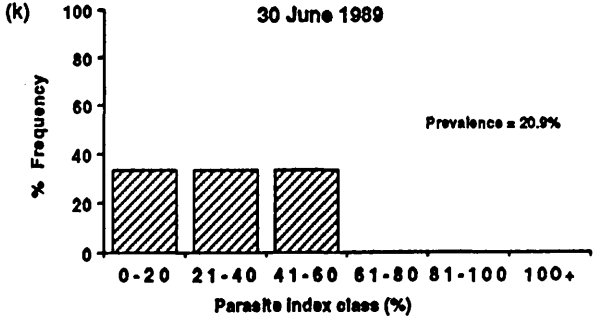

(f)
2 February 1989
Prevalence = 25.4%
% Frequency
0-20 21-40 41-60 61-80 81-100 100+
Parasite index class (%)

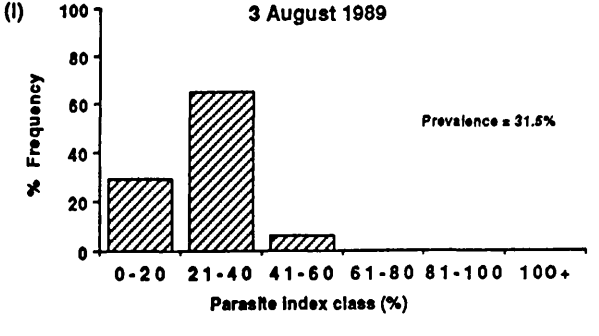

(g)
21 February 1989
% Frequency
100
80
60
40
20
0
Prevalence = 26.9%
0-20
21-40
41-60
61-80
81-100
100+
Parasite index class (%)

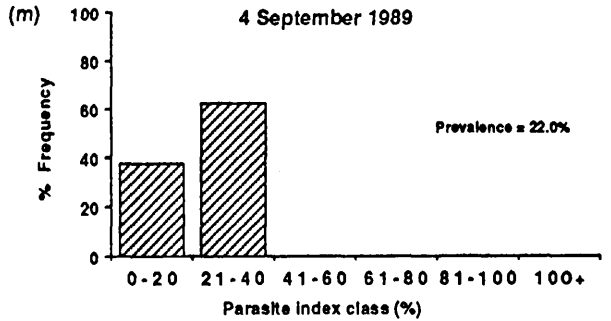

(h)
13 April 1989
% Frequency
100
80
60
40
20
0
Prevalence = 25.0%
0-20
21-40
41-60
61-80
81-100
100+
Parasite index class (%)


(i)
16 May 1989
% Frequency
100
80
60
40
20
0
Prevalence = 20.6%
0-20
21-40
41-60
61-80
81-100
100+
Parasite index class (%)


(j)
7 June 1989
% Frequency
100
80
60
40
20
0
Prevalence = 31.1%
0-20
21-40
41-60
61-80
81-100
100+
Parasite index class (%)


(k)
30 June 1989
% Frequency
100
80
60
40
20
0
Prevalence = 20.9%
0-20
21-40
41-60
61-80
81-100
100+
Parasite index class (%)


(l)
3 August 1989
% Frequency
100
80
60
40
20
0
Prevalence = 31.5%
0-20
21-40
41-60
61-80
81-100
100+
Parasite index class (%)


(m)
4 September 1989
% Frequency
100
80
60
40
20
0
Prevalence = 22.0%
0-20
21-40
41-60
61-80
81-100
100+
Parasite index class (%)

decided to investigate the relationship between stickleback size and the relative weight of plerocercoids, by comparing the median parasite indices across fish length classes (Figure 3.7). With increasing stickleback length, the relative weight of the parasite burden decreases (Kruskal Wallis ANOVA, $H=20.03$, $d.f=4$, $P<0.01$) and so there is a possibility that the parasite has a more acute effect on young fish.

3.3.5 PREVALENCE OF UNINFECTIVE / INFECTIVE PLEROCERCOIDS OF *SCHISTOCEPHALUS SOLIDUS* IN STICKLEBACKS

An examination of the infectivity of plerocercoids may directly indicate when sticklebacks were likely to be susceptible to predation. The proportion of fish harbouring plerocercoids at different stages of infectivity varied with season (Chi-square, $X^2=40.313$, $d.f.=6$, $P<0.001$). The trend was for all types of plerocercoid to be common in autumn, but throughout winter, uninfective worms were predominant and infective worms and mixed infections accounted for a smaller proportion of the total prevalence (Figure 3.8). This would seem to confirm that sticklebacks harbouring infective plerocercoids are more susceptible to predation than those with uninfective worms. Not until the spring and summer samples did almost all infections consist of infective plerocercoids.

3.3.6 GROWTH OF *SCHISTOCEPHALUS SOLIDUS* PLEROCERCOIDS IN STICKLEBACKS

Having gained some insight into when sticklebacks (Chapter 2) and *S.solidus* plerocercoids appear to be recruited and lost, the growth of the parasite in sticklebacks from Inverleith pond could be interpreted more clearly.

Growth in single and double infections

To test in a natural situation, whether individual plerocercoid growth varied with infection intensity, the mean weights of individual worms from single and double infections were compared within seasons (Figure 3.9). This was because single and double infections were the only intensities that were represented throughout the year and they were compared within seasons to provide adequate sample sizes. As acquisition of *S.solidus* seems to occur mainly in autumn, it was hoped that age-related differences in size would be limited.

In the autumn sample, plerocercoids from single infections had attained a higher mean

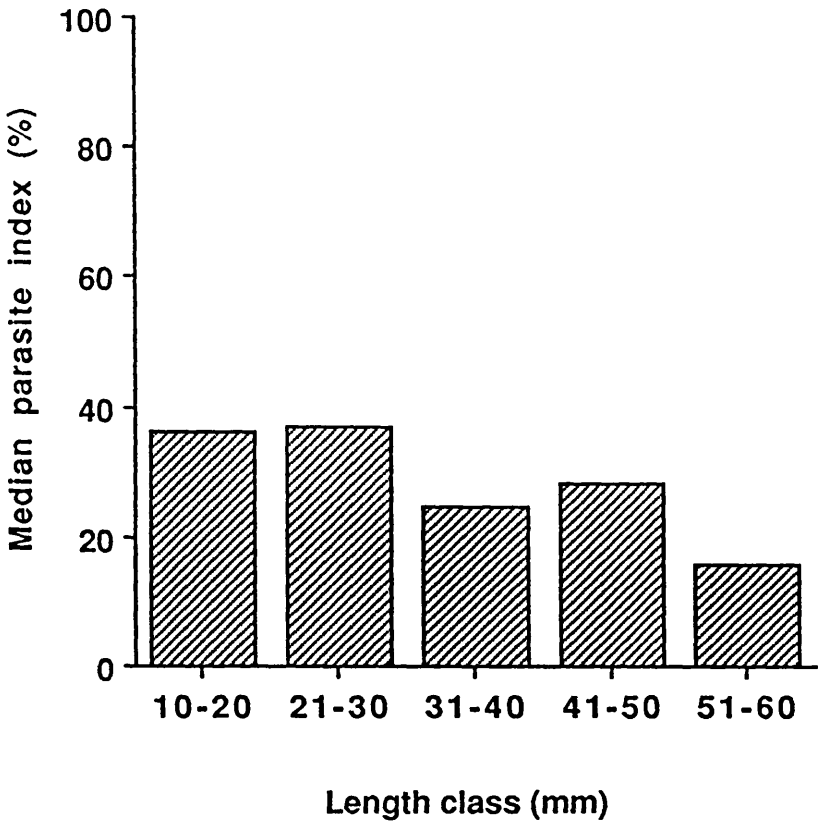


Figure 3.7: Changes in the median parasite index with length class of *S.solidus*-infected, 0+ stickleback.

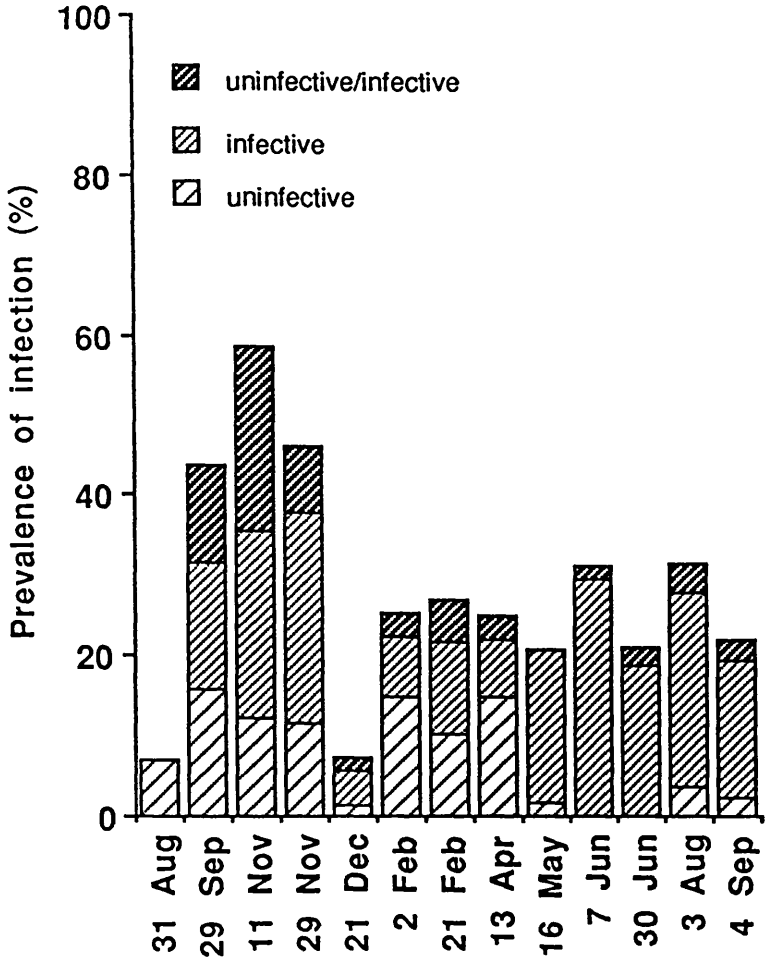
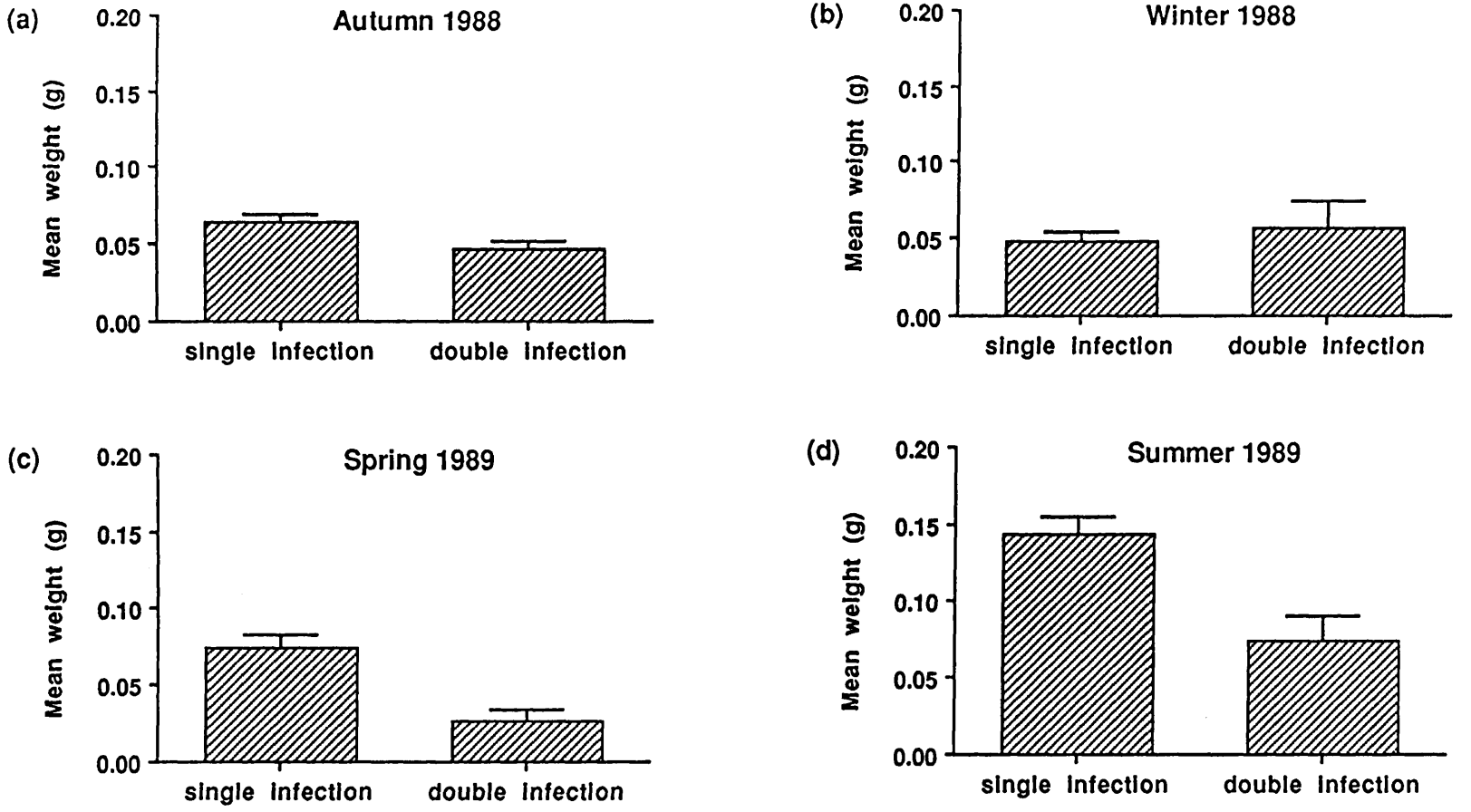


Figure 3.8: Monthly prevalence of infection with uninfective, infective and both uninfective and infective plerocercoids of *S.solidus*, in 0+ sticklebacks.

Figure 3.9: Mean wet weight (± S.E.) of individual *S.solidus* plerocercoids from single and double infections of 0+ sticklebacks, for 4 seasons.


(a)
Autumn 1988
Mean weight (g)
0.20
0.15
0.10
0.05
0.00
single infection
double infection
(b)
Winter 1988
Mean weight (g)
0.20
0.15
0.10
0.05
0.00
single infection
double infection
(c)
Spring 1989
Mean weight (g)
0.20
0.15
0.10
0.05
0.00
single infection
double infection
(d)
Summer 1989
Mean weight (g)
0.20
0.15
0.10
0.05
0.00
single infection
double infection

weight than those from double infections (t-test, $t=2.35$ d.f.$=104$, $P<0.05$). This could be the result of double infections consisting of variable aged worms, at different stages of growth. Alternatively, the growth of individual worms may have been reduced in double infections because the resources for growth were limited and shared between the two larvae.

Winter appears to be a time when infected fish are lost, especially those fish with either high intensity infections and/or high parasite indices. Also in winter no difference was detected between the mean weights of plerocercoids in single and double infections (t-test, $t=-0.56$, d.f$=42$, $P>0.05$, N.S). The general reduction in the prevalence of fish with large worms of all intensities may have been responsible. Additionally, low winter temperatures could have limited fish and parasite growth.

During spring and summer, the mean weight of plerocercoids from single infections was found to be significantly higher than those from double infections (t-test, spring: $t=3.96$, d.f$=35$, $P<0.001$; summer: $t=2.73$, d.f.$=48$ $P<0.01$). Thus, there is some evidence for a naturally occurring density-dependent limitation on individual growth of the plerocercoids, as found experimentally by Meakins and Walkey (1973).

Monthly pattern of growth in single infections

Growth was assessed in greater detail by examining the rate of change in the mean plerocercoid weight per day, from single infections only. Plerocercoids from multiple infections were omitted owing to the above results and the fact that the most common intensity of infection was 1. Since Meakins & Walkey (1973) found that growth was maximal in single infections, it is expected that the weight changes displayed in these single infections also represent the maximum for the conditions during the survey period.

The specific rate of change in mean weight of plerocercoids from each month of the survey is shown in Figure 3.10. Initially, in early autumn, the rate of growth was observed to be very high followed by a much slower rate of change for the remainder of autumn. Over winter, the specific growth rate became negative (drop in mean weight of plerocercoids), indicative of either shrinking plerocercoids, an increase in numbers of small plerocercoids or a reduction in numbers of large plerocercoids as is hinted in parasite index data. In the spring and summer samples there was a resurgence of plerocercoid growth and the final loss of weight in August

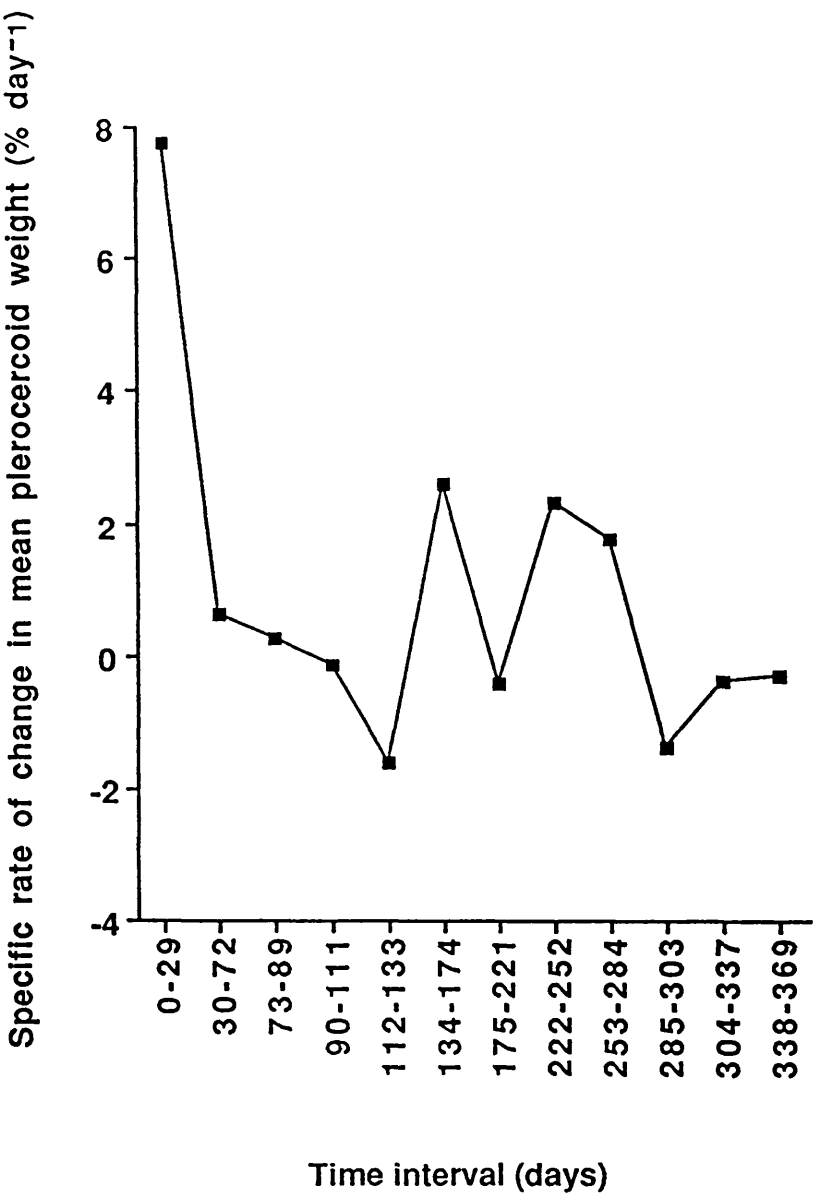


Figure 3.10: Rate of change in mean weight of *S.solidus* plerocercoids from single infections, in monthly samples of 0+ sticklebacks from 31 August 1988 to 4 September 1989.

1988 and September 1989 is likely to have been the result of death of heavily infected fish.

Growth and size of sticklebacks

Bearing in mind that fish size increases with time, the mean weights of single plerocercoids from different size classes were examined (Figure 3.11). Small sticklebacks harboured significantly smaller parasites than large sticklebacks (ANOVA, $F=26.71$, d.f.$=4$, 157, $P<0.001$), suggesting that the size of the stickleback and hence its capacity for growth may limit the growth of plerocercoids or *vice versa*.

3.4 DISCUSSION

3.4.1 EPIDEMIOLOGY OF *SCHISTOCEPHALUS SOLIDUS* INFECTION IN STICKLEBACKS FROM INVERLEITH POND

Shortly after the emergence of new fry in the summer, there was a low prevalence and intensity of *S.solidus* infection, followed by an autumnal wave of infection. Many factors could have contributed to this pattern, but essentially for susceptible sticklebacks to become infected there must be a supply of copepods with infective procercoids. This in turn requires a supply of fertile eggs that will develop, hatch and release coracidia to infect susceptible copepods. These processes are all subject to constraints of host and parasite origin and in addition, to environmental factors.

Fertile eggs will develop optimally at 26°C and to a lesser extent at 21°C and 15°C. At 5°C, as long as 13 months may be required before embryonation of eggs occurs (Mason 1965). In addition, there is large variation in the rate of development between eggs even from the same adult *S.solidus* (Chapter 6). Therefore, even if eggs were present in Inverleith pond throughout the year, their embryonation would be controlled by the water temperature, such that seasonality in the occurrence of developed eggs is bound to occur. Assuming that embryonated eggs were present continuously, coracidia would also only be produced when the light intensity was sufficient to stimulate hatching. Furthermore, the eggs are dense, likely to be lying on the pond bottom and perhaps under detritus, so it is possible that hatching only takes place at times of prolonged exposure to bright sunlight. Thus, simply the restrictions of temperature and sunlight could have produced an autumnal wave of infection, whereby warm summer temperatures and long daylight hours produced a wave of hatching, followed by a wave

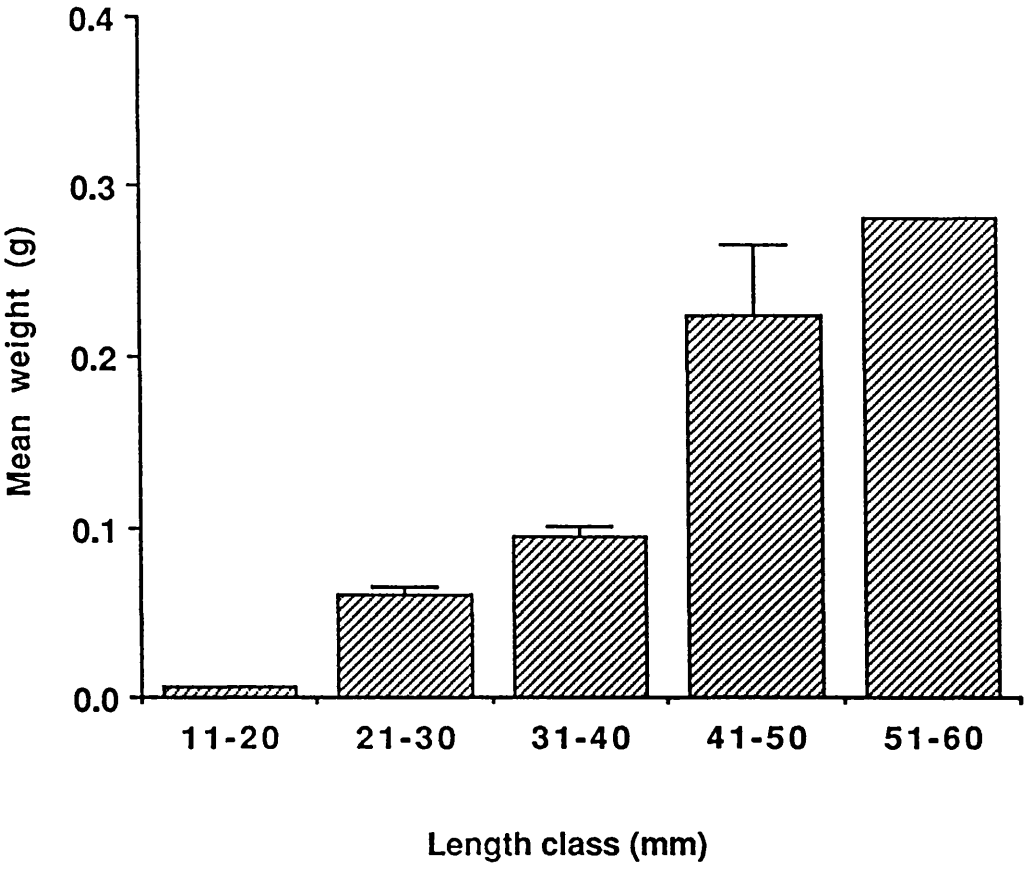


Figure 3.11: Changes in mean weight (± S.E.) of *S.solidus* plerocercoids from single infections, with length class of 0+ stickleback.

of infections in copepods and finally culminating in the observed infection pattern in sticklebacks.

At Inverleith pond, cyclopoid copepods were present and indeed eaten all year round. Not all the species found need have been susceptible to *S.solidus* infection. This could arise if they were either physiologically refractory or if they did not come into contact with coracidia as a consequence of temporal (seasonality) or spatial (habitat choice) segregation. A study of the crustacea in a shallow lake (Adalsteinsson 1979) revealed that three of the species of copepod that have been cited as intermediate hosts of *S.solidus* (see Orr & Hopkins 1969) occurred seasonally. Populations abundances of *Eucyclops serrulatus* and *Megacyclops viridis* (= *Acanthocyclops viridis*) each peaked from July until August whilst the numbers of *Cyclops* c.f *abyssorum* Sars had peaks in May, July and September. In addition, *Schistocephalus pungitii* (the species of *Schistocephalus* found in the nine-spined stickleback *Pungitius pungitius*) was found to occur from June to September in *Mesocyclops oithonoides*, with a maximum prevalence in the copepod being found in July and August. If such patterns of occurrence and infection of copepods are applicable to Inverleith pond, then an autumnal peak of infection is hardly surprising. Thus, even without restrictions on the availability of coracidia, a limited supply of infected copepods might generate a wave of infection.

During the period when new infections were occurring, the prevalence increased, but not to 100%. The intensity also increased reaching a maximum of twelve, but the modal intensity was consistently one. This is despite very high prevalences and intensities of infection being described in other studies (Arme & Owen 1967; Pennycuick 1971a). If only a proportion of Inverleith pond sticklebacks were susceptible to *S.solidus* infection then this could result in less than maximal prevalence and intensity. Experimental studies have shown that sticklebacks from Inverleith pond will not necessarily develop a plerocercoid after ingesting a copepod with a visibly mature procercoid (personal observation). Even if this were simply a reflection of variable infectivity of procercoids, it could explain why some sticklebacks from this population remain uninfected. However, it is improbable that these mechanisms would be population-specific and so the discrepancies in infection levels between populations remain largely unexplained. Furthermore, if either heterogeneity in host susceptibility or parasite infectivity

were strongly influencing the prevalence and intensity of infection, then a high degree of over-dispersion would be expected (Anderson & Gordon 1982). Only limited over-dispersion was found in the survey and so it appears that these are not strong forces in the dynamics of the parasite population, unless the stickleback population is very genetically similar or particularly resistant to infection.

An extremely similar pattern of infection with the closely related *Ligula intestinalis* in roach was described by Kennedy (1985). He also found autumnal wave of infection resulting in a peak prevalence of 30%, with a modal intensity of 1. The variance-to-mean ratios were consistently close to unity, suggesting a random distribution of plerocercoids in the fish host. Again this was in contrast to the high prevalences and intensities found by other workers on *L.intestinalis*. Kennedy (1985) ascribed these differences to the relatively short-lived nature of his host population (maximum of 18 months) compared to the others studied, causing considerable inter-population differences in the transmission dynamics.

A similar trend is observed across *S.solidus/G.aculeatus* populations. Pennycuick (1971a) found a high prevalence and intensity of infection in a population with 3 year-classes of sticklebacks. Although Arme & Owen (1967) did not describe the age structure of their study population, the observed size range of the sticklebacks (up to 500mg) strongly suggests that more than one year class was represented and again infection levels were very high. In contrast, Chappell (1969a) found prevalences and intensities of infection in an annual population that were akin to those found in Inverleith sticklebacks. A long-lived population of sticklebacks would be exposed to infected copepods for a longer period of time, perhaps allowing infections to accumulate. Also, it is envisaged that in short-lived populations of *G.aculeatus*, the annual death of sticklebacks would result in a large reduction in the parasite population. Such a decrease in plerocercoid numbers would be less dramatic in a longer-lived population. It seems then that long-lived host populations harbour larger and more stable *S.solidus* populations, which perhaps ensure a more extensive input of infective stages and further new infections. This is in contrast to *S.solidus* at Inverleith, where the parasite population is smaller and subject to large annual changes.

Later work by Wyatt and Kennedy (1989) at Slapton Ley led them to suggest that the

narrow transmission period of *L.intestinalis* infection imposed another limit on the accumulation of parasite numbers. It appeared that new infections were confined to fry probably because they spent a limited period foraging on copepods. Again an analogous wave of infection was evident in fry from Inverleith although in this case other factors may be responsible (see above). Nevertheless, the expansion of *S.solidus* population seems to be further controlled by the extent of transmission to the young sticklebacks.

The essentially random dispersion pattern of *S.solidus* numbers in sticklebacks from Inverleith pond is perhaps a consequence of strong density-independent reductions in the parasite population, resulting from the annual death of infected fish. This would prevent a build up of parasite numbers and mask the processes that produce over-dispersion. The high level of infection and over-dispersion described by Pennycuick (1971 a & c) would lend itself more to density-dependent regulation e.g by parasite-induced host mortality, of which she found evidence.

Associated with winter were dramatic changes in the structure of the parasite population. Fewer infected fish were apparent and those that were infected harboured fewer and smaller plerocercoids . This suggests that there has been loss of infected fish carrying heavy and/or many plerocercoids. Pennycuick (1971d) observed that fish found dead had significantly higher mean parasite indices and a lower body condition than those caught live, leading her to conclude that *S.solidus* was contributing to the mortality of sticklebacks. Thus, the heavily infected sticklebacks from Inverleith pond may have died from poor health. Also, Pennycuick (1971d) found dead fish most frequently in the autumn and winter and suggested that the poor condition of fish may have been exacerbated by food scarcity. As it has been found that dietary restriction results in a higher death rate of infected sticklebacks (Walkey & Meakins 1970; Pascoe & Mattey 1977), this may indeed have been the case. However, there was no evidence of dead sticklebacks to suggest that poor condition was a cause of winter mortality at Inverleith pond.

Alternatively, or perhaps additionally, the heavily infected sticklebacks may have been lost as result of predation. Information given in Chapter 2 already gives some indication that Black-headed gulls (*Larus ridibundus*) may take sticklebacks from the pond during winter, but

for them to have been responsible for the decrease in the prevalence and intensity of *S.solidus* infection they would have to have selectively predated infected sticklebacks. From the literature, it appears that there are two possible mechanisms by which could this could have occurred.

Firstly, the greater oxygen requirement of infected compared to uninfected sticklebacks (Lester 1971; Meakins & Walkey 1975), may have forced infected sticklebacks into oxygen-rich surface and marginal areas of Inverleith pond, with associated higher risks of avian predation. Indeed, Lester (1971) found a much higher prevalence of *S.solidus* infection in littoral-caught (88%) compared with pelagic-caught (12%) fish from a Canadian lake. However, he recognised that this may have resulted from the existence of two differentially susceptible groups of fish, rather than a parasite-induced choice of habitat. Furthermore, a laboratory study by Giles (1987a) revealed that infected sticklebacks react more quickly to hypoxia and do seek out surface waters, but in the field the threshold of dissolved oxygen which initiated this reaction occurred only in midsummer. Nevertheless, Giles suggested that the most heavily infected fish could suffer hypoxic stress. Therefore, an oxygen gradient may have occurred in Inverleith pond during winter, forcing infected fish (especially heavily infected fish) into the pond surface and margins. This would have segregated them from their uninfected counterparts and made them easier prey for the gulls.

A number of authors have reported alterations in stickleback anti-predator behaviour as a consequence of infection with *S.solidus* plerocercoids and such deficient anti-predator behaviour may give further scope for selective predation of infected sticklebacks at Inverleith pond. For example, Giles (1983b) found that infected sticklebacks recovered more quickly following an attack with a heron model and that the length of the time taken to recover was shorter in the most heavily infected. Also, Milinski (1985) observed that infected fish were less responsive to the presence of a potential predator and foraged closer to it, than uninfected fish. The implication from these studies is that the larvae of *S.solidus* suppress the sticklebacks' response to predators and makes them more likely to be eaten, thereby increasing the chances of parasite transmission.

Despite having a poorly developed repertoire of anti-predator behaviour, uninfected

Inverleith sticklebacks do respond to a predatory stimuli (Huntingford 1982), but infection with large (>50mg) plerocercoids of *S.solidus* appears to suppress this response, whilst infection with small (<=50mg) plerocercoids seems to enhance it (Tierney, Huntingford & Crompton 1991, in prep.). This is thought to be a parasite adaptation to ensure that only plerocercoids capable of establishing and maturing into adults are transmitted to the avian definitive host. If such behavioural changes are operating in the wild population, it is possible that the loss of heavily infected fish that is seen at Inverleith pond during the winter, is parasite-induced. The possibility of death due to morbidity or predation of infected fish is further supported by the fact that *Larus ridibundus* is a known definitive host of *S.solidus* and that adults have been recovered from the intestinal tract of winter-killed birds (Pemberton 1963).

If avian predation and not parasite-related mortality is responsible for the loss of infected hosts, then the adults in the gull intestines will have produced a large, but temporally restricted, influx of eggs into the pond. Embryonation and hatching of these eggs would probably not have been possible until the following spring or summer when temperatures were higher and day lengths longer.

The prevalence of infection remained stable throughout spring and early summer, further indicating that infection occurs mainly autumn. However, a further decrease in the intensity of infections was accompanied by slight under-dispersion in the early summer samples. This would suggest that infected fish are again being lost to greater extent than uninfecteds. As this is an annual population, death of post-breeding fish is a common occurrence, but if infected fish experienced even poorer body condition and/or the summer temperatures created a more hypoxic environment, then the situation may have been have exacerbated for infected fish.

3.4.2 GROWTH OF *SCHISTOCEPHALUS SOLIDUS* PLEROCERCOIDS IN STICKLEBACKS FROM INVERLEITH POND

Parasite growth parallels fish growth (Chapter 2) in being high during autumn. The apparent loss of large plerocercoids in winter complicates an investigation of the growth of the parasite, but it is likely to be low at this time. A resurgence of growth took place in spring and summer and this is again in accordance with the timing of fish growth. However, the intensity of

infection did appear to influence individual plerocercoid growth, it being slower in multiple infections. This suggests that nutrient resources for parasite growth were somehow restricted. It is improbable that this would have had regulatory effects on this parasite population because the intensity of infection was commonly 1, but extrapolation to high intensity infections suggests that the potential is there for reduced (regulated) transmission to the definitive host. This may be have been an important regulatory mechanism in e.g. the study of Pennycuick (1971) where the maximum intensity recorded from a single stickleback was 106.

3.5 CONCLUSIONS

During the life span of a single cohort of sticklebacks from Inverleith pond, marked changes in the population of *S.solidus* were observed. These have been interpreted with respect to the complete life history of the parasite. Ultimately, as result of infected copepods being available to sticklebacks, new infections were restricted to autumn. However, many environmental, physiological and behavioural mechanisms of either sticklebacks or copepods may have contributed. The dynamics of the stickleback population may have been an important factor in limiting the levels of infection. Despite this regulation of the parasite population and perhaps in response to it, the plerocercoids grew and may have enhanced their chances of transmission by modifying the anti-predator behaviour or habitat selection of their stickleback hosts. If the result was transmission, then the input of fertile eggs i.e. the basis for the following years wave of infection, was largely confined to the winter months. The remainder of the parasite population resumed growth at higher temperatures, but this appeared to be optimal in single infections. Further reductions in the parasite population were evident late in summer and may simply have been the result of a normal annual course of stickleback death.

CHAPTER 4: THE IMPACT OF *SCHISTOCEPHALUS SOLIDUS* ON THE STICKLEBACK POPULATION OF INVERLEITH POND, EDINBURGH

4.1 INTRODUCTION

A high density of sticklebacks exists at Inverleith pond and so the potential competition for resources such as food and mates is probably strong, especially as there is a single opportunity to breed. Therefore, any deleterious effects of *Schistocephalus solidus* on the capability of *Gasterosteus aculeatus* to find and utilise resources is likely to have serious consequences for the successful completion of their life history. This chapter is concerned particularly with comparing aspects of the diet, growth and breeding of uninfected and infected sticklebacks.

4.1.1 FORAGING AND DIET CHOICE IN *GASTEROSTEUS ACULEATUS*

Optimal foraging theory predicts that animals should adopt foraging strategies that will maximise their net energy gain per unit of time. In order to forage optimally an animal must be able to select highly profitable (food energy value per unit handling time) prey and be more selective when such prey is abundant. However, if high quality prey are sparse then it becomes more optimal to consume less profitable, but more frequently occurring prey (see Krebs & Davies 1991 for a full description of optimal foraging).

In laboratory experiments, where the time and energy expended were the principle costs to the sticklebacks, the most profitable prey items were chosen (Gibson 1980). Field studies also revealed a tendency for sticklebacks to select the most profitable prey items (Ibrahim & Huntingford 1989) and for bluegill sunfish to utilise the most profitable food patches (Werner & Mittlebach 1981) and these choices were affected by prey availability. However, foraging decisions are likely to be constrained by other factors such as competition and the need for vigilance, which will constitute additional costs.

Prior to an investigation of stickleback diet selection, Milinski (1982) first assessed each stickleback's individual competitive ability. Presented with both large and highly profitable *Daphnia* and small, less profitable *Daphnia*, increasingly poor competitors progressively selected the smaller prey. This was likely to be the most energy efficient strategy under these conditions because although the prey taken were relatively less profitable, there was reduction in costly attempts at the larger prey in the presence of a better competitor. Furthermore, Milinski (1984) found that parasites can influence foraging decisions by altering the fishes'

competitive ability. Since *Schistocephalus solidus*-infected sticklebacks are often in poorer condition (Pennycuick 1971d) and have a greater oxygen requirement (Lester 1971; Meakins & Walkey 1975) than uninfected sticklebacks, Milinski (1984) predicted that they would also be poorer competitors for food. Indeed, he found that parasitized sticklebacks attacked less profitable prey and he proposed that hunger (which was not investigated during the experiment) may have been the mechanism, since selectivity is reduced in hungry fish (Beukema 1968). However, this hypothesis requires that infected sticklebacks are generally hungrier than uninfected sticklebacks, but this has yet to be demonstrated.

Predation risk should be an additional consideration when foraging (Milinski & Heller 1978) which must be weighed against the quality of a food patch. Ibrahim and Huntingford (1989) observed a trade-off between optimal prey selection and vigilance in both laboratory and field studies on sticklebacks. In field experiments, the presence of a predator coincided with reduced food intake and the selection of less profitable, but more easily handled prey. Also, the noticeable switch from benthos to plankton probably allowed for greater vigilance, because the head-down feeding posture (which causes a stickleback to lose sight of a predator) would not have been required. Similarly, laboratory studies revealed a tendency for sticklebacks to take prey indiscriminately when exposed to a predation risk, rather than select them according to their profitability (Ibrahim & Huntingford 1989).

Risk of predation might be expected to affect differentially the foraging behaviour of *S.solidus*-infected and uninfected sticklebacks, because the presence of the parasite appears to have a suppressive effect on the anti-predator response (Giles 1983b, 1987b). Following a simulated predatory attack, Giles (1983b, 1987b) noticed that parasitized sticklebacks had a quicker recovery and fed more voraciously (Giles 1987b) than their uninfected counterparts. In addition, infected sticklebacks were found to forage closer to a predator and in its presence compete for food more effectively than uninfected sticklebacks (Milinski 1985). Thus the presence of a predator switched the competitive advantage to the infected sticklebacks, probably as a result of their poor vigilance. There was complete suppression of the fright response in infected fish after 72h food deprivation, but not in the uninfected controls (Giles 1987b). As food deprivation results in higher mortality of infected compared with uninfected

sticklebacks (Walkey & Meakins 1970; Pascoe & Mattey 1977); perhaps the costs of predation when balanced against the need for food, are less for infected fish.

If harbouring *S.solidus* does influence stickleback foraging decisions, then field data should highlight dietary differences between infected and uninfected sticklebacks. Some evidence of such dietary segregation was obtained in a study by Jakobsen, Johnsen and Zarrson (1988). They suggested that the preference for chironomids over cladocera shown by infected females in the summer, was the result of a foraging strategy that would minimise energy expenditure. This was based on the assumption that pelagic prey would be difficult for slow infected fish to capture. However, such a dietary switch from chironomids to cladocerans could be interpreted as a response to a predation hazard, because cladocera though less profitable are the more easily handled of the two prey types (Ibrahim & Huntingford 1988). It may be that the uninfected fish in this population had modified their foraging strategy in response to predators unlike infected females, which displayed risky foraging behaviour.

4.1.2 GROWTH AND CONDITION OF *GASTEROSTEUS ACULEATUS*

Assessing growth and body condition

As seen in Chapter 2, the growth of fishes can be expressed as the specific rate of change in weight or length. Changes in length provide a measure of axial growth, whilst weight changes are indicative of growth in bulk. Despite the two parameters being highly correlated, weight changes can occur without corresponding changes in length, and *vice versa*. The equation:

$$W = aL^b$$

typically describes the relationship between length and weight, where W is the weight, L is the length and a and b are constants. The linear form of the equation is:

$$\log W = \log a + b \log L$$

where b is the slope of the line and log a is the intercept on the y-axis. The change in fish weight with length is thought to be cubic so that a slope of 3 illustrates isometric fish growth (a

proportional change in weight with length) and any significant deviation from 3 suggests that allometric growth is occurring. Where the deviation from 3 is significant, then a value for the slope of greater than 3, represents fish that are becoming increasingly heavier for their length. A value of less than 3 shows that the fish in the sample are becoming increasingly light for their length (see Wootton 1990).

Thus the length-weight relationship gives a convenient estimate of the 'fatness' of a fish of a certain length and so provides an index of condition. In addition, the condition factor can serve as a predictor of stickleback body lipid, protein and glycogen (Chellappa 1988). If the slope of a linear relationship does not deviate from 3, then it can be substituted in the first equation:

$$W = aL^3$$

and rearranged to give:

$$a = W/L^3$$

The constant a is thus a measure of condition. To give workable numbers the condition factor is usually derived thus:

$$CF = W/L^3 \times 10^6$$

where W is the weight (g) and L the length (mm).

Problems arise with the application of this measure, because changes in the condition factor could result from changes in the weight of somatic tissue, gonadal tissue, liver tissue or all three. The somatic condition factor alleviates this difficulty in interpretation, because it is an index of condition based on the relationship between the carcass weight and length:

$$SCF = CW/L^3 \times 100$$

It is therefore an index that is independent from changes in size of other body components.

A final technique for comparing linear relationships of length and weight, both within and between fish populations, is covariance analysis. It allows a statistical comparison of the slopes and elevations of two or more length-weight regression lines. A difference in elevation would result from two fish groups growing at the same rate, but with one attaining a greater weight for length than the other. Slope differences would indicate that one fish group is experiencing a faster rate of change in weight for its length, than the other.

In addition to changes in absolute body size, growth of individual organs can also be investigated. This usually involves an examination of the percentage of the body weight that is accounted for by the organ. The hepatosomatic index describes the relative weight of the liver and also can be an indicator of levels of liver glycogen (Chellappa 1988). Similarly, the percentage of body weight taken up by the gonads is termed the gonadosomatic index and at least for male sticklebacks can predict well the lipid, protein and glycogen content of the gonads (Chellappa 1988). Two studies have examined the annual fluctuations in somatic, hepatic and gonadal growth. Wootton, Evans and Mills (1978) undertook a comparative study on female sticklebacks from two different Welsh habitats. Chellappa (1988) and Chellappa, Huntingford, Strang and Thomson (1989) carried out a similar survey of growth on a Scottish population of male sticklebacks. Other authors have examined changes in gonadosomatic index (Meakins 1974; Borg 1982) and some have considered a possible effect of *S.solidus* on these growth parameters (Arme & Owen 1967; Pennycuick 1971d; Meakins 1974).

Patterns of change in body condition

Seasonal changes in the condition factor of female sticklebacks were shown for two Welsh populations (Wootton *et al.* 1978). In both cases, there was drop in autumn, followed by a sharp rise in spring and with a peak in May (Wootton *et al.* 1978). Chellappa (1988) observed an initial increase in the condition factor of male sticklebacks in late summer and early autumn, followed by a drop in late autumn and winter. An earlier improvement in body condition was found in these males compared with the above females studied by Wootton *et al.* (1978), but again the peak of condition was in spring.

Pennycuick (1971d) could find no differences in condition factor between sexes or age

classes of stickleback, but seasonal patterns were evident. The main trends were, a period of poor stickleback condition in winter and the development of good condition in the spring. In addition, regression analysis revealed that the parasite index (referring to *S.solidus*) was a significant predictor of the condition factor in infected fish, suggesting that the presence of the parasite had a deleterious effect on condition.

The only discrepancy between the annual changes in somatic condition factor and the condition factor in the studies of Wootton *et al.* (1978) and Chellappa (1988), was that the spring increase in the somatic condition factor was less pronounced. It is probable that the incorporation of gonadal and hepatic growth in the condition factor is responsible for the difference.

Patterns of change in the hepatosomatic index

Relative liver weights in both male and female sticklebacks were found to be stable throughout autumn and winter, but increased sharply in spring and peaked in April-May (Wootton *et al.* 1978; Chellappa 1988). The end of the breeding season coincided with steady declines in the hepatosomatic indices of both sexes. Arme and Owen (1967) detected a strong inverse relationship between the hepatosomatic index and the parasite index, in most seasons and attributed this to a parasite-induced reduction in absolute liver weight.

Patterns of change in the gonadosomatic index

Alterations in ovarian size relative to body size are very consistent between studies (Wootton *et al.* 1978; Borg & van Veen 1982). Minimal changes in the gonadosomatic index were noted throughout autumn, winter and most of spring and the ovary contributed only a small part of the total body weight (approximately 2-6%). Just before the onset of the breeding season however, the relative weight of ovarian tissue rose dramatically and peaked at approximately 20% in May-June. Thereafter, the gonadosomatic index declined, marking the end of the breeding season. Meakins (1974) found that during the breeding season, the mean gonadosomatic index of *S.solidus*-infected females was depressed to around half of that found in uninfected females and he postulated that it was the result of an energy deficit in infected sticklebacks.

The gonadosomatic index was found to be more variable between surveys of male

sticklebacks. Chellappa *et al.* (1989) described changes in the gonadosomatic index of males that were akin to those observed in females i.e. they were consistent for most of the year until the breeding season brought about an increase. Contrastingly, Borg (1982) observed high relative gonad weights in autumn that decreased until the end of the breeding season.

4.1.3 CONTROL OF REPRODUCTION IN *GASTEROSTEUS ACULEATUS*

The effects of photoperiod and temperature

Female sticklebacks undergo a single phase of sexual development, which results in the production of mature ova via oogonia (germ cells) and oocytes. A histological investigation of oogenesis, revealed heavy vacuolisation of oocytes between November and March, commencement of yolk deposition in April, which increased during the breeding season and a decline in ovarian development thereafter (Borg & van Veen 1982).

Sexual maturation in male sticklebacks occurs in two phases. The first phase is spermatogenesis, the process by which functional spermatozoa are produced via spermatogonia (germ cells), spermatocytes and spermatids. During the second phase the interstitial or Leydig cells of the testes become increasingly active and the androgens that they secrete stimulate the characteristic hypertrophy of the nephronic tubule cells of the kidney (Mourier 1970, 1976), the development of nuptial colouration and the display of reproductive behaviour.

Owing to their temperate distribution, sticklebacks experience large seasonal changes in photoperiod and water temperature. Such variability seems to be the key to the initiation and timing of sexual maturation. These abiotic factors activate the release of gonadotrophic hormones from the pituitary gland which are responsible for initiating the development of male and female gonadal tissue (females: Baggerman 1957, 1972; Borg & van Veen 1982: males: Borg 1981; Borg 1982; De Ruiter & Mein 1982; Borg, Paulson & Peute 1986; Borg, Peute, Reschke & van den Hurk 1987; Borg, Peute & Paulson 1988) culminating in a season of breeding sometime between March and early August. Despite all sticklebacks within a population being subject to the same light and temperature regimes, not all reach maturity and reproduce and therefore other factors must contribute.

The importance of food ration and energy reserves

Experimental studies have revealed that increased food rations promote greater

proportions of mature females and result in a higher body weight at maturity (Wootton 1973a). Although body size was found to be the major determinant of fecundity at individual spawnings (Wootton 1973b), total egg production and the number of spawnings were directly related to the level of food (Wootton 1977). When food was limited, egg production was found to induce somatic weight loss (Wootton & Evans 1976) and following food deprivation, females were able to spawn once at the most (Wootton 1977). This is hardly surprising when it is considered that for spawning females to maintain their body weight, it is estimated that they would have to consume a ration 5 times that of a non-spawning female (over 40 day period, Wootton 1977). Furthermore, a survey of females from two populations found that the breeding season was a time of depletion of lipid and glycogen from the liver, but not the ovaries (Wootton *et al.* 1978).

Manipulation of food rations also has consequences for reproduction in male sticklebacks. Low food rations (2% of body weight for 41 days) had an inhibitory effect on the establishment of territories and nest building (Stanley & Wootton 1986). In addition, Chellappa *et al.* (1989) found that the protein, lipid and glycogen reserves of males from a natural stickleback population were extensively depleted as a result of breeding activity. Therefore, breeding is a nutritionally demanding time for both male and female sticklebacks and their ability to reproduce is directly affected by exogenous food availability and their endogenous reserves.

4.1.4 PARASITES AND SEXUAL SELECTION

A recent model of sexual selection was proposed by Hamilton and Zuk (1982), whereby elaborate secondary sexual characteristics in males may have evolved through female choice for indicators of a males resistance to disease. The model was based on the assumptions that parasite resistance is heritable; that host fitness is deleteriously affected by the parasite in question and that there is a relationship between parasite load and expression of the secondary sexual characteristics. Inter-specifically the model predicts that species with a large diversity of parasite genera will have a greater level of sexual showiness and indeed there is evidence to support this in passerine birds (Hamilton & Zuk 1982) and in freshwater fish (Ward 1988) but such studies are fraught with phylogenetic and ecological confounding. Read (1988) examined

the model further and stressed the need for intra-specific experiments which would investigate how parasites can affect the cues used by females in mate selection and whether there is active choice of males depending on their parasite burden. He also realised the need for assessing the costs of parasites in natural host populations. Endler and Lyles (1989) suggested that future studies should concentrate on the heritability of secondary sexual characteristics, whether they are parasite indicative and the sensory mechanisms involved in perceiving these indicators by potential mates. Indeed, many of these points have now been investigated experimentally.

Parasites have been found to negatively affect the male sexual ornaments of three-spined sticklebacks (Milinski & Bakker 1990) and red jungle fowl, *Gallus gallus* (Zuk, Thornhill, Ligon 1990) and the male sexual behaviour of guppies, *Poecilia reticulata* (McMinn 1990), Rock Doves, *Columba livia* (Clayton 1990) and Sage Grouse (Boyce 1990). In addition, parasite-associated reductions in male reproductive success have been detected (Boyce 1990; Clayton 1990; Zuk *et al.* 1990). Although, active female mate choice for unparasitized males is evident (Clayton 1990; Milinski & Bakker 1990; Zuk *et al.* 1990), heritable resistance to parasites has been less frequently demonstrated (Borgia & Collis 1990; Hillgarth 1990).

It seems that under experimental conditions some of the assumptions of the Hamilton and Zuk (1982) hypothesis have been met, but there remains a need to examine the costs of parasites in natural host populations, their adaptive significance and their possible role in the process of sexual selection.

4.1.5 AIMS

This part of the field study aimed to investigate the interactions of *S.solidus* and *G.aculeatus* at Inverleith pond against a detailed knowledge of the life history of both species in this environment. Specifically, the diet composition and stomach fullness were monitored to determine whether natural feeding was affected by *S.solidus* infection. Also, the body condition and reproductive condition of uninfected and infected sticklebacks were compared to ascertain whether the parasite had any long term effect on the growth and attainment of breeding condition of sticklebacks and their mating potential. Finally, the findings were used to interpret how stickleback life history adaptations may be altered by the presence of *S.solidus*.

4.2 MATERIALS AND METHODS

4.2.1 DATA COLLECTION

Diet

The diet of infected sticklebacks was investigated in the same way as for uninfected csticklebacks (Chapter 2), using the occurrence and points methods. This provided measures of the percentage occurrence of food items in stickleback stomachs, the percentage occurrence of different levels of stomach fullness and the percentage contribution of various food items by bulk to the total food volume in stomachs. However, to compare the diet structure of uninfected and infected sticklebacks only the occurrence method and the stomach fullness were used.

Growth

The growth of uninfected and infected sticklebacks was compared on a number of levels. Firstly, the log-linear relationships of total weight upon length and carcass weight upon length were compared, whereby total weight in both cases means the weight of fish tissue (i.e. excluding the weight of *S.solidus*). Having, assessed the absolute relationship between length and weight for both uninfected and infected sticklebacks, two estimates of the condition of fish were calculated, which would also serve as indicators of energy reserves:

$$\text{Condition factor} = \frac{\text{weight of fish tissue}}{\text{length}^a} \times 10^6$$

$$\text{Somatic condition factor} = \frac{\text{weight of carcass}}{\text{length}^a} \times 10^6$$

where 'a' is dependent on the observed relationship between weight and length. It should be noted that the weight of *S.solidus* is also excluded from these calculations.

Another index of growth and predictor of energy reserves used was the hepatosomatic index:

$$\text{Hepatosomatic index} = \frac{\text{weight of liver}}{\text{weight of fish tissue}} \times 100$$

which is simply the percentage of fish weight that is accounted for by the liver. The parasite index was calculated as in Chapter 3:

$$\text{Parasite index} = \frac{\text{weight of } \textit{S.solidus}}{\text{weight of stickleback}} \times 100$$

and could be meaningfully compared with all of the above indices, because the denominator excluded the weight of *S.solidus*.

Breeding

Several aspects of reproductive condition were examined in uninfected and infected sticklebacks within the breeding season. In order to encompass both late and early breeders, the four survey samples from April 1989 - June 1989 were included. Fish were classified as mature or immature according to the criteria described in Chapter 2. The relative weights of the ovaries and testes to the body weight of fully mature fish were calculated:

$$\text{Gonadosomatic index} = \frac{\text{weight of gonads}}{\text{weight of fish tissue}} \times 100$$

as was the relative weight of the kidneys to body weight of mature males:

$$\text{Renosomatic index} = \frac{\text{weight of kidneys}}{\text{weight of fish tissue}} \times 100$$

Kidneys that had been removed from the bodies of mature males and preserved in 10% formalin were dehydrated through an ethanol series, halved and embedded in paraffin wax for sectioning. Thus, sections (6μm) were consistently taken from the central region of kidney trunk, stained in haematoxylin and eosin, cleared in Histoclear and mounted in Histomount (National Diagnostics).

A good quality slide was selected and a graticule with random dots was placed in the microscope eyepiece and used to select five tubule sections per kidney. The epithelial cell height was taken as the distance from the outside edge of the tubule to the lumen and was measured at x40 magnification using an ocular micrometer. An average of the kidney tubule cell heights from five observations was derived for each male.

As photographs of the ventral surface of male sticklebacks seemed to give the best representation of the extent and intensity of nuptial colouration, they were used to derive a numerical index of redness. Four type specimens with ranks from 1 to 4, were selected to depict the range of colour extent and intensity in mature male sticklebacks from Inverleith pond. Copies of all available mature male photographs, along with type specimens were then given to eight students (undergraduates and postgraduates unrelated to the project) to be classified from 1 to 4 for both extent and intensity of red colouration.

4.2.2 DATA ANALYSES

Diet

As the diet and stomach fullness of uninfected sticklebacks frequently varied across seasons (Chapter 2), all dietary comparisons between uninfected and infected fish were made within seasons:

December - February	= winter
March - May	= spring
June - August	= summer
September - November	= autumn

The frequency of occurrence of stomach fullness categories were compared between uninfected and infected sticklebacks using a Chi-Square test of independence. To investigate the diet composition of infected sticklebacks relative to that of uninfected sticklebacks, the

frequency of occurrence of these dietary items were compared between uninfected and infected sticklebacks using Chi-Square tests of independence.

Growth

To determine whether there were basic differences in the log-linear length/weight (of fish tissue) relationships of uninfected and infected sticklebacks, analysis of covariance was used. In order to obtain good estimates of these relationships, samples had to be combined into seasons as defined earlier. The analyses of covariance were then used to compare the slopes and the elevations of the regression lines for uninfected and infected fish from autumn 1988 to summer 1989. Also, the 95% confidence levels for each regression coefficient was calculated to test if the slopes of any of the regressions differed significantly from 3.

Each of the condition factors and the hepatosomatic index appeared normally distributed, allowing the use of a parametric one-way ANOVA to compare between months of the survey. The student's t-test was then used to ascertain how these indices may vary between uninfected and infected sticklebacks within samples. However, the parasite indices were not normally distributed and so the relationship between the parasite index, the condition factors and the hepatosomatic index was explored, using Spearman rank correlations.

Breeding

To establish whether infection had any influence on the maturation of fish, the occurrence of maturity/immaturity in uninfected and infected fish was compared within samples, using Fisher's exact probability test. This was preferred over the Chi-Square test of independence, because small cell frequencies were common.

The growth of fish as described by the condition factor, the somatic condition factor and the hepatosomatic index, were checked for monthly differences, to enable pooling of months that did not differ statistically and thereby increase the size of samples. Sticklebacks were then classed according to a combination of their maturity and infection status and the indices compared using parametric two-way ANOVAs.

The kappa statistic K, the coefficient of agreement for nominally scaled data, was calculated for each of the colour extent and intensity rankings (Siegel & Castellan 1988). K directly indicates the level of agreement between observers and can range from 0 to 1, where 0

represents no agreement between the observers (other than that due to chance) and 1 represents complete agreement. To test whether the derived values of K differed significantly from 0 (i.e. significantly more agreement than that due to chance), z was estimated from the ratio of K to the variance of K. An average of the ranks was assigned to each fish for colour extent and intensity, providing two colour indices per fish.

Neither the gonadosomatic, renal and colour indices nor the average kidney tubule heights were normally distributed invalidating the use of parametric tests. As the samples of mature fish were quite small (particularly of infected fish), it was again decided to pool monthly samples where possible. The Kruskal Wallis, non-parametric one-way ANOVA was used to check for monthly differences in each of the variables and only those months that did not differ statistically were pooled for further analysis. Comparisons of the indices and colour between uninfected and infected fish were then made using the Wilcoxon-Mann-Whitney test. A similar pooling procedure was undertaken for the parasite indices before they were compared between infected breeding and non-breeding sticklebacks using the Wilcoxon-Mann-Whitney test. Although the condition factor, the somatic condition factor and the hepatosomatic index were normally distributed, in order to investigate how they related to the other indices and colour scores (which were not normally distributed) Spearman rank correlations were carried out.

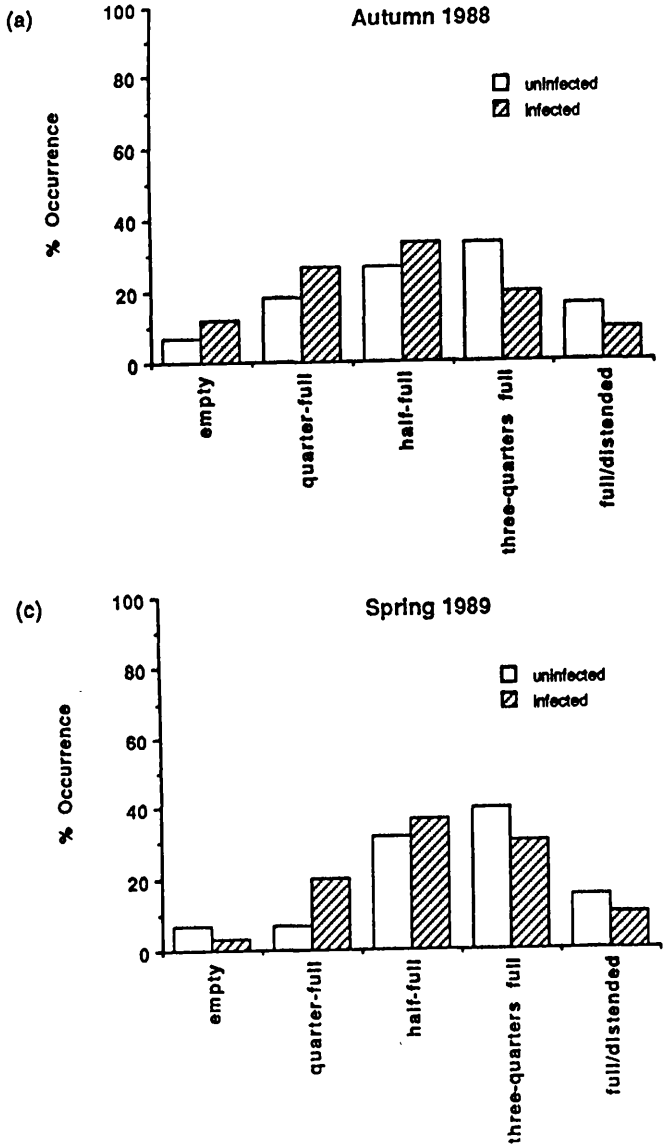
4.3 RESULTS

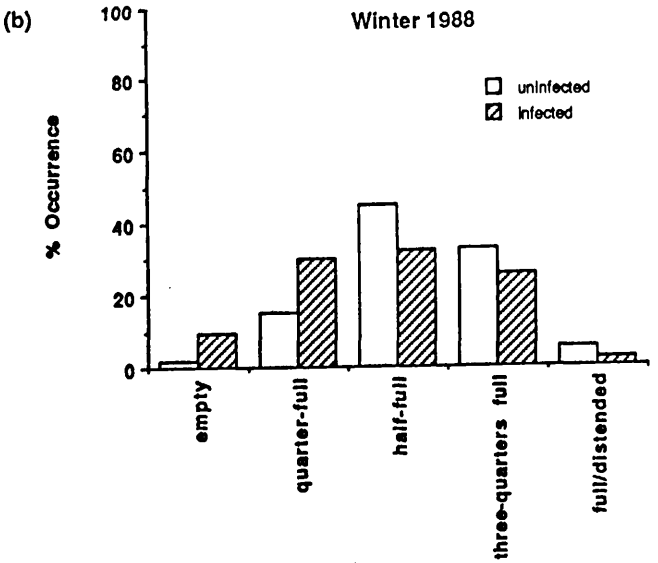
4.3.1 COMPARISON OF THE DIET OF UNINFECTED AND INFECTED STICKLEBACKS

Stomach fullness

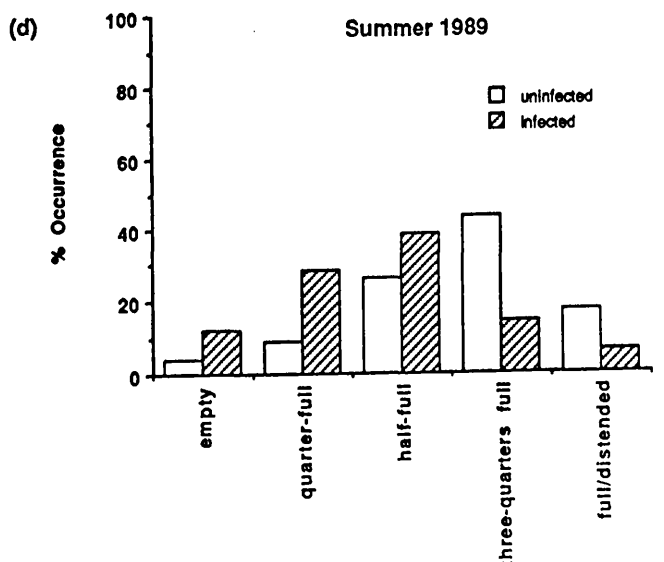
The percentage occurrence of different levels of fullness in uninfected and infected sticklebacks is given in Figure 4.1(a-d). The distribution of stomachs over the various fullness categories is essentially the same for uninfected and infected fish in autumn 1988 and spring 1989 (Chi-Square, autumn: X^2=7.912, d.f=4, P>0.05, N.S.; spring: X^2=5.613, d.f=4, P>0.05, N.S), but not in both winter 1988 (Chi-Square, X^2=12.827, d.f=4, P<0.05) and summer 1989 (Chi-Square, X^2=26.130, d.f=4, P<0.001). In these seasons there was a tendency for infected sticklebacks to be found with less full stomachs than their uninfected counterparts.

Figure 4.1: Occurrence, by season, of stomach fullness categories in 0+ sticklebacks, uninfected and infected by *S.solidus*.


(a)
Autumn 1988
% Occurrence
100
80
60
40
20
0
uninfected
infected
empty
quarter-full
half-full
three-quarters full
full/distended


(b)
Winter 1988
% Occurrence
100
80
60
40
20
0
uninfected
infected
empty
quarter-full
half-full
three-quarters full
full/distended

(c)
Spring 1989
% Occurrence
100
80
60
40
20
0
uninfected
infected
empty
quarter-full
half-full
three-quarters full
full/distended


(d)
Summer 1989
% Occurrence
100
80
60
40
20
0
uninfected
infected
empty
quarter-full
half-full
three-quarters full
full/distended

The occurrence of dietary items

The percentages of uninfected and infected fish stomachs containing the main dietary items are given in Table 4.1 along with the probabilities from the Chi-Square analyses. In autumn 1988, a significantly smaller proportion of infected fish were eating rotifers, invertebrate eggs, nematodes and algae, but a higher proportion consumed arcellidae. In winter 1988, even greater divergence in the diet of uninfected and infected sticklebacks was observed. Significantly fewer infected compared with uninfected fish were consuming cladocerans, rotifers, ostracods and nematodes but significantly more were eating higher plants. During spring 1989, the diet structure of uninfected and infected sticklebacks was very similar, but again in summer 1989 dietary discrepancies were detected. Chironomids were significantly less apparent whilst rotifers and arcellidae were significantly more apparent in the stomachs of infected fish compared with uninfected fish.

4.3.2 COMPARISON OF THE GROWTH AND CONDITION OF UNINFECTED AND INFECTED STICKLEBACKS

The length/weight relationship

The regressions of log-weight on log-length were highly significant in each season and for both uninfected and infected sticklebacks (Table 4.2). In addition, the R^2 values indicate that the variation in the log-weight of fish tissue was largely explained by the variation in log-length. Therefore, any disparity between the log-linear relationships of weight on length for uninfected and infected fish should be readily detected.

The regression lines of uninfected and infected fish are given for each of the four seasons in Figure 4.2. A difference in the elevation of the lines was evident in autumn 1988 (ANCOVA, $F_{elevation}=7.60$, d.f.=1,180, $P<0.01$), but the slopes of the lines were similar (ANCOVA, $F_{slopes}=2.63$, d.f.=1,179, $P>0.05$, N.S.). In the winter sample, the regression lines for uninfected and infected sticklebacks were identical (ANCOVA, $F_{elevation}=0.56$, d.f.=1,212, $P>0.05$, N.S.; $F_{slopes}=0.00$, d.f=1,211, $P>0.05$, N.S.) and during spring there was no significant divergence of relationships (ANCOVA, $F_{elevation}=0.46$, d.f.=1,128, $P>0.05$, N.S.; $F_{slopes}=2.13$, d.f.=1,127, $P>0.05$, N.S.). However, in the summer of 1989 infected sticklebacks had a significantly lower weight for length than uninfected sticklebacks (ANCOVA,

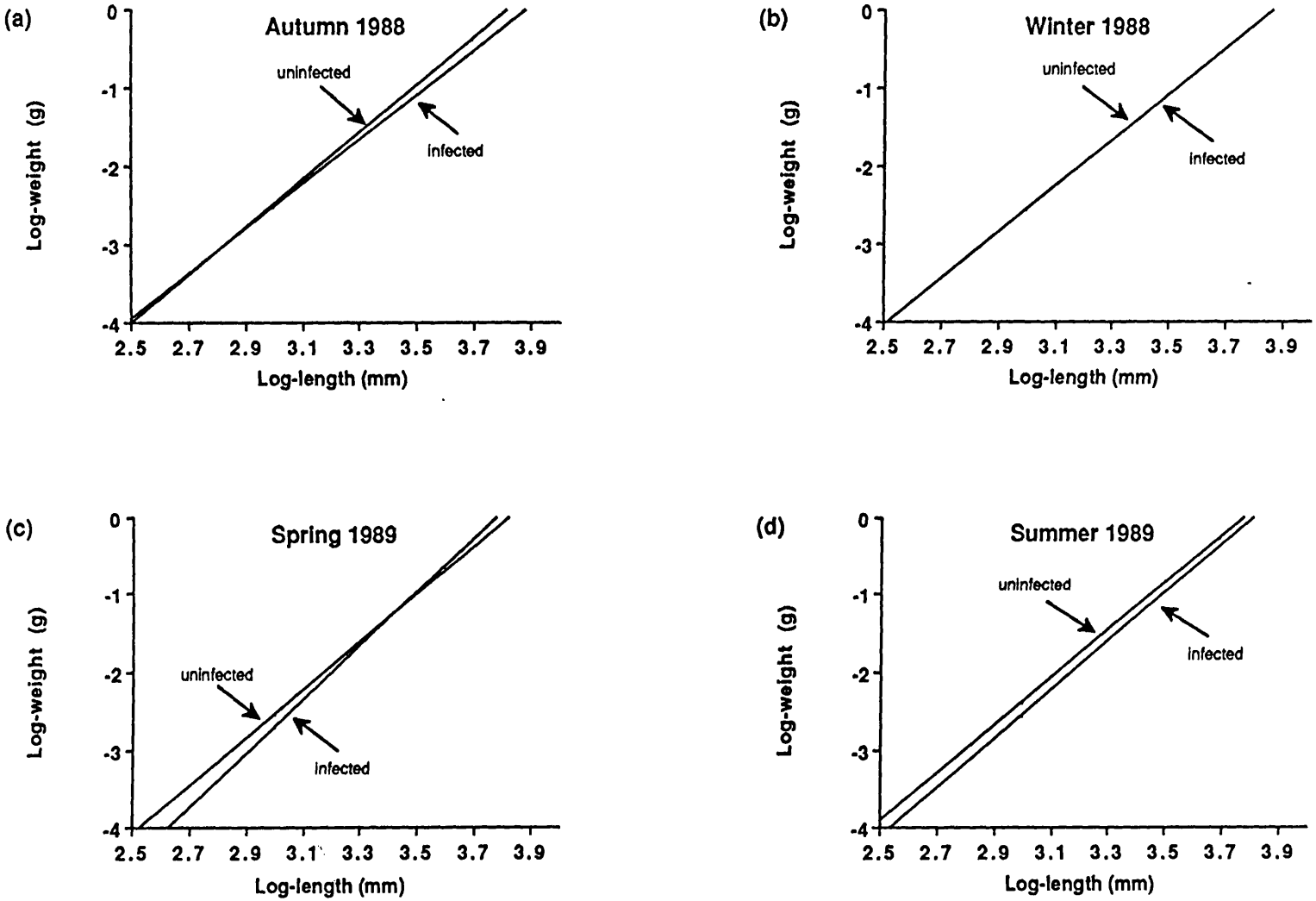
Table 4.1: The percentage occurrence of dietary items in the stomachs of sticklebacks uninfected and infected by *S.solidus*.

Season	Food item									
	cyclopoidea	cladocera	rotifera	chironomidae	ostracoda	nematoda	arcellidae	invertebrate eggs	higher plants	algae
Autumn 1988										
uninfected	25.0	45.2	59.5	21.4	23.8	44.0	20.2	31.1	23.8	70.2
infected	29.9	42.9	31.2	32.5	27.3	27.3	36.4	11.7	32.5	39.0
	N.S.	N.S.	P<0.001	N.S.	N.S.	P<0.05	P<0.05	P<0.001	N.S.	P<0.001
Winter 1988										
uninfected	20.0	54.4	62.7	29.0	66.9	76.3	12.4	7.7	24.3	78.1
infected	23.1	35.9	33.3	33.3	38.5	51.3	12.8	6.9	48.7	56.4
	N.S.	P<0.05	P<0.001	N.S.	P<0.01	P<0.01	N.S.	N.S.	P<0.01	P<0.01
Spring 1989										
uninfected	58.5	58.5	19.4	73.4	63.8	33.0	28.7	7.4	24.5	38.3
infected	44.8	48.3	24.1	55.2	69.0	27.6	10.3	5.1	34.5	41.4
	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Summer 1989										
uninfected	41.0	61.5	5.1	91.5	54.7	2.6	31.6	5.1	11.1	8.5
infected	25.6	60.5	20.9	69.8	46.5	4.7	30.2	20.9	18.6	18.6
	N.S.	N.S.	P<0.001	P<0.001	N.S.	N.S.	N.S.	P<0.001	N.S.	N.S.

Table 4.2: Log-linear regression analysis of weight on length for sticklebacks uninfected and infected by *S.solidus*.

Season		Regression equation	ANOVA summary			R^2(%)
autumn 1988						
	uninfected	log(weight) = 3.07 X log(length) - 11.7	F=1738.12,	d.f.=1, 90,	P<0.001	95.0
	infected	log(weight) = 2.86 X log(length) - 11.1	F= 845.64,	d.f.=1, 89,	P<0.001	90.4
winter 1988						
	uninfected	log(weight) = 2.98 X log(length) - 11.5	F=2340.80,	d.f.=1,170,	P<0.001	93.2
	infected	log(weight) = 2.98 X log(length) - 11.5	F= 144.05,	d.f.=1, 41,	P<0.001	77.3
spring 1989						
	uninfected	log(weight) = 3.50 X log(length) - 13.2	F=1066.24,	d.f.=1, 99,	P<0.001	91.4
	infected	log(weight) = 3.09 X log(length) - 11.8	F= 147.59,	d.f.=1, 28,	P<0.001	83.5
summer 1989						
	uninfected	log(weight) = 3.15 X log(length) - 12.0	F= 478.03,	d.f.=1, 47,	P<0.001	90.9
	infected	log(weight) = 3.07 X log(length) - 11.6	F= 798.44,	d.f.=1,120,	P<0.001	86.8

Figure 4.2: Log-linear relationship, by season, between the weight and length of 0+ sticklebacks, uninfected and infected by *S.solidus*.


(a)
Autumn 1988
uninfected
infected
Log-weight (g)
0
-1
-2
-3
-4
2.5 2.7 2.9 3.1 3.3 3.5 3.7 3.9
Log-length (mm)
(b)
Winter 1988
uninfected
infected
Log-weight (g)
Log-length (mm)
(c)
Spring 1989
uninfected
infected
Log-weight (g)
Log-length (mm)
(d)
Summer 1989
uninfected
infected
Log-weight (g)
Log-length (mm)

$F_{elevation}$=22.28, d.f.=1,168, P<0.001), but there was no difference in the rate of change in weight with length (ANCOVA, F_{slopes}=1.62, d.f.=1,167, P>0.05, N.S.).

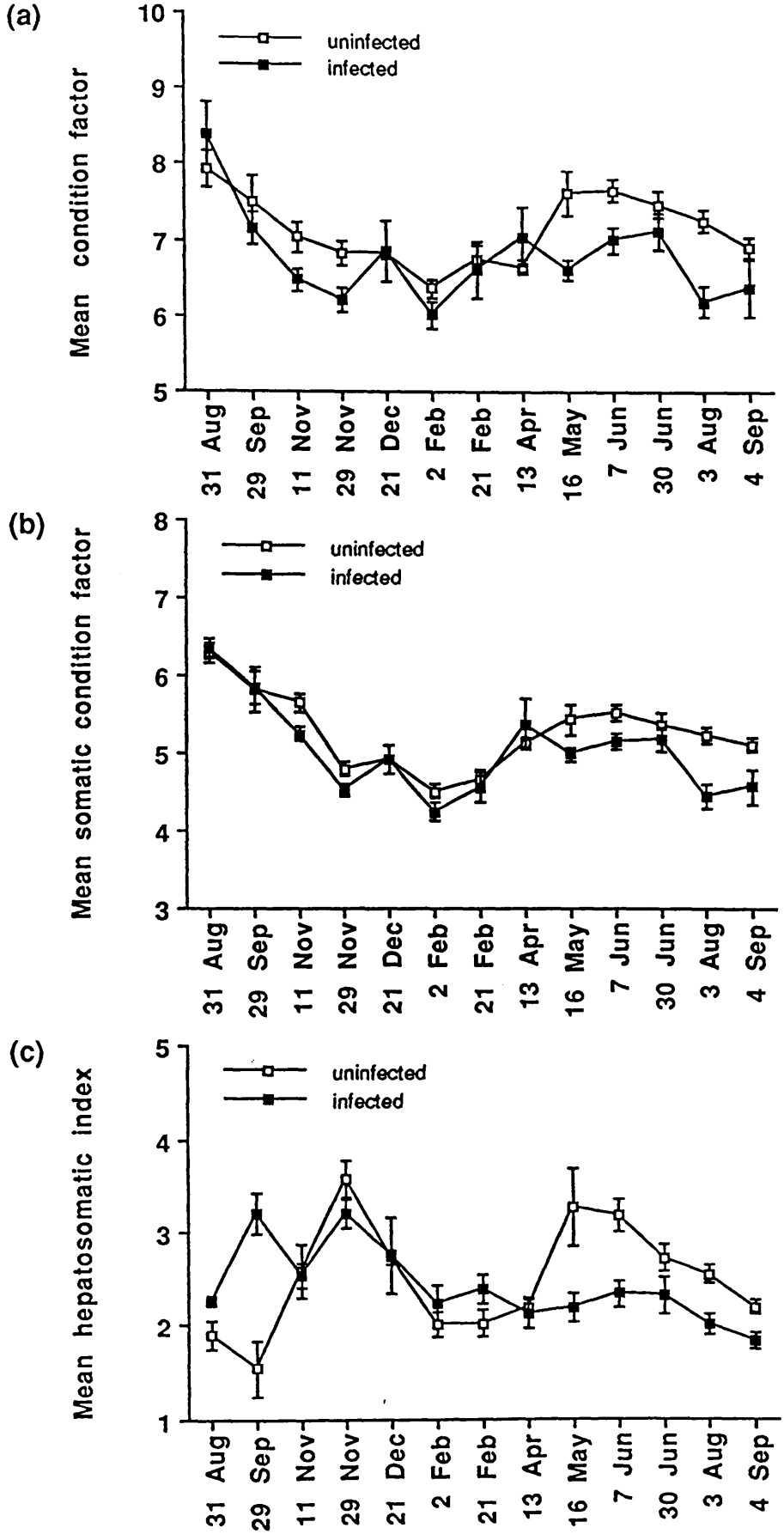
The monthly pattern of growth and condition

As none of the slopes of the log-linear regressions of weight on length were significantly different from 3 (P>0.05) or significantly affected by infection with *S.solidus*, a combined regression line was derived for all young-of-the-year sticklebacks. Again the slope of this line (3.12) did not differ statistically from 3 (P>0.05) and so it appears that growth in sticklebacks from Inverleith pond is largely isometric. Furthermore, the value 3.12 was used in the calculation of the condition factor and somatic condition factor, enabling a more accurate and population-specific assessment of the condition of Inverleith pond sticklebacks (i.e. a=3.12 in section 4.2.1).

Plots of the mean condition factor and mean somatic condition factor by month and for uninfected and infected fish are given in Figure 4.3 (a-b). Both these indices of body condition were found to vary with the time of sampling (ANOVA, CF: F=8.42, d.f.=12,772, P<0.001; SCF: F=20.83, d.f.=12,759, P<0.001). The observed decline in body condition throughout autumn was accelerated in sticklebacks harbouring *S.solidus* and by the beginning of November they were in significantly poorer condition than their uninfected counterparts (t-test, CF: t=2.39, d.f.=50, P<0.05; SCF: t=2.71, d.f.=52, P<0.01). This trend continued for the remainder of November (t-test, CF: t=2.80, d.f.=58, P<0.01; SCF: t=2.17, d.f.=55, P<0.05).

Over winter, the body condition of all sticklebacks was low irrespective of infection status and did not begin to increase until spring. The rate of improvement was less in infected sticklebacks, such that in May and early June they had a significantly lower mean condition factor (t-test, 16 May 1989: t=3.20, d.f.=60, P<0.01; 7 June 1989: t=3.06, d.f.=53, P<0.01) and somatic condition factor (t-test, 16 May 1989: t=2.05, d.f.=59, P<0.05; 7 June 1989: t=2.49, d.f.=52, P<0.05) than those sticklebacks that were free from infection. In late June, the condition of uninfected sticklebacks had begun to decline, whilst that of infected sticklebacks was still increasing and no differences between the condition of uninfected and infected sticklebacks were evident. Finally in late summer, both condition factors approached the levels found in winter, but were significantly lower for infected sticklebacks (t-test, CF: t=4.40,

Figure 4.3: Monthly changes in the mean (± S.E.) a) condition factor b) somatic condition factor and c) hepatosomatic index of 0+ sticklebacks, uninfected and infected by *S.solidus* from 31 August 1988 to 4 September 1989.


(a)
Mean condition factor
10
9
8
7
6
5
uninfected
infected
31 Aug
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
13 Apr
16 May
7 Jun
30 Jun
3 Aug
4 Sep
(b)
Mean somatic condition factor
8
7
6
5
4
3
uninfected
infected
31 Aug
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
13 Apr
16 May
7 Jun
30 Jun
3 Aug
4 Sep
(c)
Mean hepatosomatic index
5
4
3
2
1
uninfected
infected
31 Aug
29 Sep
11 Nov
29 Nov
21 Dec
2 Feb
21 Feb
13 Apr
16 May
7 Jun
30 Jun
3 Aug
4 Sep

d.f.=32, P<0.001; SCF: t=4.16, d.f.=31, P<0.001).

The observed fluctuations in the relative liver weight (Figure 4.3(c)), like body condition, were influenced by the time of year (ANOVA, F=7.89, d.f.=12,768, P<0.001). A significantly higher mean hepatosomatic index was detected in infected compared with uninfected sticklebacks around the time when many new infections were being acquired (t-test, HSI: 31 August 1989, t=-2.05, d.f.=40, P<0.05; 29 September 1989, t=t=-4.45, d.f.=53, P<0.0001). By November and throughout winter, the mean hepatosomatic indices of each group of sticklebacks were indistinguishable. In addition, the relative liver weights of all fish declined during winter and did not increase markedly until May. However, there were much smaller increases in the hepatosomatic indices of infected fish and consequently they had a significantly smaller mean hepatosomatic index in May (t-test, t=2.42, d.f.=58, P<0.05) and early June (t-test, t=3.93, d.f.=68, P<0.001). Late in June there was an apparent reduction in the relative liver weights of uninfected sticklebacks such that they were similar to those of infected sticklebacks. Thereafter, a faster decline in the relative liver weights of infected fish, resulted in them having a significantly smaller mean hepatosomatic index than uninfected fish in August (t-test, t=3.68, d.f.=39, P<0.001) and September (t-test, t=2.94, d.f.=21, P<0.01) of 1989.

The relationship between the condition factor, the somatic condition factor, the hepatosomatic index and the parasite index

Late in autumn 1988 and in the summer/autumn of 1989, a significant negative correlation was detected between the condition factor and the parasite index (Table 4.3). A similar relationship was also observed between the somatic condition factor and the parasite index, but only in early November and late June. However, there appeared to be no particular relationship between the hepatosomatic index and the parasite index.

4.3.3 COMPARISON OF MATURITY AND CONDITION IN UNINFECTED AND INFECTED STICKLEBACKS

The occurrence of maturity

For each month of the breeding season, the numbers of uninfected and infected sticklebacks that were found to be either immature or mature are given in Table 4.4. In the

Table 4.3: The correlation coefficients (N) and probabilities for Spearman rank correlations of the condition factor, the somatic condition factor and the hepatosomatic index with the parasite index.

Month	condition factor v parasite index	somatic condition factor v parasite index	hepatsosomatic index v parasite index
31 Aug 1988	-1.000 (3) N.S.	-0.500 (3) N.S.	-0.500 (3) N.S.
29 Sep 1988	-0.058 (25) N.S.	-0.012 (25) N.S.	-0.184 (25) N.S.
11 Nov 1988	-0.509 (38) P<0.01	-0.425 (38) P<0.01	-0.235 (37) N.S.
29 Nov 1988	-0.411 (28) P<0.05	-0.346 (26) N.S.	-0.290 (28) N.S.
21 Dec 1988	-0.200 (5) N.S.	-0.400 (5) N.S.	-0.900 (5) N.S.
2 Feb 1989	-0.110 (17) N.S.	-0.049 (17) N.S.	-0.370 (17) N.S.
21 Feb 1989	0.087 (21) N.S.	-0.046 (21) N.S.	0.104 (21) N.S.
13 Apr 1989	0.074 (17) N.S.	0.029 (16) N.S.	-0.551 (17) P<0.05
16 May 1989	-0.082 (13) N.S.	0.260 (13) N.S.	0.170 (13) N.S.
7 Jun 1989	-0.639 (23) P<0.01	-0.357 (21) N.S.	0.150 (23) N.S.
30 Jun 1989	-0.717 (9) P<0.05	-0.733 (9) P<0.05	-0.400 (9) N.S.
3 Aug 1989	-0.103 (17) N.S.	-0.137 (17) N.S.	-0.225 (17) N.S.
4 Sep 1989	-0.783 (9) N.S.	-0.500 (9) N.S.	-0.167 (9) N.S.

early months of the breeding season, no significant differences were observed in the proportions of uninfected and infected sticklebacks that had attained sexual maturity. However, when the numbers of mature males and females peaked in the June 7th sample, sticklebacks harbouring *S.solidus* were significantly under-represented. There followed an overall decline in the numbers of mature fish, such that the relative proportions of uninfected and infected fish in this category did not differ significantly.

Table 4.4: The occurrence of sexual maturity in sticklebacks uninfected and infected by *S.solidus*.

Month		Males		Females	
		immature	mature	immature	mature
13 Apr 1989					
	uninfected	10	3	2	7
	infected	3	2	0	0
		N.S.		N.S.	
16 May 1989					
	uninfected	9	5	4	18
	infected	2	0	1	0
		N.S.		N.S.	
7 Jun 1989					
	uninfected	8	19	5	17
	infected	10	2	5	1
		P<0.01		P<0.05	
30 Jun 1989					
	uninfected	8	10	4	9
	infected	0	1	4	2
		N.S.		N.S.	

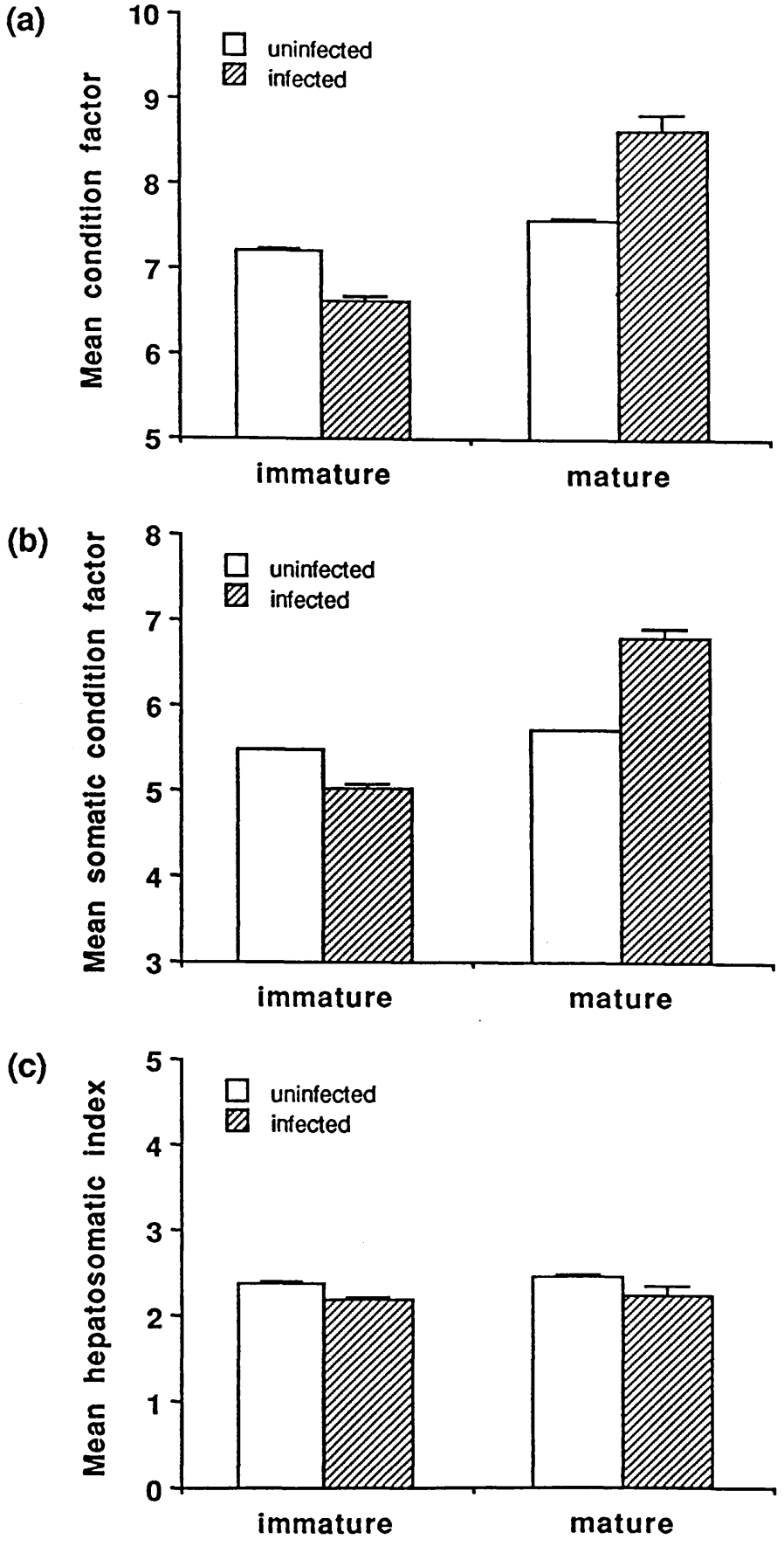
Body condition and sexual maturity

The condition factor, the somatic condition factor and the hepatosomatic index of male sticklebacks was not found to vary significantly across the months of the breeding season (ANOVA, condition factor: $F=1.28$, d.f.$=3,88$, $P>0.05$, N.S.; somatic condition factor: $F=0.48$,

d.f.=3,87, P>0.05, N.S.; hepatosomatic index: F=2.27, d.f.=3,87, P>0.05, N.S.). However, the level of maturity had a significant impact on both the condition factor (ANOVA, F=22.43, d.f.=1,88, P<0.001) and the somatic condition factor (ANOVA, F=32.34, d.f.=1,87 P<0.001). There was no significant overall effect of infection status on body condition (ANOVA, condition factor: F=0.85, d.f.=1,88, P>0.05 N.S.; somatic condition factor: F=3.22, d.f.=1,87, P>0.05, N.S.), but there was a significant interaction between maturity and infection status with respect to condition (ANOVA, condition factor: F=10.84, d.f.=1,88, P<0.01; somatic condition factor: F=18.64, d.f.=1,87, P<0.001). The trend was for infected immature males to have the lowest body condition, comparable with winter levels (Figure 4.4(a-b)). Uninfected immature and mature males had higher and similar levels of body condition, but infected mature males were in the best condition. Neither, maturity (ANOVA, F=0.23, d.f.=1,87, P>0.05, N.S.) nor infection status (ANOVA, F=1.67, d.f.=1,87, P>0.05, N.S.) had a significant influence on the hepatosomatic index and moreover, there was no interaction between these two categories (ANOVA, F=0.000, d.f.=1,87, P>0.05, N.S.) (Figure 4.4(c)). The mean hepatosomatic index was fairly consistent across the four categories (Figure 4.4(c)) (ANOVA, F=0.60, d.f.=3,87, P>0.05, N.S.)

As with males, the mean condition factor and somatic condition factor of females were similar in each of the months of the breeding season (ANOVA, condition factor: F=1.97, d.f.=3,75, P>0.05 N.S.; somatic condition factor: F=0.88, d.f.=3,74, P>0.05, N.S.). Contrastingly, the hepatosomatic index of females did vary within the breeding season (ANOVA, F=3.23, d.f.=3,75, P<0.05), being lower in April than in any other month. Unlike the situation observed in male sticklebacks, females were found to have similar levels of body condition irrespective of their level of maturity (ANOVA, condition factor: F=0.71, d.f.=1,75, P>0.05, N.S.; somatic condition factor: F=0.05, d.f.=1,74, P>0.05, N.S.) and infection status (ANOVA, condition factor: F=0.84, d.f.=1,75, P>0.05 N.S.; somatic condition factor: F=0.13 d.f.=1,74, P>0.05, N.S.) and in addition, there was no interaction effect (ANOVA, condition factor, F=0.01, d.f.=1,75, P>0.05, N.S.; somatic condition factor: F=0.08, d.f.=1,74, P.0.05, N.S.). However, the extent of maturity almost had a significant effect on the hepatosomatic index of females (ANOVA, F=3.89, d.f.=1,66, P=0.053, N.S.) and the infection status did have

Figure 4.4: Mean (± S.E.) a) condition factor b) somatic condition factor and c) hepatosomatic index of immature and mature, 0+ male sticklebacks, uninfected and infected by *S.solidus* from 9 April 1989 to 30 June 1989.


(a)
Mean condition factor
10
9
8
7
6
5
uninfected
infected
immature
mature
(b)
Mean somatic condition factor
8
7
6
5
4
3
uninfected
infected
immature
mature
(c)
Mean hepatosomatic index
5
4
3
2
1
0
uninfected
infected
immature
mature

a significant effect (ANOVA, $F=5.93$, d.f.$=1,66$, $P<0.05$). There was also a significant interaction between the level of maturity and the infection status (ANOVA, $F=5.09$, d.f.$=1,66$, $P<0.05$). Overall, uninfected mature females had by far the largest relative liver weights (Figure 4.5(a-c)).

Male and female sticklebacks also differed in respects other than body condition and the hepatosomatic index. The median parasite index of infected immature males was also found to be significantly higher than that of infected mature males (Wilcoxon-Mann-Whitney, $W=185.0$, $n=15$, $m=5$, $P<0.05$), but there was no significant difference (Wilcoxon-Mann-Whitney, $W=77.0$, $n=10$, $m=5$, $P>0.05$, N.S.) detected between the median parasite indices of mature and immature infected females (Figure 4.6(a-b)).

4.3.4 COMPARISON OF THE GONADS, KIDNEYS AND NUPTIAL COLOURATION OF UNINFECTED AND INFECTED MATURE MALE STICKLEBACKS

The kidneys and gonads

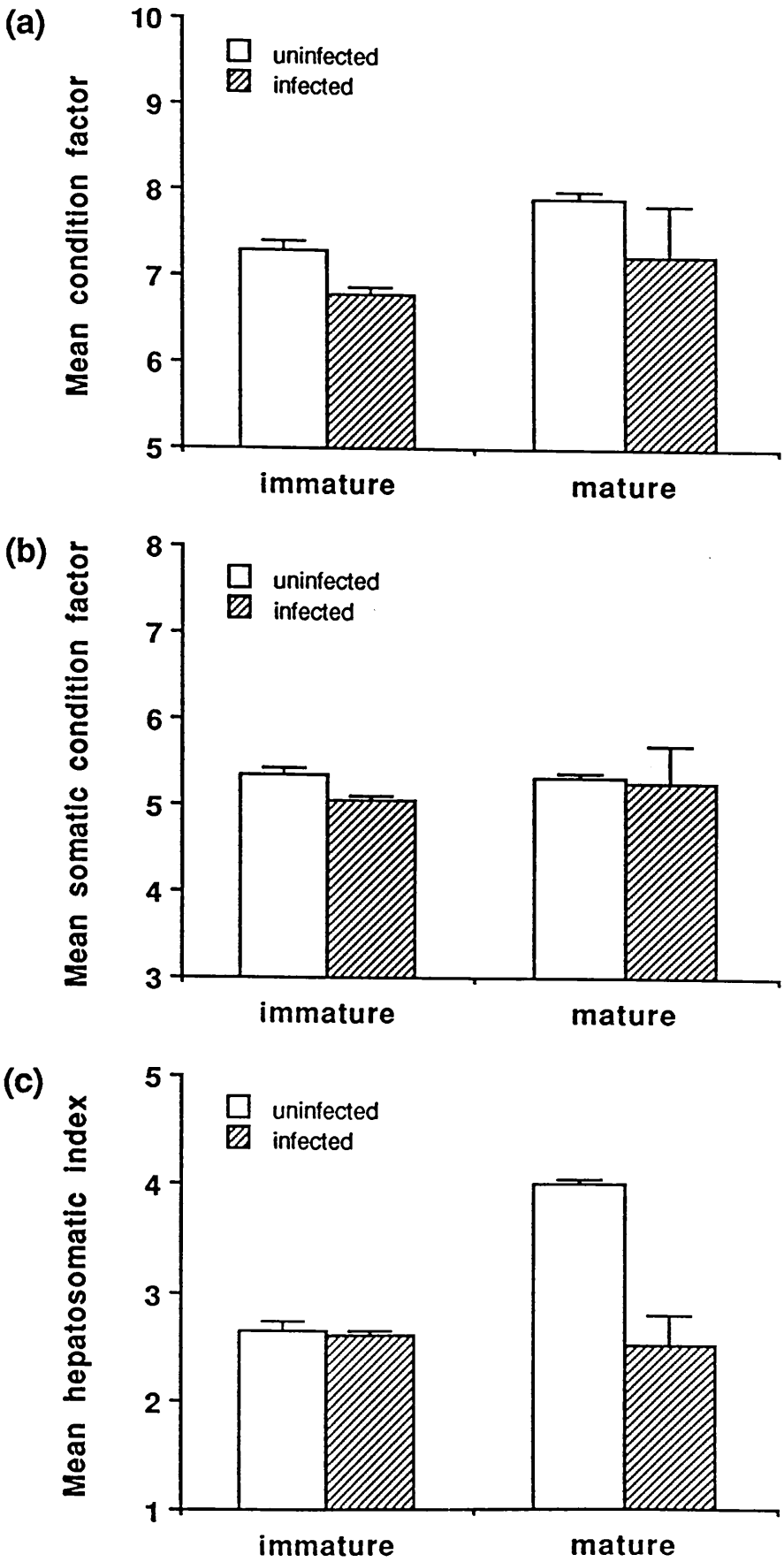
The median gonadosomatic indices of mature male sticklebacks were observed to be similar throughout the breeding season (Kruskal Wallis ANOVA, $H=2.78$, d.f.$=3$, $P>0.05$, N.S.), but the median renosomatic index and the median cell height of the kidney tubule epithelium did differ significantly across months (Kruskal Wallis ANOVA, renosomatic index: $H=13.06$, d.f.$=3$, $P<0.01$; cell height: $H=15.41$, d.f.$=3$, $P<0.01$). April-caught males had much lower relative kidney weights and smaller kidney epithelial cell heights.

Despite there being only five infected mature males in all the samples from the breeding season, the median gonadosomatic index of these males was significantly higher (Wilcoxon-Mann-Whitney, $W=777.0$, $n=38$, $m=5$, $P<0.05$) than that of the uninfected mature males (Figure 4.7(a)). Uninfected and infected mature males however, did not differ with respect to their renosomatic indices or kidney epithelial cell heights (Wilcoxon-Mann-Whitney, renosomatic index: $W=667$, $n=34$, $m=5$, $P>0.05$, N.S.; cell height: $W=623$, $n=33$, $m=5$, $P>0.05$, N.S.) (Figure 4.7(b-c)).

Nuptial colouration

The agreement between observers was significantly higher than that expected by chance for both the extent ($P<0.00001$) and intensity ($P<0.00001$) of nuptial colouration. However,

Figure 4.5: Mean (± S.E.) a) condition factor b) somatic condition factor and c) hepatosomatic index of immature and mature, 0+ female sticklebacks, uninfected and infected by *S.solidus* from 9 April 1989 to 30 June 1989.


(a)
Mean condition factor
10
9
8
7
6
5
uninfected
infected
immature
mature
(b)
Mean somatic condition factor
8
7
6
5
4
3
uninfected
infected
immature
mature
(c)
Mean hepatosomatic index
5
4
3
2
1
uninfected
infected
immature
mature

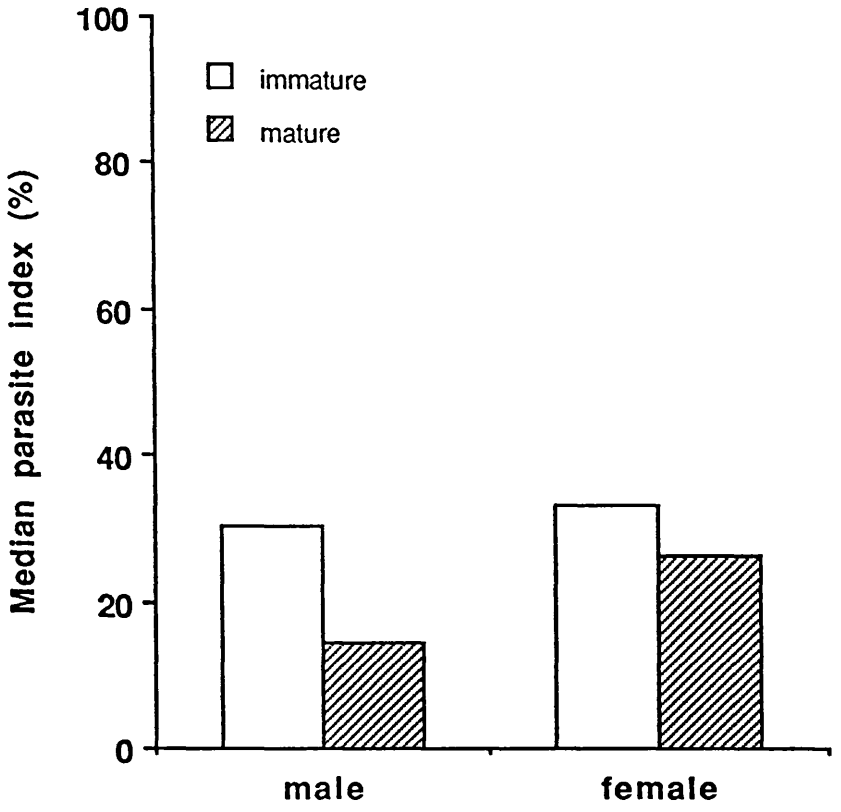
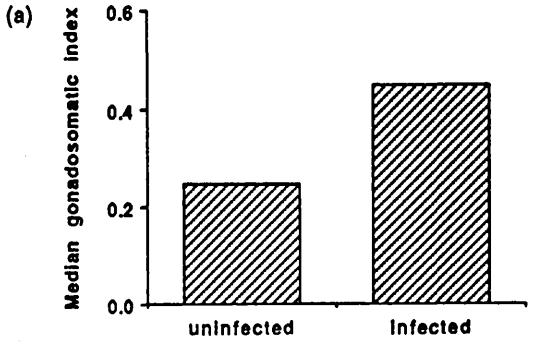
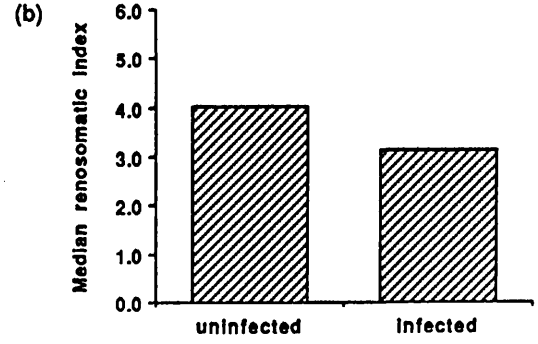
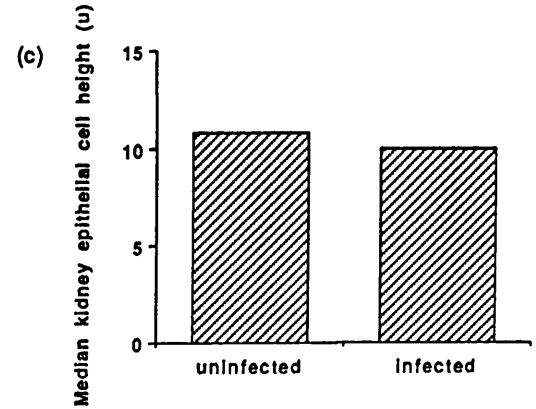


Figure 4.6: Median parasite index of *S.solidus*-infected immature and mature, male and female, 0+ sticklebacks from 9 April 1989 to 30 June 1989.

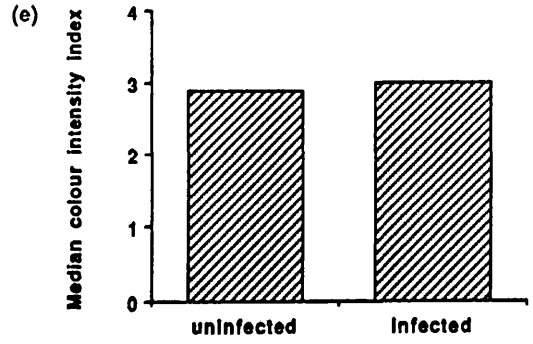
Figure 4.7: Median a) gonadosomatic index b) renosomatic index c) kidney epithelial cell height d) colour extent index and e) colour intensity index of uninfected and infected, mature male 0+ sticklebacks from 9 April 1988 to 30 June 1989.


(a)
Median gonadosomatic index
0.6
0.4
0.2
0.0
uninfected
infected


(b)
Median renosomatic index
6.0
5.0
4.0
3.0
2.0
1.0
0.0
uninfected
infected


(c)
Median kidney epithelial cell height (u)
15
10
5
0
uninfected
infected


(d)
Median colour extent index
4
3
2
1
0
uninfected
infected


(e)
Median colour intensity index
4
3
2
1
0
uninfected
infected

the degree of agreement as indicated by the kappa statistic differed for each of the two colour parameters. There was a moderate level of agreement with respect to colour extent (K=0.411) compared with a much lower level of agreement for colour intensity (K=0.230). Indeed, the observers did state that they found colour intensity more difficult to rank.

Nuptial colouration did not vary during the course of the breeding season (Kruskal Wallis ANOVA, extent of colour: H=3.13, d.f.=2, P>0.05, N.S.; intensity of colour: H=2.72, d.f.=2, P>0.05, N.S.), but may have done had data been available for April. Nevertheless, there was no detectable influence of *S.solidus* infection on either the extent (Wilcoxon-Mann-Whitney, W=626, n=43, m=5, P>0.05, N.S.) or intensity (Wilcoxon-Mann-Whitney, W=637, n=34, m=5, P>0.05, N.S.) of red colouration (Figure 4.7(d-e)).

The relationship between the indices of condition, gonad and kidney weight and nuptial colouration

A correlation matrix of all the indicators of male body condition and breeding condition for uninfected mature males (Table 4.5) revealed that there were significant positive relationships between the condition factor and the somatic condition factor, the relative kidney weight and the kidney tubule epithelial cell height and the extent and intensity of red colouration.

The same comparisons of female gonadosomatic indices could not be made because there were significant differences between the months of the breeding season and when the appropriate months were excluded only one infected female remained.

4.4 DISCUSSION

Coinciding with the wave of *S.solidus* infection in autumn 1988 were a number of consequences for infected sticklebacks. There was a tendency for fewer parasitized than unparasitized fish to consume rotifers, invertebrate eggs, nematodes and algae. Although the stomach fullness was similar in both groups of fish, infected sticklebacks did not seem to be exploiting alternative foodstuffs, with the exception of arcellidae to a small degree. Instead, there may have been poorer competitive ability in infected compared with uninfected fish, as described by Milinski (1984). However, the results need not represent differences in the actual foraging decisions of fish. A greater physiological oxygen requirement by *S.solidus*-infected

Table 4.5: Spearman rank correlation matrix of indicators of mature male body condition, breeding condition and nuptial colouration, including resultant probabilities.

N=37	CF	SCF	HSI	GSI	RSI	KEH	extent
SCF	0.826 P<0.001						
HSI	0.182 N.S.	0.143 N.S.					
GSI	0.006 N.S.	0.160 N.S.	0.089 N.S.				
RSI	0.010 N.S.	-0.117 N.S.	0.208 N.S.	-0.132 N.S.			
KEH	0.228 N.S.	0.144 N.S.	0.265 N.S.	-0.077 N.S.	0.600 P<0.001		
extent	0.185 N.S.	0.266 N.S.	0.058 N.S.	0.133 N.S.	0.111 N.S	0.163 N.S.	
intensity	0.228 N.S.	0.090 N.S.	0.265 N.S.	0.104 N.S.	0.192 N.S.	0.113 N.S.	0.778 P<0.001

CF - condition factor
SCF - somatic condition factor
HSI - hepatosomatic index
RSI - renosomatic index
KEH - kidney tubule epithelial cell height
extent - extent of red colouration
intensity - intensity of red colouration

sticklebacks could have forced them to seek out oxygen rich surface waters (Lester 1971), where they may have experienced slightly different prey availability. If this were the case, infected fish would be expected to have a more planktivorous diet, yet this was not observed.

Also during autumn, the body condition of infected sticklebacks declined at a faster rate than that of uninfected sticklebacks and in some months at least, body condition was related to the relative weight of the *S.solidus* plerocercoids. It would appear that the high parasite growth found at this time (Chapter 3), resulted in fewer resources being available to the infected fish and so condition was more difficult to maintain. Despite the condition factor and the somatic condition factor being significant if weak predictors of total stickleback body lipid, protein and glycogen (Chellappa & Huntingford pers. comm.), it is probably only carbohydrates that are being assimilated by the plerocercoids. This is because carbohydrate is the single most important energy substrate of cestodes (Smyth & McManus 1990), including *S.solidus* plerocercoids (Körting & Barrett 1977).

By contrast, the relative liver weights of infected fish were far greater than those of uninfected fish in late summer and early autumn. As this phenomenon was restricted to the early part of the wave of infection, it may represent a pathological or other physiological response to the acquisition of new infections. Thereafter, the hepatosomatic indices of uninfected and infected fish rose at similar rates. In autumn, the hepatosomatic index is a significant, but weak predictor of total liver glycogen (Chellappa & Huntingford pers. comm.) and thus the presence of the plerocercoid does not seem to interfere with the accumulation of liver glycogen reserves in the infected sticklebacks. This is a surprising result because the percentage increase in the glycogen content of plerocercoids of the size found in late autumn (mean wet weight = approx 70mg) is thought to be exponential (McCaig & Hopkins 1965) and the hepatosomatic index has previously been found to be negatively correlated with the parasite index at this time of the year (Arme & Owen 1967). It may be that the energy reserves available to the population of sticklebacks at Inverleith is not very limited in autumn such that the presence of *S.solidus* infection has a negligible effect on the liver and a small effect on the rest of the body.

Associated with winter was a greater divergence in the diet of sticklebacks harbouring

S.solidus, from those that did not. Fewer infected fish consumed rotifers, cladocerans, ostracods, nematodes and algae, but more ingested higher plants. In addition to the differences in occurrence of these dietary items, infected fish also had less full stomachs. It is expected that prey would have been less abundant and foraging time greatly restricted by short day lengths and this may have led to intra-specific competition for food. Infected sticklebacks may have competed poorly for food compared with uninfected sticklebacks (Milinski 1984) resulting in their food intake being reduced and further reflected in their diet composition. However, it has been proposed that predation acts upon both uninfected and infected sticklebacks in winter, but selectively on heavily infected individuals (Chapters 2 and 3). Such a risk of predation may have resulted in uninfected sticklebacks adopting a foraging strategy that incorporated greater vigilance either by choice of habitat or the type of prey selected. Although it has been found that infected sticklebacks respond less to a risk of predation (Giles 1983b, 1987b; Milinski 1985), during winter at Inverleith most plerocercoids harboured were small (Chapter 3) and uninfective (Chapter 6). There is evidence to suggest that infections with this worm size increases the host response to predators (Tierney, Huntingford & Crompton, in prep.) and therefore may also have the opposite effect on foraging. Perhaps in winter infected sticklebacks are the most vigilant and thus the poorest competitors for food resulting in the observed reduced food intake and the greater reliance on higher plants.

Throughout winter, the condition factors of uninfected and infected fish were similar, despite infected fish having depressed body condition in autumn. Such uniform body condition was the result of an apparent initial increase in the condition of infected fish. It is unlikely that this reflects an actual improvement in the condition of infected sticklebacks, but is probably due to the differential loss of those heavily infected fish in the very poorest condition (Chapter 3). Nevertheless, the condition of the remaining fish whether infected or not was also low. During winter, the condition factor and somatic condition factor is generally a poor indicator of energy reserves (Chellappa & Huntingford pers. comm.). and so it would appear that sticklebacks are existing with minimal stores of lipid, protein and glycogen at this time. The lack of available host energy stores could also explain the suppression of parasite growth apparent at this time.

The steep decrease in the hepatosomatic indices of both uninfected and infected sticklebacks over winter, probably represents increased breakdown and utilisation of liver glycogen relative to accumulation. A lack of both stickleback and parasite growth suggests that this carbohydrate source is necessary to supplement stickleback energy requirements at this time of reduced food intake.

Greater food availability, longer daylight hours, the absence of a predation hazard and large plerocercoids in spring, seemed to minimise dietary discrepancies between uninfected and infected sticklebacks, with stomach fullness and diet composition being almost identical in each group. This suggests that changes in some, if not all of these factors contribute to the dietary variability seen in autumn and winter.

In contrast to the negligible effect of *S.solidus* infection on diet during spring, was a large the impact on the growth and energy reserves of infected sticklebacks. The condition factor rose rapidly in uninfected sticklebacks, but was virtually unchanged in infected sticklebacks. The somatic condition factor of infected fish did appear to increase in spring, but quickly dropped to a level much lower than that found in uninfected fish. In spring, these indices of condition tend to predict well the total body lipid and protein (Chellappa & Huntingford pers. comm.) and so it would appear that these stores have been used more extensively in infected fish. However, far more dramatic, was the complete absence of relative liver growth in infected sticklebacks, despite a large increase in the hepatosomatic index of uninfected fish. Bearing in mind that percentage glycogen content rapidly increases in plerocercoids of 15-100mg (McCaig & Hopkins 1965) and that the mean weight of plerocercoids rose from 56mg in February to a peak of 168mg in June 7, it appears that the lack of weight increase in the major glycogen storage organ is the result of assimilation, by *S.solidus*, of the sticklebacks available carbohydrate. Indeed, the hepatosomatic index has been found to be accounted for largely by total liver glycogen in spring (Chellappa & Huntingford pers. comm.).

The negative association between condition and parasite burden in late spring and early summer suggests that low fish growth is a direct consequence of high plerocercoid growth. However, no such relationship was discernible between the hepatosomatic and parasite indices, thus demonstrating that there is not a progressive reduction in liver weight with

plerocercoid growth. Instead, the plerocercoids seem to divert the available carbohydrate away from the infected fish and store it as glycogen such that there is complete cessation of relative liver growth in infected sticklebacks, regardless of parasite burden. Again, this is not in agreement with the findings of Arme and Owen (1967) who found that the reduction in liver weight was correlated with an increase in the relative parasite weight.

In spring, mature fish of both sexes were still rather infrequent irrespective of *S.solidus* infection status. However, at the peak of breeding almost no infected sticklebacks showed signs of maturity and this applied to both males and females. Similar low proportions of infected mature males were found in other populations (Pennycuick 1971d). Also, few infected females were found to be in breeding condition in other studies (Pennycuick 1971d; McPhail & Peacock 1983). It was argued by McPhail and Peacock (1983) that these results need not represent an alteration in the breeding potential of infected fish, but could indicate that mature fish are more susceptible to infection. At least in this study, the latter explanation can be ruled out, because infections are largely confined to autumn when all young-of-the-year sticklebacks are immature. Furthermore, the body condition and relative liver weights of infected fish were consistently lower around the time of breeding. A closer look at these factors gives greater insight into the impact of *S.solidus* on the breeding of sticklebacks from Inverleith.

Attainment of sexual maturity in uninfected male sticklebacks at Inverleith does not seem to be limited entirely by their body condition or by their liver size, but infected immature males were in the poorest condition, comparable to that found in winter. The few infected males that were fully mature were in the best condition. As these mature, infected sticklebacks harboured smaller parasite burdens than immature infected sticklebacks, this might explain why they were able to breed, but it does not account for their good body condition. Perhaps only high quality males can sustain an infection with *S.solidus* plerocercoids and reach maturity, whilst uninfected males can become mature over a range of body conditions. This suggest that the expression of the secondary sexual characteristics represents a condition-dependent handicap (Maynard Smith 1985). The high quality of infected mature males is further emphasised by the fact that neither their testosterone production (which is correlated with the kidney tubule epithelial cell height) nor the expression of nuptial colouration were impaired

and the testes of these sticklebacks were larger than those of uninfected mature males. From these data, it would appear that such infected mature males would be attractive to females because of their bright nuptial colouration (Milinski & Bakker 1990) and *S.solidus*-induced large body size (Rowland 1988).

Arme & Owen (1967) also observed expression of the secondary sexual characteristics in heavily infected males, but found them to be deficient in their ability to build nests. Experiments carried out in 1988 to monitor the aggressiveness of size-matched, uninfected and infected, mature males from Inverleith (unpublished data), also revealed a significantly lesser tendency for infected males to build nests (Fisher's Exact Probability test, $P<0.01$). It is unlikely that such infected males would be selected as mates because in spite of their well-developed secondary sexual characteristics, their reproductive behaviour and perhaps their competitive ability is impaired. However, a repeat of these tests in 1990 indicated that infected males that did build nests were able to compete as effectively as uninfected males, but generally harboured small worms. Therefore, although heavily infected mature males initially appear to be a good potential mate this signal may be misleading if their reproductive behaviour is inferior. On the other hand, a lightly infected mature male appears to be as good a choice of mate (if not better) as an uninfected mature male, in terms of viability and the potential for good paternal care. Hence, although females by selecting mature males are likely to choose the more abundant parasite-free males there is the chance that infected males will produce offspring. Assuming that parasite resistant genes did exist there would be selection both for and against them. However, an examination of other populations suggests that few if any sticklebacks are completely resistant to *S.solidus* because high prevalences and intensities are common (Chapter 3). Nevertheless, resistance may be more subtly expressed a e.g. behavioural avoidance of infection.

An entirely different effect of *S.solidus* on the reproductive potential of females was evident. There was uniform body condition in uninfected and infected sticklebacks at each stage of maturity and the only trend in the hepatosomatic indices was for uninfected mature females to have much larger livers than all other groups. As a few infected females had mature ovaries, it would appear that infection does not completely remove the breeding capability of

females. However, gravid ovaries were not detected in these few infected females and it is possible that the large livers of uninfected mature females are representative of the energy reserves needed to sustain the production of mature ova. Indeed, Allen and Wootton (1982) have observed that ovarian development is fairly insensitive to exogenous resources, because of a buffering effect of the liver. When female sticklebacks were maintained on low rations in the pre-spawning period, a reduction in liver size coincided with increased ovary size. It can be envisaged that the low liver weight and constant energetic drain of the parasite does not allow the ovaries of the infected sticklebacks to pass the pre-spawning phase. Further support for this notion comes from a histological examination of the ovaries of female sticklebacks following the breeding season (Arme & Owen 1967). In contrast to those of uninfected and lightly infected sticklebacks, the ovaries of heavily infected, female sticklebacks contained numerous pre-ovulatory *corpora atretica* which are indicative of unshed eggs. As the parasite indices of uninfected and infected mature females did not differ, it may be that only those females with sufficient liver reserves are able to spawn successfully and infected females do not meet this requirement. Nevertheless, the distension of the body in infected females may have evoked a courtship response from males, although this probably would not have been reciprocated as they were not ready to spawn.

The discrepancies in sexual maturity and growth between uninfected and infected sticklebacks may have been influenced by the resurgences of dietary differences in the summer. Stomach fullness was depressed in infected sticklebacks and they relied more heavily on rotifers and invertebrate eggs, but perhaps more importantly they had a lesser tendency to consume chironomids. Certainly chironomids are profitable food items for large sticklebacks relative to some others in the diet (Ibrahim 1988a) and the failure of infected sticklebacks to exploit them more fully may have exacerbated the effects of *S.solidus* on breeding. As the infections were comprised mostly of large worms the observed dietary differences may have been due to variable competitive ability as found by Milinski (1984).

4.5 CONCLUSIONS

Infection with *Schistocephalus solidus* appears to have a strong and variable impact on its stickleback hosts at Inverleith. Food intake was both qualitatively and quantitatively influenced

by infection status, although the mechanisms and effects of these changes on the host are difficult to interpret. Plerocercoid growth seems to be at the expense of fish growth and has two seasonal consequences. The first seems to be mortality of fish that have high parasite burdens and are in poor condition, probably as a result of predation. The second is an impaired ability to reproduce, which seems to be most marked in heavily-infected sticklebacks of both sexes. The consequences for the mating success of the few mature infected males is less clear, but there are indications that they may father offspring.

Such serious interference with the life history of sticklebacks is also bound to have longer term adaptive significance. It is thought to be a selective advantage for sticklebacks to breed early in the season, in order to maximise the time available for the growth and development of their offspring and therefore ensure their survival during the winter. Furthermore, if inherited resistance to infection exists whether it be immunological, physiological or behavioural (foraging behaviour, anti-predator behaviour), it is likely to be perpetuated when infection-free sticklebacks breed. However, the selective advantage of early breeding may be lost, because fry would be exposed for longer to a wave of *S.solidus* infection and have more chance of accumulating high intensity infections (Chapter 3). This would in turn make it more likely that they succumb over winter. Of those infected fish that did survive the winter, probably only high quality fish with lighter infections would breed and produce susceptible, but perhaps more resilient fry. Finally, there would be no continuation of the genes of fish that were not able to sustain an infection and also breed.

CHAPTER 5: THE MAINTENANCE AND MANIPULATION OF *SCHISTOCEPHALUS SOLIDUS* IN THE LABORATORY

5.1 INTRODUCTION

Using field observations to study the interactions in a host-parasite system provides valuable data on the effects of the parasite on its hosts in the natural habitat, but limits the information that can be collected and the extent of the interpretation. Another approach is to maintain the life history of the parasite in the laboratory and to manipulate the various host-parasite systems experimentally. Initially, attempts were made to set up the life history of *Schistocephalus solidus* by adopting the methods of Orr and Hopkins (1969) and those used for the infecting the definitive and for harvesting and embryonating the eggs were only slightly modified. However, the techniques employed for culturing copepods failed to provide an adequate turnover for the reliable maintenance of the parasite and so alternative methods had to be developed. Also, the procedures for exposing copepods and sticklebacks to infective stages were inappropriate for the desired experimental manipulations. Hence, the methods for infecting the avian host and retrieving and developing eggs detailed below are similar to those used by Orr and Hopkins (1969). Alternative methods are described for maintaining and infecting the copepod and stickleback hosts some of which are utilised in Chapters 6 and 7.

5.2 MAINTENANCE OF HOSTS IN THE LIFE HISTORY OF *SCHISTOCEPHALUS SOLIDUS*

5.2.1 DEFINITIVE HOST: CHICKEN, *GALLUS GALLUS*

Male chickens, *Gallus gallus*, were chosen as laboratory hosts for adult *S.solidus* because they are easy to infect, parasite eggs can be readily recovered from their faeces (Orr and Hopkins 1969) and they would be killed otherwise. Chickens were obtained, as hatched, from Marshall's Hatcheries (Whitburn, East Lothian) and reared at Yorkhill Hospital (Glasgow) on Goldstart chick crumbs, for at least one week, before plerocercoids were administered. During this time the chicks were heated continually with lamps and were therefore, under a constant light regime. Orr and Hopkins (1969) found that the use of a bulky, high protein diet led to the eggs being less concentrated in the chicken faeces and a high level of uric acid production. Thorough washing and sieving of the faeces counteracted both problems whilst enabling the use of a commercial diet.

5.2.2 SECOND INTERMEDIATE HOST: THREE-SPINED STICKLEBACK, *GASTEROSTEUS ACULEATUS*

An urban pond in Inverleith public park, Edinburgh (74N 27E, O.S. map Second series, Sheet 66) was utilised as a source of *S.solidus*-infected sticklebacks (for more details see Chapter 2.2.1). Visibly infected fish were collected, using long handled nets and were transported to the laboratory in sealed plastic buckets. They were housed until required in glass aquaria at a density of about 1 fish per 2l of water and the controlled light and temperature conditions were set to approximate normal for the time of year.

In the meantime, pathogenic organisms, such as white spot (*Ichthyopthirius multifillis*) and skin and gill flukes (*Gyrodactylus* sp), were minimised by the use of filtration and chemical disease treatments. It was necessary to give sticklebacks food that would not introduce new ecto- and endo-parasitic species. The only live food used was bloodworm (chironomid larvae) and a range of frozen, sterilised feed was employed (*Daphnia*, brine shrimp, bloodworm, plankton etc.).

5.2.3 FIRST INTERMEDIATE HOST: COPEPOD, *ACANTHOCYCLOPS VIRIDIS*

Acanthocyclops viridis (jurine) was chosen as the cyclopoid host because it is known to act as an intermediate host of *S.solidus* (Callot and Desportes 1934) and also because it can be acquired as a monoculture from a biological supplier (Sciento, Manchester).

A.viridis are herbivorous as nauplii, omnivorous as copepodites and carnivorous when adult (Khan 1965; Adalsteinsson 1979; Laybourn-Parry & Tinson 1985; Kurashov 1989). They were therefore fed on a diet of mixed protozoa and algae. To procure protozoan cultures, grain infusions were used. Dried maize grains (one per 30ml of final culture medium) were boiled for ten minutes in about 100ml of water and a slit was made in the side of each (Page 1981). They were then added to Kilner jars of water (approximately 600ml) followed by an inoculation with 10-20 full pipettes of pond water or dense, mixed species, existing cultures. A high density of protozoa was reached after about 2 weeks at 20-25^{o}C and cultures were used for as long as two months.

New copepod cultures were established by adding 1 part protozoan culture to 4 parts water or spent medium (at the equivalent temperature) in 250 or 500ml conical flasks at a

density of approximately 1-2 per ml. The size of flasks used depended on the space and number copepods available. At this copepod density male-female contact and hence the level of breeding was optimal, without rates of growth or brood size being compromised by overcrowding. Feeding took place once a week and involved decanting one fifth of the medium through a sieve with 150μm mesh (to retain copepods) and replacing it with an equal volume of protozoan culture. This was a four-fold increase in the level of feeding used by Orr and Hopkins (1969) and although this may dramatically alter the pH and the oxygen concentration of the culture medium, the copepods nevertheless flourish. Aluminium foil covers were placed on the flask openings to limit pathogenic contamination. The copepods were then stored at a constant temperature of 25^{O} C to maximise growth (Khan 1965) and with a 12L/12D photoperiod of fluorescent light to encourage the natural growth of algae in the culture vessels. Under these conditions growth and expansion of copepod numbers was high.

When it became apparent that cannibalism of young by adults (described by Khan 1965) was reducing the potential expansion of the copepod cultures, egg separation chambers similar to that of Yassen (1981) were employed. An added advantage was that the resultant cultures contained similar-aged copepods, conducive to controlled experimental manipulation (see Chapter 7). A 250ml or 500ml flask (depending on the availability of gravid females) was filled to the neck with medium as described above and a universal tube with plankton mesh bottom (around 200μm) was suspended from the mouth of the vessel by wire. Gravid females were enclosed in the universal tube, allowing eggs and nauplii to pass through the mesh and feed without risk of predation.

Existing cultures were checked periodically for the presence of females with egg sacs. By pouring the cultures on to a 150μm mesh sieve (submerging in carbonated water can be used to anaesthetise the copepods) and washing the copepods into a crystallising dish with a small volume of medium, such females could be extracted using a Pasteur pipette. Approximately 30-50 (250ml flask) or 70-100 (500ml flask) gravid females were housed for 2-3 days in the separation chamber and subsequently returned to mass culture. The new copepods were thenceforth fed as described above.

5.3 ACQUISITION OF EMBRYONATED EGGS OF *SCHISTOCEPHALUS SOLIDUS*

5.3.1 INFECTION OF CHICKENS AND COLLECTION OF FAECES

Infected sticklebacks from Inverleith pond (see Section 5.1.1) were killed as needed by exposure to an overdose of Benzocaine anaesthesia. They were dissected and any plerocercoids found, were released into 0.9% sodium chloride solution. Three to four plerocercoids (at least 100mg fresh weight) were fed to each chicken, by placing them individually at the back of 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 cages, under which trays of wet paper towelling were placed, to catch faeces (Orr & Hopkins 1969). From days 2-7 post-infection (*p.i.*), the towelling was removed daily, folded into polythene bags and refrigerated until all faeces had been collected. Chickens were killed on day 7 *p.i.*, by inhalation of CO_2.

5.3.2 CONCENTRATION OF EGGS

Once they had been removed from the paper towelling, the faeces were macerated in tap water with the aid of a hand-held blender, until a very thin paste was formed. This was poured in small quantities on to a series of sieves of decreasing pore diameter (300μm, 53μm, 38μm) and flushed thoroughly under a running tap. The eggs (and minor amounts of faecal debris) retained in the bottom sieve were rinsed into foil covered conical flasks and stored in tap water in the refrigerator for as long as six months (modified from Orr & Hopkins 1969).

5.3.3 EMBRYONATION OF EGGS

Eggs were allowed to embryonate in small (8cm diameter) crystallising dishes that had been previously blacked out with paint or covered in aluminium foil and had lids that had been similarly treated. This inhibited the occurrence of light-activated hatching during incubation. Thin coatings of eggs were applied to the bottom of the dishes which were then two-thirds filled with tap water, covered and placed in an incubator set at 25^{o}C for a minimum of two weeks (modified from Orr & Hopkins 1969).

5.4 PRODUCTION OF *SCHISTOCEPHALUS SOLIDUS* CORACIDIA

Prior to hatching, the embryonated eggs were concentrated in a 38μm mesh sieve and rinsed with a little water into a small petri dish. They were then exposed at 15^{o}C, to a high

intensity light source to induce rapid hatching. At this temperature the eggs can be stimulated for up to 4h, promoting maximum hatching, without much deterioration of the coracidia. This procedure gives a concentrated mixture of empty egg cases, unhatched eggs, faecal debris and coracidia. Centrifugation (MSE Centaur 2) at 400 r.p.m. for 1min sediments the debris and leaves most coracidia in the supernatant.

5.5 INFECTION OF *ACANTHOCYCLOPS VIRIDIS* WITH *SCHISTOCEPHALUS SOLIDUS*

5.5.1 OBTAINING A KNOWN CONCENTRATION OF CORACIDIA

A process of limiting dilution was employed, whenever it was necessary to achieve a situation where each drop of the coracidial suspension (see above) contained approximately one coracidia. Initially, the number of coracidia was estimated by examining a single drop of the mixture (from a Pasteur pipette), under a high power dissecting microscope. As this number was often high, the mixture was transferred to a small conical flask, magnetically stirred at slow speed and progressively diluted with water. After each dilution, the number of coracidia per drop was more accurately reassessed by examining 30 individual drops until finally a mean of approximately one resulted.

5.5.2 GROUP INFECTIONS OF COPEPODS

Having acquired a single coracidium per drop, the simplest but least predictable way of obtaining infected *A.viridis* was to add coracidia directly to the copepod cultures. The quantity of coracidia in a full pipette was estimated by counting the drops and the infective stages were added at a rate of 2-5 per copepod. Uptake of coracidia was maximised by adding coracidia when the level of protozoa was low and the copepods were actively seeking food. However, this resulted in copepods of many ages and stages becoming infected with very variable numbers of procercoids.

5.5.3 CONTROLLED INFECTIONS OF COPEPODS

When a large quantity of lightly infected copepods was required, an alternative method for infecting *A.viridis* was employed. Single copepods were placed in each well of a 96 well, cell culture plate (flat-bottomed), together with 2 drops of water. One drop of coracidial suspension with a mean of one coracidia per drop (see above) was then added. The plates were placed in the light for at least 3-4h and stored overnight at 25^{o}C. By the following day, no live

coracidia were detected and the copepods were rinsed from the plates, washed in a 150μm mesh sieve (such that eggs and other debris passed through) and transferred into normal culture (see Section 5.1.1).

5.5.4 MONITORING THE INTENSITY OF INFECTION IN COPEPODS

Infection intensity can be determined in both living and dead *A.viridis*. A high level of accuracy was achieved after 5-7 days *p.i.* when the large procercoids were easily recognisable. Copepods were isolated on glass slides in a small volume of water, then teased apart with dissecting needles. The procercoids tended to move out of the copepod haemocoel into the surrounding fluid and were counted under a high-power dissecting microscope.

Living copepods had to be immobilised to facilitate examination with a compound microscope. This was achieved by placing a copepod on a cavity slide, drawing off the culture fluid and adding a drop of viscous methyl cellulose, to restrict major movements. Alternatively, a drop of carbonated water was added to the slide, thereby anaesthetising the copepod. When a coverslip was in position, it was gently moved so that the copepod would roll over exposing all its sides. This ensured that any procercoids were clearly visible and allowed an estimate of the numbers of procercoids to be made. Only when the intensity of infection exceeded four did the accuracy of this technique decrease dramatically. For example, the intensity of infection of a live copepod could be estimated as 5, but if were dissected it could have as many as 7 procercoids.

5.6 INFECTIONS OF *GASTEROSTEUS ACULEATUS* WITH *SCHISTOCEPHALUS SOLIDUS*

5.6.1 CONTROLLED INFECTIONS OF STICKLEBACKS

Sometimes it was necessary to offer sticklebacks a precise dose of procercoids by the natural oral route. Recipient sticklebacks were individually placed in small plastic tanks (22.5 x 12 x 7cm), painted black to allow the fate of an infected copepod, in the presence of a single stickleback to be followed. Sticklebacks will rarely feed under such unnatural conditions and had to undergo previous training. This involved a few days of feeding with planktonic invertebrates (frozen), from a Pasteur pipette. During pre-training the fish were fed in groups, but in their stock tanks and most came to equate a pipette with food. Finally, food was withheld

for 12-24h, before sticklebacks were allowed to settle in a small tank.

A pipette containing a copepod, with a known intensity of infection, was held in the tank such that the fish could see the copepod's movements. Commonly, the stickleback would make bites at the end of the pipette and when the copepod was released, it was quickly eaten. If the copepod was not ingested immediately, it was either watched until it had been eaten or it was caught by the observer and reintroduced. Care was also taken to check that the copepods had not been regurgitated.

5.6.2 GROUP INFECTIONS OF STICKLEBACKS

Group infections of sticklebacks were carried out when knowledge of the transmission dynamics was not required. After switching off the filtration, to lessen the water turbulence, infected copepods (3-5 per fish) and uninfected copepods, were added simultaneously to stock tanks of hungry fish. The resultant swarm of copepods was more conspicuous to the sticklebacks, than infected copepods alone and encouraged many to feed and develop infections. They were then housed as described above.

CHAPTER 6: *SCHISTOCEPHALUS SOLIDUS* IN A DEFINITIVE HOST, *GALLUS GALLUS*: INFECTIVITY OF THE PLEROCERCOIDS AND REPRODUCTIVE CAPACITY OF THE ADULTS

6.1 INTRODUCTION

6.1.1 ESTABLISHMENT OF *SCHISTOCEPHALUS SOLIDUS* IN THE DEFINITIVE HOST

There is both inter-parasite and inter-host variability in the level of establishment of *S.solidus* in definitive hosts. From the data of McCaig & Hopkins (1963), it would appear that plerocercoids of *S.solidus* are very likely to establish as adults in herring gulls (*Larus argentatus*) and black-headed gulls (*Larus ridibundus*), but the probability of establishing decreases through ducklings (*Anas boschas* = *Anas platyrhynchos*?), chickens (*Gallus domesticus* = *Gallus gallus*), pigeons (*Columba livia*), rats (*Rattus norvegicus*) and hamsters (*Mesocricetus auratus*). In the same study, the positions occupied by the worms in the intestine varied amongst host species, adults being found in the mid- to hind regions of the intestine in ducks, chickens and rats and in the anterior of hamster and pigeon intestines. These differences in establishment and the subsequent intestinal position of the adults perhaps results from variable gut morphology and physiology across the host species.

Within a single host species, host age and plerocercoid size appear to be important determinants of establishment. Greater numbers of adult worms were recovered from ducks less than one month old compared to older ducks and infectivity to similar-aged ducks was correlated with size of the plerocercoid (Hopkins & McCaig 1963). Only 3% of plerocercoids less than 10mg established, compared with 40% of 10-30mg plerocercoids and 80% of plerocercoids exceeding 30mg (Hopkins & McCaig 1963). It was postulated that such size-linked infectivity of *S.solidus* reflects a differential ability of worms to be retained in the intestine. As the scolex comprises little more than a shallow groove, it is unlikely to function as an attachment organ. Smyth and McManus (1989) suggested that the well developed longitudinal musculature of the parasite enables it to flex against the intestinal wall and be retained against peristaltic flow.

6.1.2. FACTORS AFFECTING THE MATURATION AND FECUNDITY OF *SCHISTOCEPHALUS SOLIDUS* IN THE DEFINITIVE HOST

The plerocercoids of *S.solidus*, in common with those of some other pseudophyllidean cestodes (*Ligula intestinalis* and *Digramma interrupta*) are progenetic (having advanced genital development without maturation, Smyth 1946) and have large glycogen reserves (Hopkins

1950; McCaig & Hopkins 1965). Thus a stimulus such as a rise in temperature to 40^{o}C is sufficient to stimulate rapid maturation (Smyth 1946). Progenesis commences at an early stage in the development of the plerocercoid, so that once they have reached a weight of 19mg the genitalia are already formed (Hopkins & McCaig 1963). Therefore, provided establishment takes place, a small worm can mature and produce eggs in a definitive host (Clarke 1954, Hopkins & McCaig 1963).

In a variety of experimentally infected definitive hosts, egg production in *S.solidus* was consistently found to begin at 2 days post-infection (*p.i.*) and from day 7 *p.i.* the number of animals bearing eggs in their faeces declined (McCaig & Hopkins 1963). However, as these were multiple infections there was no indication of the pattern of egg output or the quantity of eggs produced from individual adults. Following 48h in a duck and 24h in culture, Mason (1965) found that adult *S.solidus* (55-390mg) produced between 2000-11000 eggs; longer periods in a definitive host and culture resulted in an increased production of abnormal eggs (Mason 1965). This suggests that the age of the adult worm may have qualitative and quantitative effects on egg production. There is little information on other features of the adult that may affect fecundity. However, studies on the cyclophyllidean cestode *Hymenolepis diminuta* have revealed that egg production per worm and individual worm weight (wet and dry) decrease with increased infection intensity, suggesting that fecundity is related to adult size (Hesselberg & Andreassen 1975).

6.1.3 IMPROVING ON PREVIOUS STUDIES

Although size-correlated infectivity of plerocercoids has been demonstrated in the past (Hopkins & McCaig 1963), there was heavy reliance on data obtained from feeding intact infected fish to pigeons and ducklings. This required that the initial number and weight of plerocercoids be estimated by examining the intensity of infection and the weights of plerocercoids from a sub-sample of sticklebacks. Also, only a relatively small size range of pre-weighed plerocercoids were administered to ducklings and as they were not given individually, there was difficulty in determining those which had established. This was especially problematic, because of an apparent weight loss in the transition from the plerocercoid to adult stage (Hopkins & McCaig 1963). To overcome some of these difficulties, the infectivity to

chickens (*Gallus gallus*) of individual plerocercoids spanning a range of weights was investigated. As the modal intensity of *S.solidus* infection in sticklebacks from Inverleith pond was 1 (Chapter 3), it is probable that single-worm infections of the definitive host are a common, naturally-occurring phenomenon. Furthermore, the effects of host heterogeneity were minimised by using chickens of the same age and sex and from the same source.

Egg production by adults has largely been investigated in multiple infections (McCaig & Hopkins 1963; Mason 1965) and sometimes using a combination of *in vivo* maturation and subsequent *in vitro* culture (Mason 1965). By using single worm infections of chickens and faecal egg count techniques, the pattern of *in vivo* egg production from adults could be investigated quantitatively. Information on the initial weight of the plerocercoid and adult longevity and weight were also recorded to assess whether they could be related to fecundity. In addition, the use of single-worm infections allowed the quality of eggs from individual adult-host interactions to be quantified.

6.1.4 AIMS

The work described in this chapter was aimed at examining further the infectivity of plerocercoids to a definitive host, concentrating particularly on the effect of plerocercoid weight on establishment. Emphasis was placed on examining the infectivity of a wide size-range of plerocercoids in a single definitive host species (*Gallus gallus*) of homogeneous age and sex. Quantitative aspects of *in vivo* egg production by adult *S.solidus* were also examined against a background of host homogeneity and in both cases, single worm infections were used to avoid the possible confounding effects of multiple infections. Furthermore, morphometric characteristics of the infecting plerocercoids and surviving adults were recorded in order to assess their relationship to adult fecundity. Finally, using the degree of embryonation as an index of egg quality, the effects of the parent worm age on this feature of reproductive capacity was investigated.

6.2 MATERIALS AND METHODS

6.2.1 INFECTIVITY OF *SCHISTOCEPHALUS SOLIDUS* PLEROCERCOIDS TO CHICKENS, *GALLUS GALLUS*

Maintenance of chickens

Day-old chickens were obtained from Marshall's hatcheries (Whitburn, East Lothian). They were reared in communal cages with constant heat, light, water and food (Goldstart Chick crumbs) for 7-8 days before plerocercoids were administered.

Acquisition of plerocercoids

Sticklebacks from Inverleith pond (see Chapter 2) were consistently used as a source of plerocercoids. Infected sticklebacks were collected using long-handled nets, transported back to the laboratory in sealed plastic buckets and housed in glass tanks until required (see Chapter 2 for detailed housing regime). Samples were collected in spring, early autumn and late autumn, in order to extend the size range of plerocercoids that could be fed to chickens (see Chapter 3).

Infection procedure

Before plerocercoids were administered, each chicken was fitted with a wing tab for identification purposes. Intact plerocercoids were obtained by making ventral incisions in sticklebacks which had been killed by exposure to an overdose of Benzocaine anaesthesia. Plerocercoids were held until needed in 0.9% sodium chloride solution. To minimise damage to the plerocercoid, which might possibly alter their infectivity to chickens, small and large plerocercoids were handled differently. Large worms were blotted and then transferred to aluminium foil, whilst small, delicate worms were first transferred to foil and then the excess saline drawn off with a pipette, before being weighed in mg. A single plerocercoid was weighed and given to each chicken by gavage. Small worms were released into the back of the chicken throat by pouring them off the foil in some saline, whilst large worms had to be placed at the back of the throat with blunt forceps. In each case, the chicken was encouraged to swallow the plerocercoid (by closing the bill and gently rubbing the throat) and checked for regurgitation before being returned to its communal cage. The infectivity of 166 plerocercoids (2-659mg) was followed in this way, over three experiments.

Investigation of establishment

Approximately 24h *p.i.*, the chickens were killed by inhalation of carbon dioxide. Their intestines were removed and the whole length of the small intestine was opened and examined for adult *S.solidus*, the presence of which was regarded as evidence of establishment.

6.2.2 FECUNDITY OF ADULT *SCHISTOCEPHALUS SOLIDUS* IN CHICKENS, *GALLUS GALLUS*

Chickens were maintained and plerocercoids acquired by the same methods described above (Section 6.2.1).

Infection procedure

Fifteen plerocercoids between 160-218mg were selected to infect chickens, because they have a high degree of establishment (>70% , this Chapter) and are expected to produce eggs (Orr & Hopkins 1969). Each chicken was infected by a single plerocercoid, the weight of which was noted. After checking for regurgitation they were housed individually in wire-bottomed cages. Food and water were supplied and trays of wet paper towelling were placed under each cage to catch faeces.

Estimation of egg output

From days 1-7 *p.i.*, the paper towelling was removed from under each cage and stored temporarily in labelled polythene bags in a refrigerator. Faeces were processed by a version of the ether concentration technique (Allen & Ridley 1970). Faecal material was removed from the paper towelling (care being taken to exclude crumbs of food) and macerated in tap water with the aid of a hand-held blender, until a thin paste was formed. Approximately 100ml of the mixture was filtered through 2 layers of double-thickness cotton gauze and the remainder was retained and used to derive viable eggs (See below **Concentration of eggs**). Ten ml of the filtrate was transferred to a pre-weighed centrifuge tube and the suspension centrifuged at 3500 r.p.m. for 5 min. The supernatant fluid was discarded and the pellet was drained (by inverting the tube for 1 min) before the pellet and tube were weighed (g). The pellet was resuspended in 6ml tap water and the suspension was made up to 10ml with diethyl ether before the tube was stoppered and shaken for 30 sec. Once the stopper had been removed, the tube was immediately centrifuged for 2 min at 2000 r.p.m., resulting in the formation of four layers: an

upper layer of ether soluble material, a fatty detritus layer, an aqueous layer and a pellet at the bottom. The detritus layer was dislodged with a wooden spill and the tube quickly inverted to remove all but the pellet. The pellet was resuspended in a few drops of water and a single drop of suspension (from a Pasteur pipette) was examined, for the presence of *S.solidus* eggs, at x10 magnification. The observed number of eggs was multiplied by the total number of drops, to give an egg count per weight of pellet, from which an estimate of the number of eggs per gram of (sieved) faeces could be derived, following Hall (1981).

Concentration of eggs

Eggs were concentrated from the excess faecal paste, using a series of sieves (Chapter 5.3.2). Batches of eggs were thus obtained from the faeces of each chicken and at known times *p.i.* and stored at $5^{o}C$.

Recovery of adult *Schistocephalus solidus*

On day 7 *p.i.*, the 15 chickens were killed by inhalation of CO_2 and their intestines removed and inspected for the presence of *S.solidus* adults (see Section 6.2.1). Any found were rinsed free of debris, blotted and weighed to the nearest mg, before being dried to a constant weight in a $60^{o}C$ oven and weighed to the nearest 0.01 of a mg.

6.2.3 PATTERN OF EMBRYONATION IN EGGS FROM DIFFERENT DAYS POST-INFECTION.

Preliminary experiments revealed that repeated sampling of eggs during incubation exposed them to sufficient light to induce hatching. This resulted in egg batches from many of adult worms being wasted while the experimental technique was being developed. Eventually, the rate of development of eggs at $25^{o}C$ from days 2-7 p.i. was investigated using a randomised block design to ascertain whether adult age had any effect on fecundity. The central eighteen wells of black-painted, 96 well culture plates were used to give 3 replicates (rows) of eggs from each of the six days of egg production. Six drops of eggs suspension (approximately 100 eggs per drop) from each time point, were randomly assigned to the wells of each replicate and all wells were sealed using insulating tape, thus inhibiting evaporation and illumination. To examine the extent of egg development after 2, 4 and 6 weeks incubation, separate culture plates were required for each and unfortunately had to be limited to the eggs expelled from only two

different adult worms. Such a lack of replication will present a rather conservative view egg quality, but at least can provide some information on whether it is likely to be consistent between and within plerocercoids.

6.2.4 DATA ANALYSES

Infectivity of *S.solidus* plerocercoids

The weights of the 166 plerocercoids that had been given to chickens, were grouped into 50mg classes. The proportions that had established were compared across these weight classes, using a Chi-Square test of independence.

Fecundity of adult *S.solidus*

As egg count values (e.p.g.) were normally distributed across worms, the egg outputs from the adult worms that had survived until day seven *p.i.* were compared across the infection period using a parametric one-way ANOVA. Regression analysis was used to determine whether the final adult wet weight was related to the initial wet weight of the plerocercoid. The average egg output (mean e.p.g.) was individually regressed on the plerocercoid wet weight, the wet weight loss in the transition from plercocercoid to adult, the adult dry weight and the proportional wet weight loss from plerocercoid to adult, to assess whether the fecundity of adult *S.solidus* could be related to any of these factors.

Patterns of egg embryonation

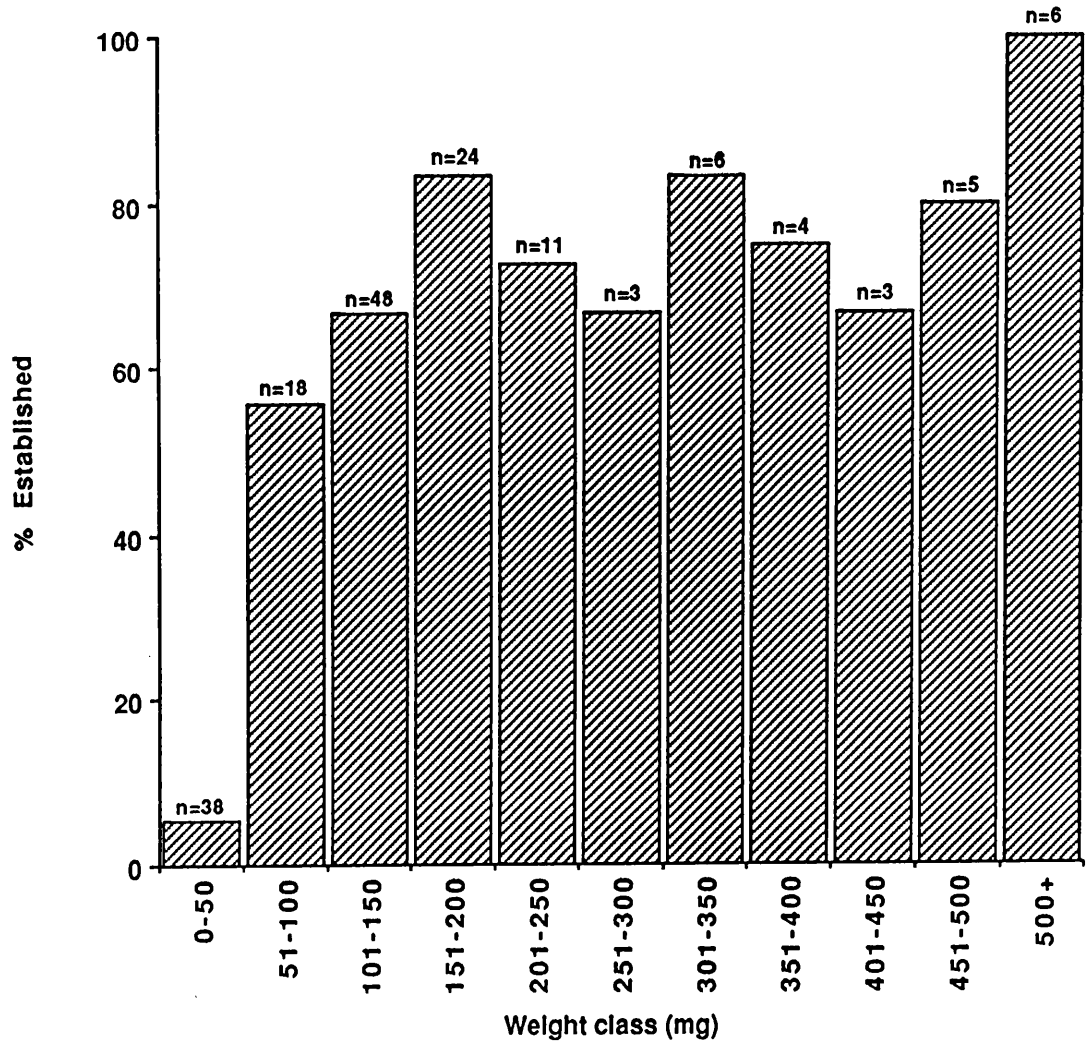
The effect of the stage in the time course of egg production on embryonation was studied separately for each adult worm by means of a Friedman two-way ANOVA by ranks. This technique allowed for the small number of replicates.

6.3 RESULTS

6.3.1 INFECTIVITY OF *SCHISTOCEPHALUS SOLIDUS* PLEROCERCOIDS TO CHICKENS, *GALLUS GALLUS*

Establishment of *S.solidus* in chickens varied greatly with plerocercoid weight (Chi-Square, $X^2=58.785$, d.f.$=5$, $P<0.001$). Notably, only 5.3% of plerocercoids up to 50mg established, compared with 55.6% in the 51-100mg weight class and in excess of 66.7% in the heavier classes (Figure 6.1). The smallest plerocercoid to establish was 26mg.

Figure 6.1: Establishment of *S.solidus* plerocercoids of different weight classes, in week-old chickens.


% Established
100
80
60
40
20
0
n=38
n=18
n=48
n=24
n=11
n=3
n=6
n=4
n=3
n=5
n=6
0-50
51-100
101-150
151-200
201-250
251-300
301-350
351-400
401-450
451-500
500+
Weight class (mg)

6.3.2 FECUNDITY OF ADULT *SCHISTOCEPHALUS SOLIDUS* IN CHICKENS, *GALLUS GALLUS*

Worm recovery and egg output

Of the 15 plerocercoids administered to chickens, 13 established (86.7%) and 10 (66.7%) survived until the termination of the experiment. The initial plerocercoid weight did not appear to influence survival to the end of the experiment, as the median initial weight of the worms that survived was not significantly different from those that did not (Kruskal Wallis ANOVA, $H=1.83$, d.f.$=1$, $P>0.05$, N.S.). The egg output of these 10 surviving adults, as described by the mean e.p.g. across the 10 infected chickens, varied significantly during the time course of the experiment (ANOVA, $F=14.74$, d.f.$=6,62$, $P<0.001$) and is presented in Figure 6.2(a). No eggs were detected in the faeces after 24h in the definitive host, but peak egg output was rapidly reached on days 2-3 *p.i.* Thereafter, the mean e.p.g. decreased and remained low until day 7 *p.i.* The pattern of *S.solidus* egg output was similar in the three adults that did not survive until day 7, but ceased earlier in the time course of infection (Figure 6.2(b)). Again, the number of eggs in the host faeces (e.p.g.) was high early in the infection and then declined, but the egg production of one of the adults was considerably lower than that of the other two.

Factors influencing adult worm weight and fecundity

The individual weights of adults seemed to be entirely unrelated to the weight of the plerocercoids from which they were derived (Linear regression ANOVA, $F=0.64$, d.f.$=1,8$, $P>0.05$, N.S., $R^2=0.0\%$) (Figure 6.3(a)). This seemed to be the result of very variable weight loss in the adults (24.3-73.9% of the initial plerocercoid weight). The average egg output into host faeces (mean e.p.g.) by each adult worm (Figure 6.3(b)) was similarly unrelated to the initial weight of the plerocercoid (Linear regression ANOVA, $F=1.08$, d.f.$=1,8$, $P>0.05$, N.S. $R^2=1.0\%$). However, as shown in Figure 6.4(a-c) the average e.p.g was predicted well by the adult dry weight (Linear regression ANOVA, $F=42.74$, d.f.$=1,8$, $P<0.001$, $R^2=83.9\%$) and to a lesser degree by the proportional wet weight loss (Linear regression ANOVA, $F=8.55$, d.f.$=1,8$, $P<0.05$, $R^2=48.6\%$), but not by the absolute wet weight loss (Linear regression ANOVA, $F=3.16$, d.f.$=1,8$, $P>0.05$, N.S., $R^2=21.3\%$).

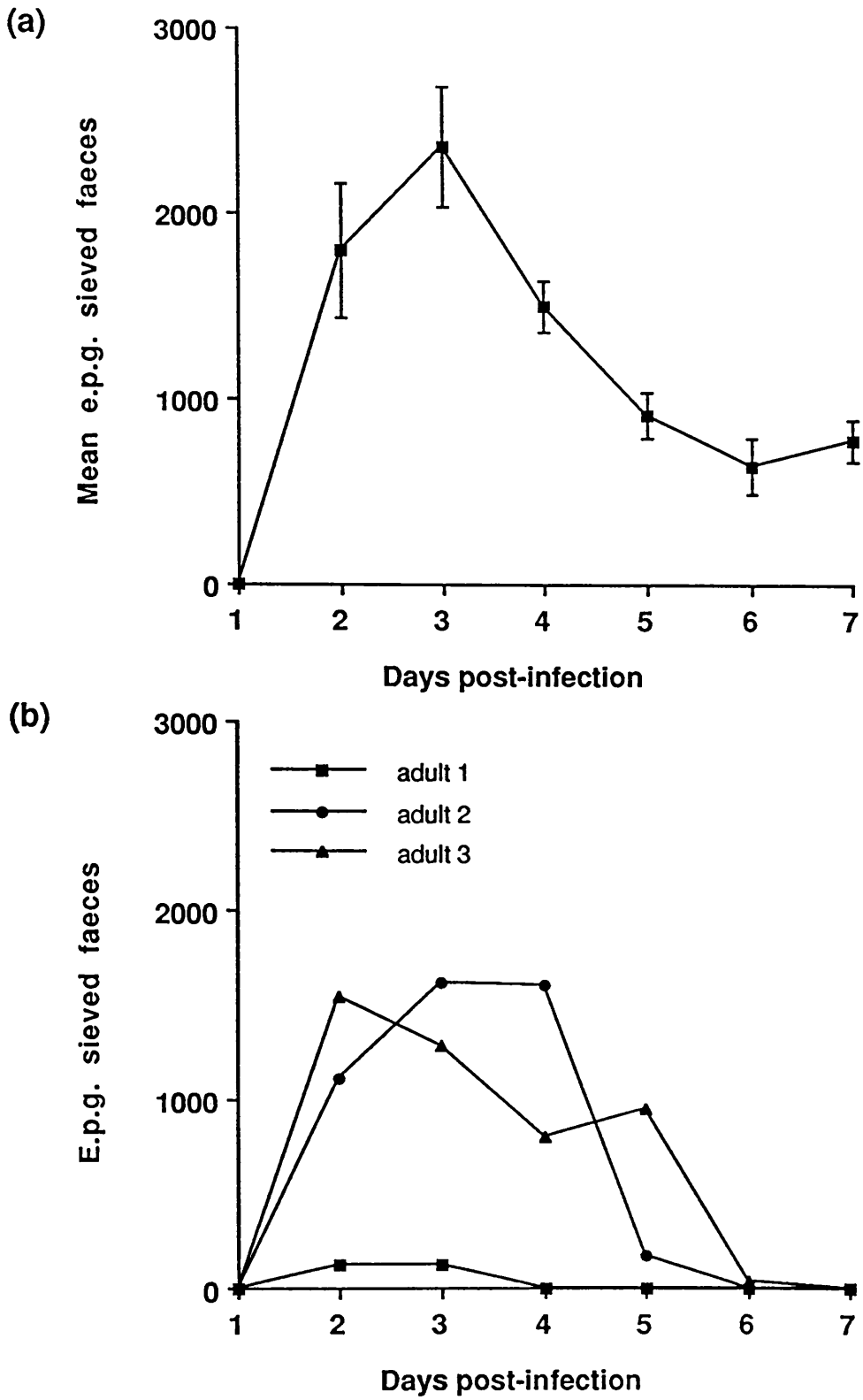


Figure 6.2: Faecal egg output of *S.solidus* adults a) surviving until day 7 *p.i.* (mean ± S.E.) and b) not recovered on day 7 *p.i.* (individual counts).

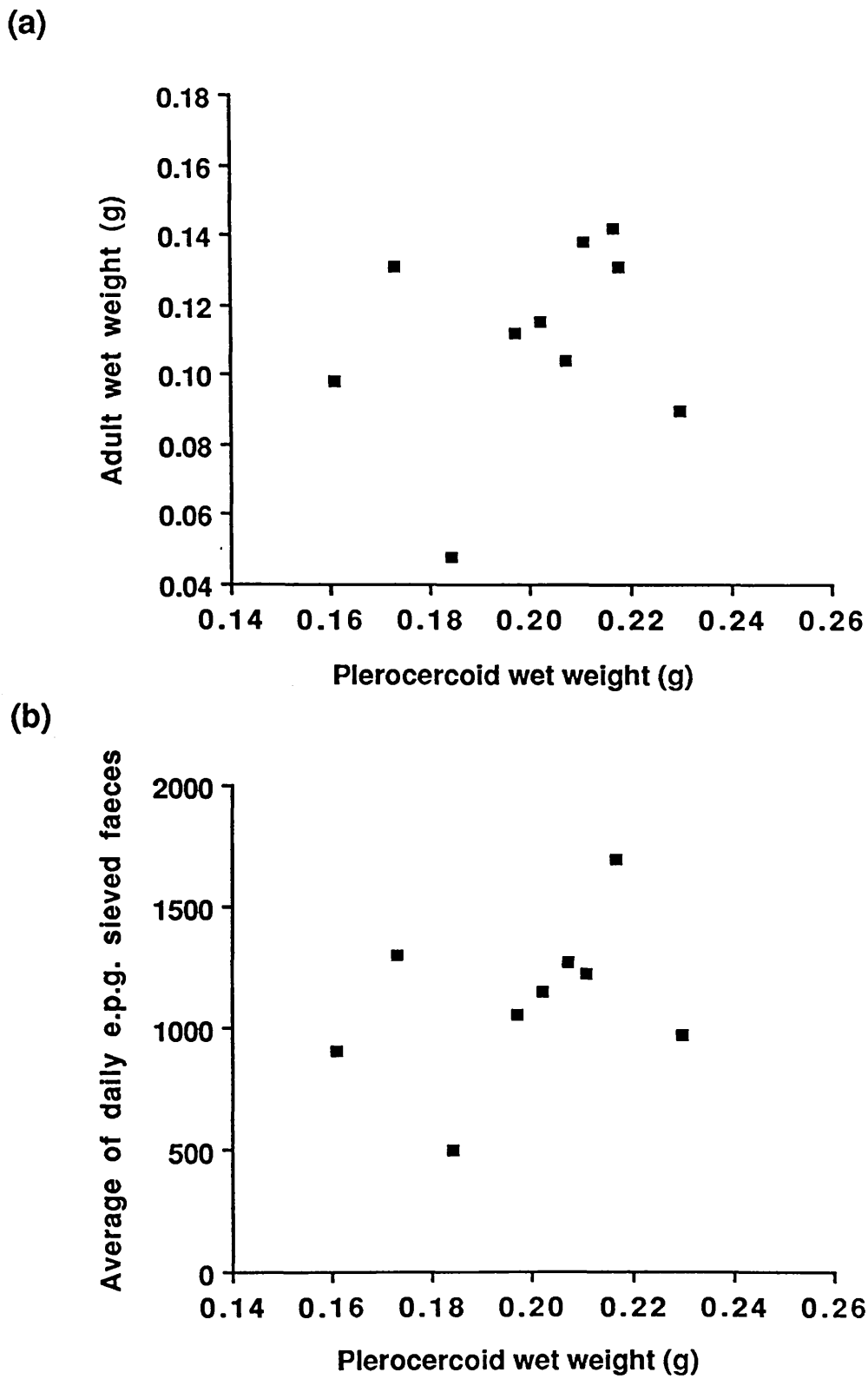
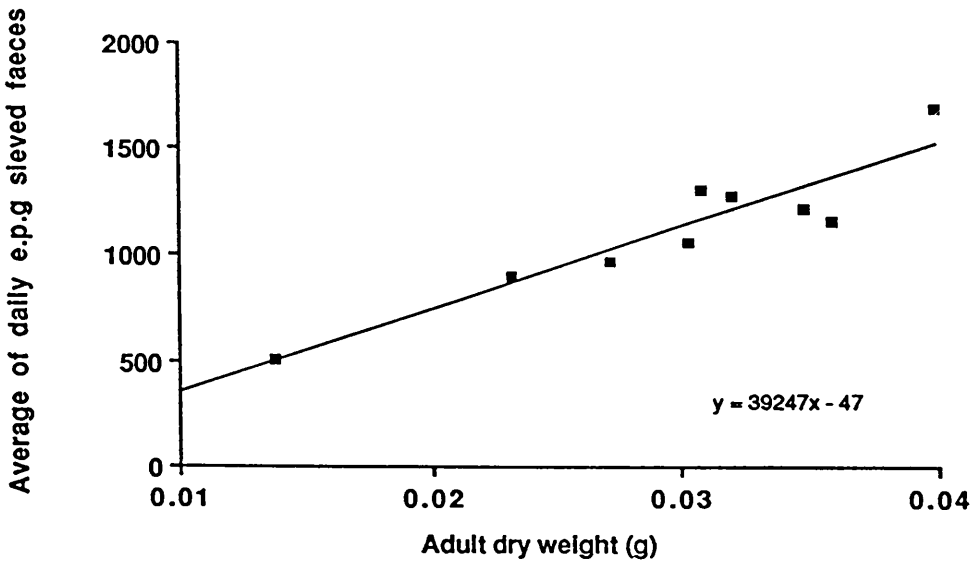


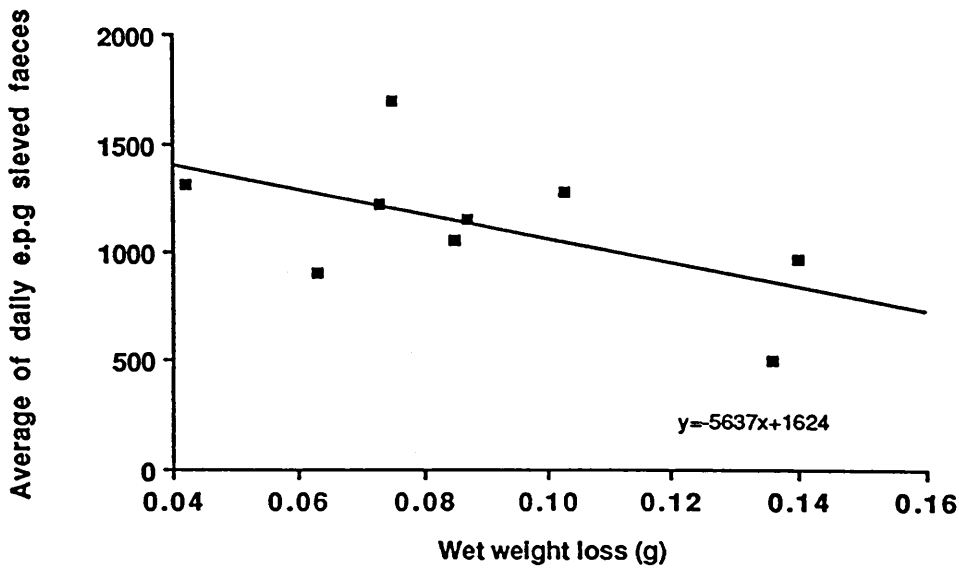
Figure 6.3: Linear regressions of a) *S.solidus* adult wet weight and b) average daily faecal egg output, on the initial wet weight of the plerocercoid.

Figure 6.4: Linear regressions of the average daily faecal egg output on *S.solidus* a) adult dry weight b) the plerocercoid wet weight loss and c) plerocercoid wet weight loss as a proportion of the initial plerocercoid wet weight.

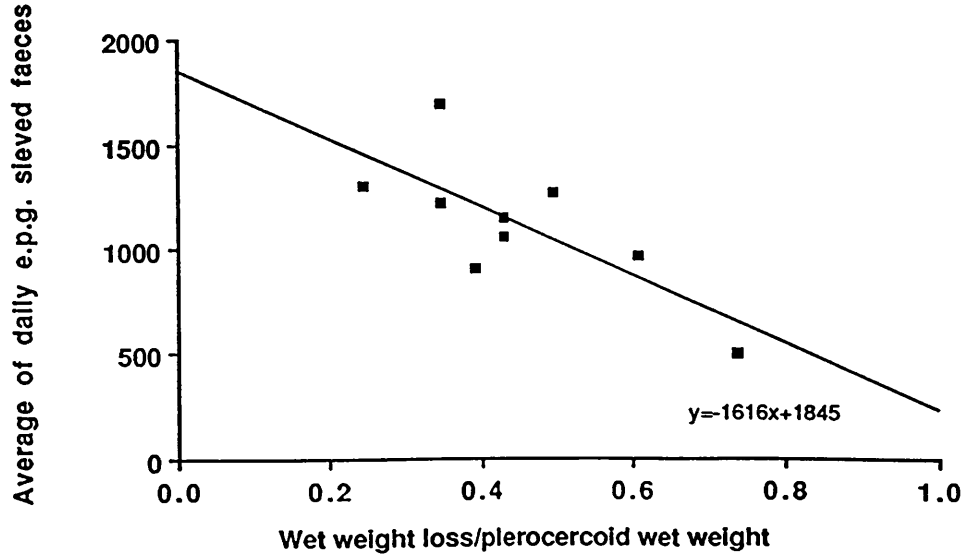
(a)


Average of daily e.p.g sieved faeces
2000
1500
1000
500
0
y = 39247x - 47
0.01
0.02
0.03
0.04
Adult dry weight (g)

(b)


Average of daily e.p.g sieved faeces
2000
1500
1000
500
0
y=-5637x+1624
0.04
0.06
0.08
0.10
0.12
0.14
0.16
Wet weight loss (g)

(c)


Average of daily e.p.g. sieved faeces
2000
1500
1000
500
0
y=-1616x+1845
0.0
0.2
0.4
0.6
0.8
1.0
Wet weight loss/plerocercoid wet weight

6.3.3 PATTERN OF EMBRYONATION IN *SCHISTOCEPHALUS SOLIDUS* EGGS FROM DIFFERENT DAYS POST-INFECTION

The pattern of embryonation in eggs derived from days 2-7 *p.i.* for two individual adults is given in Figure 6.5(a-b). Following incubation for 2 weeks, no differences were found in the proportion of embryonated eggs between batches obtained at each time point, from either of the two adult *S.solidus* (Friedman two-way ANOVA, Adult 1: $F_r=6.51$, d.f.=5, $P>0.05$, N.S.; Adult 2: $F_r=8.04$, d.f.=5, $P>0.05$, N.S.). After 4 weeks incubation, there was significant variation amongst the egg batches derived from adult 1 (Friedman two-way ANOVA, $F_r=11.33$, d.f.=5, $P<0.05$), but not in those from adult 2 (Friedman two-way ANOVA, $F_r=9.11$, d.f.=5, $P>0.05$, N.S.). This pattern was conserved after 6 weeks at 25^{O}C, with the embryonation of eggs from adult 1 varying according to when they were expelled (Friedman two-way ANOVA, $F_r=11.81$, d.f.=5, $P<0.05$), but there was an additional tendency for the extent of development to vary with the time of expulsion from adult 2 (Friedman two-way ANOVA, $F_r=9.57$, d.f.=5, $P>0.05<0.10$, N.S.). Overall there was a higher degree of embryonation in eggs derived from adult 1.

6.4 DISCUSSION

As many of the results obtained in these experiments could be the result of either parasite or host differences or both, it is important to assess the probable structure of each genome. Although plerocercoids were always derived from the same population of sticklebacks and cross-fertilisation may be limited in this species, there is reason to suspect that *S.solidus* heterogeneity does exist. The migratory life-style of the black-headed gull (*Larus ridibundus*), the proposed natural definitive host of *S.solidus* at Inverleith, makes it likely that the parasite genome will sometimes experience an influx of new genotypes, derived from other sources of the parasite. By contrast, the *G.gallus* hosts having been obtained from the same hatchery, were likely to have a rather homogeneous genetic make-up as a result of repeated inbreeding, in addition to being of similar age and the same sex.

The investigation of weight-related, infectivity of *S.solidus* to chickens extended the earlier findings of Hopkins and McCaig (1963) that small plerocercoids rarely become established in a suitable definitive host. In addition, it has been demonstrated that

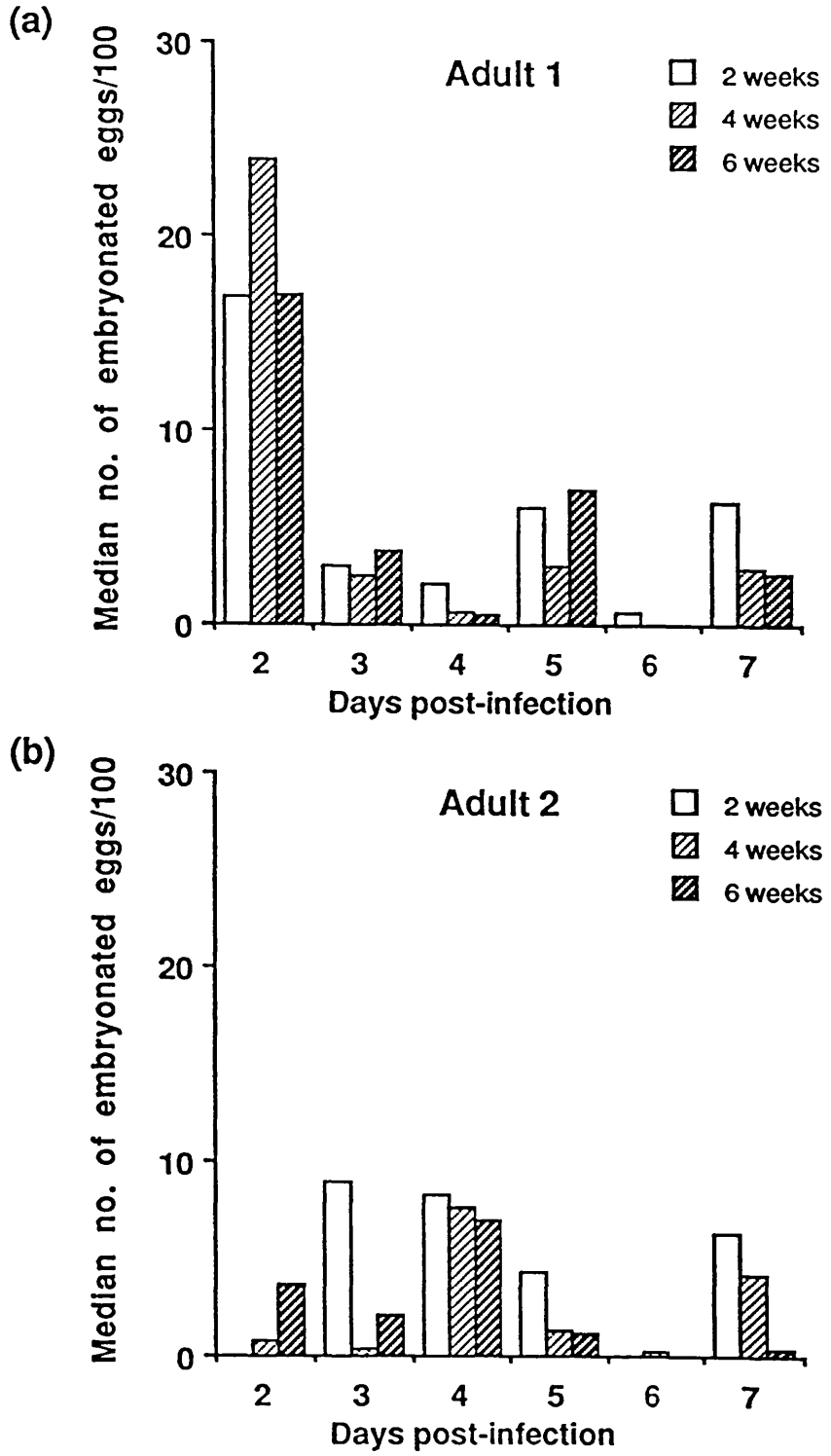


Figure 6.5: Median number of embryonated *S.solidus* eggs per 100 examined following 2, 4 and 6 weeks incubation and from faeces collected from 2-7 days *p.i.*

establishment is consistently high above a threshold weight of 50mg and not 10mg as previously implicated (Hopkins and McCaig 1963). These authors suggested that the ability of a plerocercoid to infect a final host was dependent on whether it could be retained in the host gut; Smyth and McManus (1989) proposed that the substantial longitudinal musculature of *S.solidus* enables it to brace itself against the host's intestine wall. This notion is supported by the fact that adults worms recovered in this experiment were always found to be contracted and frequently curled round the circumference of the chicken intestine, effectively forming a plug. It can be envisaged that the minimal muscular development of small worms (Clarke 1954) limits their establishment by preventing this retention mechanism. However, some small plerocercoids did establish and some large plerocercoids did not, indicating that there is an additional component to infectivity, such as the ability to survive passage to the intestine or withstand immune expulsion. The degree of establishment of plerocercoids used in the fecundity experiment was extremely consistent with that of similar-sized plerocercoids in the infectivity experiment and probably illustrates that the constraints on infectivity are intrinsic in this host-parasite relationship.

Despite the narrow size-range of plerocercoids used, much variability in the adult stage was detected. Although, the pattern of egg output was similar in all worms, beginning at day 2 *p.i.*, peaking on days 2-3 *p.i.* and declining thereafter, the quantitative output was quite variable (mean e.p.g.=504.2 - 1694.5). Secondly, weight loss was quite substantial in all adults (>20%), but in some was extreme (73.9%) and could not be attributed to obvious physical damage. The transition from plerocercoid to adult has previously been associated with weight loss (Hopkins & McCaig 1963) and regarded as inhibition of somatic growth by maturation. An *in vitro* study of plerocercoid growth lends further support to this hypothesis. Sinha and Hopkins (1967) found that somatic growth was suppressed when plerocercoids were stimulated to mature in culture and in addition they experienced weight loss which was increasingly pronounced with higher temperatures and bigger worms. They hypothesised that two enzyme systems exist in *S.solidus* with different temperature optima, one of which is responsible for somatic growth and the other for inducing reproduction. It is not known whether such weight loss is the result of glycogen depletion early in maturation, as found after 24h in a pigeon gut by Hopkins (1950).

Glycogen has been located histologically in areas undergoing late spermatogenesis in many cestode species, including the pseudophyllidean *Diphyllobothrium latum* (see Rybicka 1966) and in the vitelline cells of some other pseudophyllideans (Rybicka 1966), suggesting that embryogenesis is a candidate cause for the nutritive drain described by Hopkins (1950) and the weight loss found in this and previous studies (Hopkins & McCaig 1963; Sinha & Hopkins 1967). Also the decline in egg output during the infections suggest that the energy reserves that fuel reproduction may be progressively depleted with time.

As egg output was negatively correlated with weight loss and positively associated with the final worm weight, it would appear that the worms with the greatest reproductive output were those which lost least weight and perhaps energy reserves. Thus, for gametogenesis and egg production to have been solely responsible for the observed loss of weight, there would need to have been substantial differences in the initial levels of progenesis of the plerocercoids. Alternatively, the adults with high weight loss may have had low egg outputs, because their endogenous reserves were redirected to metabolic processes other than maturation and egg production.

Longevity of some of the adult worms was less than seven days and, judging by the results of Hopkins and McCaig (1963), the remainder of adults might have survived for as many as 15 days had the infection been allowed to continue. It has been proposed (Hopkins & McCaig 1963) that longevity is likely to be the result of intrinsic factors rather than extrinsic factors such as host immunity or physiology. The exhaustion of reserves, by reproductive output or otherwise, could have a number of consequences. If retention in the host gut is dependent on the adult musculature and the action of the muscles dependent on energy reserves (i.e. glycogen),then a depletion of metabolites could increasingly limit the retention capability causing worms to be lost. Expelled adults were never observed in the chicken faeces after egg production had ceased and so it seems that they were probably dead and degraded before being expelled. Death could also have resulted from the exhaustion of energy reserves, necessary for basic metabolism.

It is generally assumed that the diversion of energy into reproduction has consequences for the survival and/or future reproduction of the parent (Calow 1979). Many endoparasites

represent exceptions to this generalisation, for example cestodes often invest great amounts of energy into reproduction without apparent costs for future fecundity or survival and this has been coined the "parasite paradox" (Calow 1979, 1983). It has been suggested that the predominantly carbohydrate metabolism of cestodes has allowed them to evolve toward endoparasitism (Calow & Jennings 1974; Jennings & Calow 1975) and the costs of high fecundity may be offset by the rich, nutritional environment of the host intestine (Calow & Jennings 1977). However, there is evidence from studies on the cyclophyllidean cestode *Hymenolepis diminuta* to suggest that the intestinal environment has a limited carrying capacity, such that, as infection intensity is increased there is a trade-off between reproduction and the maintenance of metabolism and body size (Boddington & Mettrick 1981). As *S.solidus* adults do not extract nutrients from the definitive host, they are dependent on those assimilated by the plerocercoid stage and the consequence appears to be an expensive trade-off between reproduction and survival in the adult stage, which is extremely short-lived.

Considerable inter-worm variability in the level of egg development was apparent. Eggs expelled at different days *p.i.* from one adult *S.solidus* showed fairly uniform levels of development, suggesting that egg quality was largely unaffected by adult age. On the other hand, eggs released from another adult worm had a greater overall level of development and embryonation was maximal in eggs produced at the time of high egg output and greater overall. It may be that the latter adult with a high egg output and lower weight loss (34.6%) directed a greater proportion of its reserves into producing eggs quickly. After this initial high fecundity there may have been degeneration of the gonads, resulting in inferior quality eggs being produced later in the infection. However, given time, more eggs may have developed fully, especially as the apparent viability was low in comparison with other studies (Smyth 1946; Mason 1965). This may have been due to prolonged storage at low temperature which can result in slow development. Furthermore, having only compared two adults, the interpretation is limited although a re-examination of this aspect of *S.solidus* adult fecundity with larger sample sizes could prove instructive. Nevertheless, this experiment did reveal an intrinsic variability in both egg production and the subsequent ability of eggs to develop.

6.5. CONCLUSIONS

Using *Gallus gallus* as a definitive host, the infectivity of plerocercoids less than 50mg is extremely limited, whereas above this weight and across broad spectrum of worm sizes, the level of establishment is consistently high. This may represent a mechanical inability of small plerocercoids to be retained in the host gut. When the size range of plerocercoids administered to chickens is restricted, there is still considerable variability in the quantitative egg output from adults and in the viability of the eggs.

CHAPTER 7: FACTORS AFFECTING THE ACQUISITION OF *SCHISTOCEPHALUS SOLIDUS* INFECTION IN A FIRST INTERMEDIATE HOST, *ACANTHOCYCLOPS VIRIDIS*

7.1 INTRODUCTION

Data are available on the growth (Clarke 1954) and host specificity of the procercoid stage of *Schistocephalus solidus* in the copepod first intermediate host (Clarke 1954; Orr & Hopkins 1969). However, there is a lack of information on the acquisition of infection by copepods and the epidemiology of procercoid infections either in the field or laboratory. Such details would prove invaluable in explaining transmission of *S.solidus* to the stickleback host and the subsequent epidemiology (Chapter 3). An extensive survey for helminth procercoids in copepods in the Lakes of Karelia (Sysoev 1987), revealed that *S.pungitii* existed at a very low prevalence (maximum 0.078%) and was only observed in late summer and autumn, suggesting that the conditions under which infections take place are rare. This may also be the case for *S.solidus*, but no quantitative investigation has been made. This chapter describes a series of experiments designed to investigate factors that might influence the acquisition of *S.solidus* infection by *Acanthocyclops viridis*. This was achieved by manipulation of characteristics of both the coracidial infective stages and the copepod hosts and subsequently quantifying levels of infection.

7.1.1 PREVIOUS STUDIES OF EXPERIMENTAL EPIDEMIOLOGY

A general trend in this research has been to focus on, or manipulate, a single aspect of a particular host-parasite interaction, whilst keeping other ecological variables constant and then to monitor the effects on the resultant infection parameters such as prevalence, intensity and the distribution of parasite numbers amongst hosts. Some of the results of these studies are described below.

Manipulation of infective stages

Manipulation of the density of infective stages has been found to affect the subsequent intensity of parasitic infection and the frequency distribution of numbers of parasites per host. Following a fixed period of exposure to the cercariae of *Transversotrema patialense*, the number of cercariae attached to zebra danios (*Brachydanio rerio*) was found to be proportional to the cercarial density (Anderson, Whitfield & Dobson 1978). Similarly, the number of *Hymenolepis diminuta* recovered from flour beetles (*Tribolium confusum*) increased to a plateau in association with an increase in the density of infective eggs (Keymer & Anderson 1979). In

addition, Anderson *et al* (1978) found that the extent of over-dispersion of *T.patialense* in zebra danios was positively related to the density of cercariae to which the fish had been exposed.

The distribution of infective stages has also been found to influence the acquisition of parasitic infections. Exposing groups of *T.confusum* to the same density of *H.diminuta* eggs, but with increasingly aggregated spatial distributions, resulted in the worms being increasingly more aggregated in the beetles (Keymer & Anderson 1979). In the same study, beetles were exposed to eggs that had spent different periods of time in sub-optimal conditions. Those beetles that had been subjected to eggs maintained longest in adverse conditions subsequently harboured the lowest worm burdens, presumably as a consequence of diminishing viability of the eggs with time.

Observation and manipulation of hosts

When infective stages are held constant, the impact of the host on infection dynamics can be elucidated. A number of studies have revealed that host susceptibility plays an important role in the pattern of parasitic infection. The genetically-controlled resistance of the white-footed deer mouse (*Peromyscus maniculatus*) to *Hymenolepis citelli* provides sufficient heterogeneous host susceptibility to generate over-dispersion in this host-parasite system (Wassom, Dick, Arnason, Strickland & Grundman 1986). Anderson *et al* (1978) suggested that differences in the behaviour of zebra danios affected their susceptibility to *T.patialense* cercariae, with fish that were more active during exposure acquiring the highest infection intensities; such an effect would generate the observed over-dispersion of the parasite numbers amongst the fish. Keymer (1982) found that host age was a contributing factor in the susceptibility of *T.confusum* to *H.diminuta*.

Host density also appears to influence the acquisition of parasitic infections. An increase in the density of flour beetles exposed to *H.diminuta* resulted in an increase in the total number of *H.diminuta* cysticercoids harboured, but also provoked a simultaneous decrease in the number of parasites per beetle (Keymer 1982). The local density of the snail *Lymnaea peregra* was manipulated by altering their distribution in an infection arena before cercariae of *Echinoparyphium recurvatum* were released into the arena (McCarthy 1990). The subsequent prevalence and intensity of infection were highest in snail groups that had been clumped during

the period of exposure.

Altering the duration of exposure to infective stages has two identifiable outcomes. Keymer (1982) detected higher burdens of *H.diminuta* in flour beetles that had been in the presence of infective eggs for the longest time. However, in both zebra danios exposed to *T.patialense* (Anderson *et al.* 1978) and laboratory mice exposed to the pinworms *Syphacia obvelata* and *Aspiculuris tetraptera* (Scott & Gibbs 1989), greater over-dispersion of parasite numbers was detected as the period of exposure lengthened. In each case, this was attributed to heterogeneous host susceptibility.

Where infective stages of the parasite are ingested by the host, hunger has been found to affect infection levels. Starved flour beetles acquired much higher average worm burdens than their satiated counterparts, when exposed to eggs of *H.diminuta* (Keymer 1982).

The studies described above have examined the processes that influence host-parasite relationships in aquatic systems where infection takes place via parasite attachment to the host surface (Anderson *et al.* 1978) or by direct penetration of the host surface (Anderson 1978). Also studies have been made in a terrestrial host-parasite relationship where there is ingestion of the infective stage by the host (Keymer & Anderson 1979; Keymer 1982). The purpose of present chapter is to extend the present knowledge of one aspect of infection dynamics by investigating the acquisition of infection in a aquatic host-parasite system where the host becomes infected by ingesting a free-living and motile infective stage.

7.1.2 MODE OF INFECTION OF THE COPEPOD FIRST INTERMEDIATE HOST OF *SCHISTOCEPHALUS SOLIDUS*

Although actual ingestion of coracidia has yet to be observed in experimental infections of copepods with *S.solidus* (Clarke 1954; Mason 1965), evidence from other pseudopyllidea indicate that this is almost certainly the mode of infection. Transformation to the oncosphere in the intestine of copepods has been detected in *Bothriocephalus claviceps* (Dupont & Gabrion 1987), *Spirometra mansonoides* (Mueller 1959) and *Diphyllobothrium dendriticum* (Sharp, Secombes & Pike 1990). In addition, the oncospheres of *D.dendriticum* have been observed locating and penetrating the luminal surface of the copepod intestinal wall (Sharp *et al.* 1990). Despite prolonged observation of copepods in the presence of *S.solidus* coracidia, Clarke

(1954) failed to observe any attraction between host and parasite other than what would be expected by random movement. This was reiterated by Mueller (1959) with regard to *S.mansonoides* and he further observed that coracidia are not actively pursued by copepods. Indeed, *S.solidus* infection is not limited to carnivorous copepods, suggesting that this species may also be accidentally ingested rather than hunted as prey, especially as infection levels in the herbivorous species *Eucyclops agilis* were as high as in the carnivorous *Acanthocyclops viridis* (Mason 1965).

7.1.3 AIMS

The work detailed in this chapter utilised the *A.viridis/S.solidus* system to investigate aspects of the host-parasite relationship. Experiments were designed to alter the relative densities of copepods and coracidia to determine the conditions that are optimal for the acquisition of infection. This involved manipulation of coracidial density, *A.viridis* density and the volume of the habitat with the purpose of altering coracidia/copepod proximity. In addition, hunger was manipulated in order to see whether it correlated with the subsequent infection status of *A.viridis* and the length of the exposure period was varied to examine whether infection was cumulative. In all cases the pattern of dispersion was examined and used to give clues to the processes involved in parasite acquisition.

7.2 GENERAL MATERIALS AND METHODS

7.2.1 EXPERIMENTAL HOSTS AND PARASITES

Acanthocyclops viridis

Using egg separation chambers (Chapter 5), batches of copepods of 6-8 days old and of similar size were obtained. Only copepodites were selected for experimental infections, to prevent uncontrolled breeding within experimental groups and to limit possible effects of reproductive status on susceptibility. In order to manipulate the extent of food deprivation, copepods were removed from culture, and thus separated from their food supply at various times prior to exposure to coracidia. This involved draining cultures of similar-aged copepods in a 150μm mesh sieve and washing them with water to remove as much residual protozoa as possible (some algae invariably remained), before rinsing the copepods into a crystallizing dish in a small volume of water. Groups of similar-sized copepodites could then be selected for

experimental infections.

Schistocephalus solidus

Coracidia were derived from embryonated eggs as described in Chapter 5. Following centrifugation to remove egg debris, the suspension of coracidia was progressively diluted with water until approximately one per drop (from a Pasteur pipette) was obtained (Chapter 5). Prior to each experiment, 30 drops of coracidial suspension were examined to determine the actual range and mean (± S.E. of the mean) number of coracidia per drop.

Preparation of experimental containers

Six-well plastic culture plates with lids were used as experimental containers. Each experiment was confined to a single plate, with treatments being applied to separate wells. As the volume in the experimental chamber, and thus the concentration of coracidia, may have important effects on the acquisition of *S.solidus* infection, the initial volume was standardised at 3ml. This volume of water was added to the wells in each experimental plate and the top and bottom of the meniscus demarcated with a waterproof pen. The culture plates were then emptied and dried so that when copepods were added, the volume could be made up to 3ml. This procedure was modified for the experiment to test whether the infection volume affected acquisition of infection. In order to expose copepods to infection in the dark, a black-painted culture plate was used. The outside of the plate and lid were thoroughly covered with matt black spray paint, well in advance of the experiment so that any noxious vapours had disappeared.

At 5-7 days *p.i.*, all copepods were teased apart under a high power dissecting microscope (Chapter 5) to determine the prevalence, intensity and distribution of *S.solidus* procercoids.

7.2.2 DATA ANALYSES

To compare the prevalence of infection across treatments and within experiments, a Chi-Square test of independence was applied to the raw frequencies of uninfected and infected copepods. As the number of procercoids per infected copepod (intensity) was rarely normally distributed, and because the number of infected copepods was frequently small, a non-parametric, Kruskal Wallis, one-way ANOVA was used to compare the parasite burdens that

resulted from different treatments. The variance-to-mean ratios were calculated for the distributions of procercoid numbers per host for each treatment and values of d obtained and used to determine whether distributions were random or over-dispersed (see Chapter 3).

7.3 EXPERIMENTAL PROTOCOLS AND RESULTS

7.3.1 EXPOSURE OF *ACANTHOCYCLOPS VIRIDIS* TO DIFFERENT DENSITIES OF *SCHISTOCEPHALUS SOLIDUS* CORACIDIA

Thirty copepods were added to each well of a culture plate, the volumes were made up to 3ml with water and at least 4h allowed to pass before coracidia were added. Each successive group was exposed to a greater number of coracidia (10, 20, 30, 40, 60, 80 drops, mean=1.36 ± 0.195 coracidia per drop) and to make the final infection volumes equal, an appropriate number of water drops also was added to each well (70, 60, 50, 40, 20 and 0). Once no coracidia were visible, protozoa were added to the wells and the plate transferred to a room with constant temperature (25^{o}C) and photoperiod (12L/12D).

When the coracidial density was increased there was a resultant increase in the prevalence of *S.solidus* infection (Chi-Square, X^2=26.157, d.f.=5, P<0.001; Figure 7.1(a)). The median intensity of infection was low (Figure 7.1(b)), but did appear to be associated somewhat with the number of coracidia administered to copepods (Kruskal Wallis ANOVA, H=10.69, d.f.=5, P=0.059). Over-dispersion of procercoid numbers in *A.viridis* was only apparent in the group exposed to 60 drops of coracidial suspension, yet all other treatments led to a random pattern (Figure 7.2(a-f)).

7.3.2 EXPOSURE OF DIFFERENT DENSITIES OF *ACANTHOCYCLOPS VIRIDIS* TO *SCHISTOCEPHALUS SOLIDUS* CORACIDIA

A different density of copepods (10, 20, 30, 40, 50 and 60) was added to each well of a culture plate and the volume in each well made up to 3ml with water. After a minimum of 4h without protozoa, 30 drops of coracidial suspension (mean=1.267 ± 0.197 coracidia per drop) were added to all groups. When coracidia were no longer detectable, the copepods were fed with protozoa and maintained in a room with constant temperature and photoperiod (see above).

Manipulation of the copepod density had a highly significant effect on the prevalence of

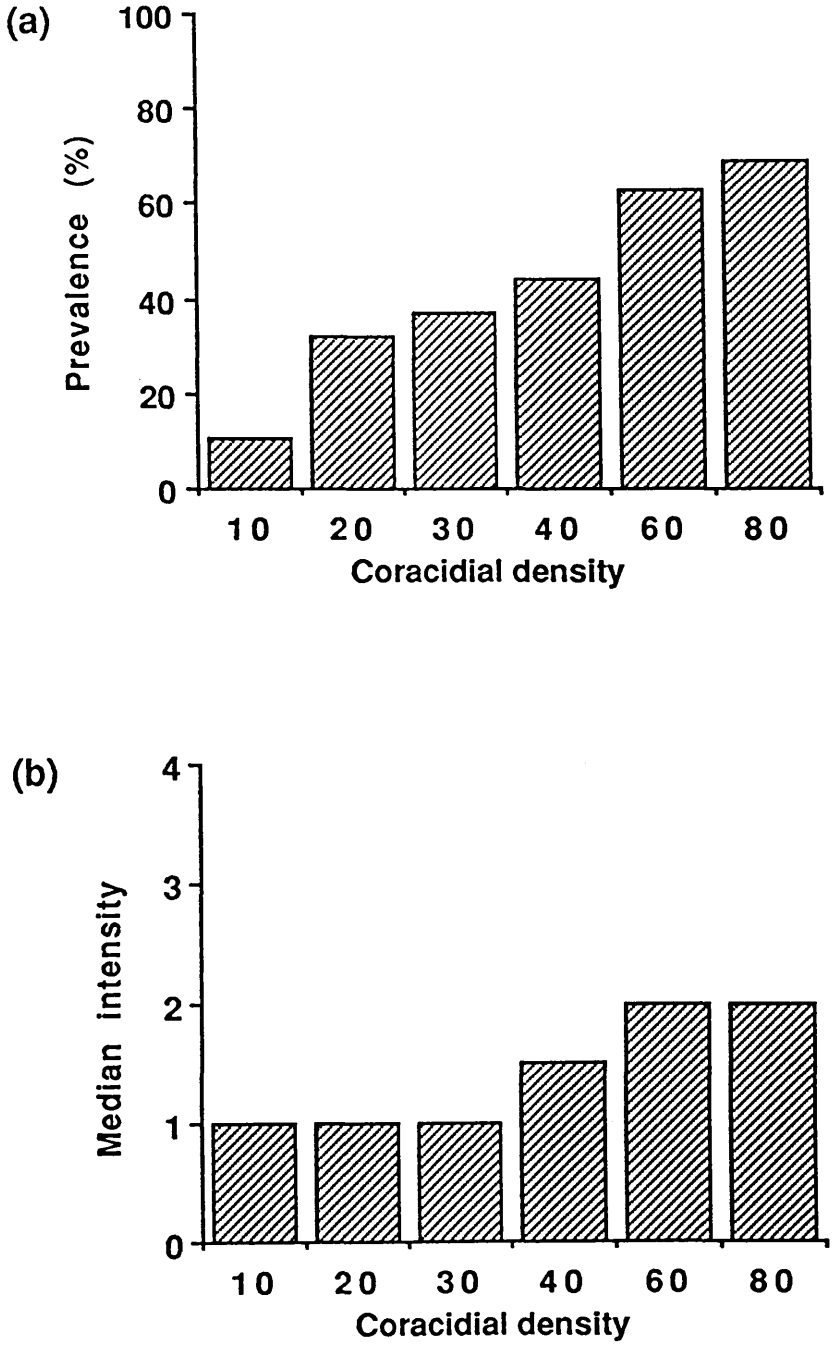
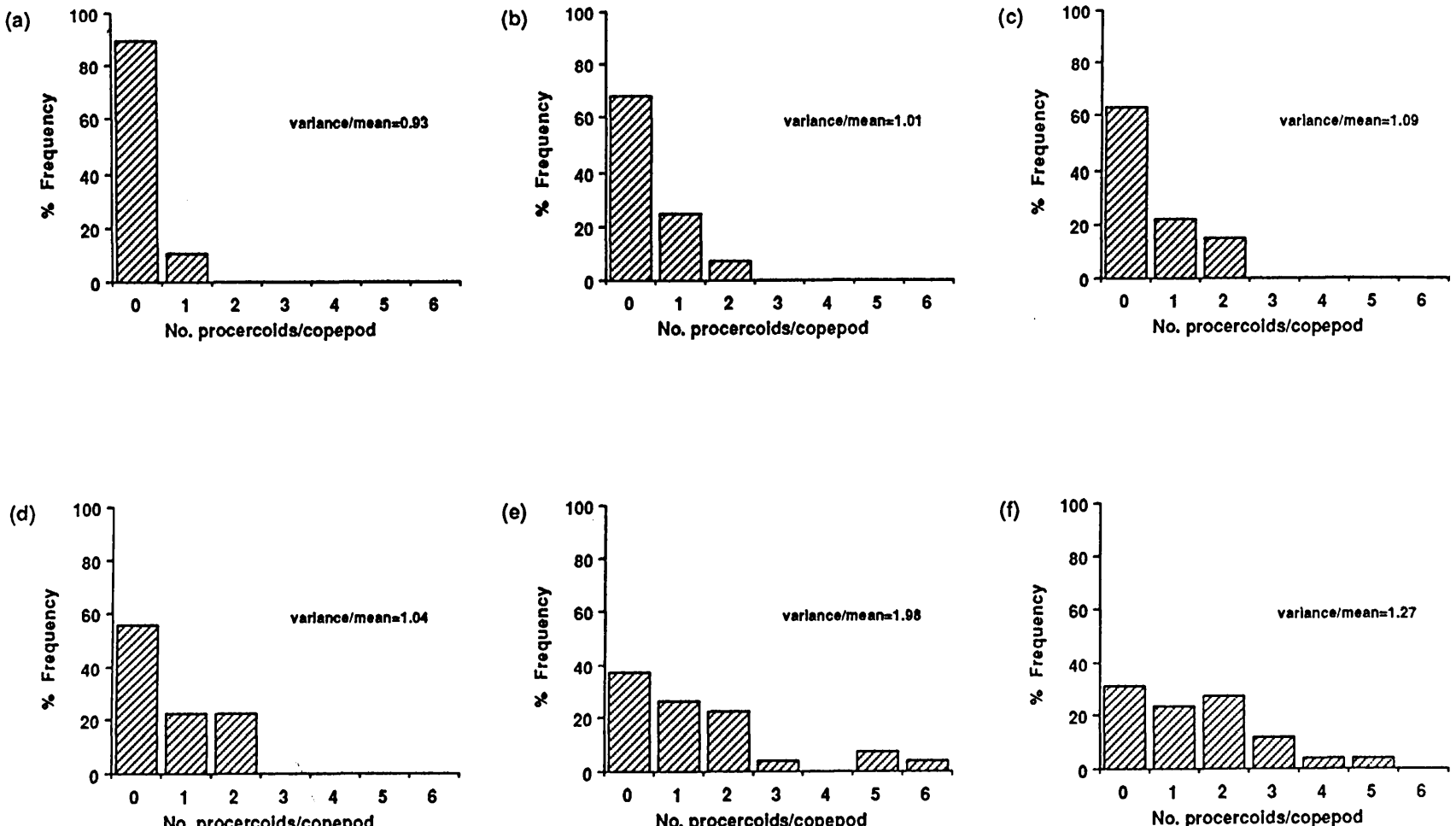


Figure 7.1: a) Prevalence and b) median intensity of *S.solidus* infection in *A.viridis* exposed to approximately 10, 20, 30, 40, 60 and 80 coracidia.

Figure 7.2: Frequency distributions of *S.solidus* procercoid numbers per host and corresponding variance-to-mean ratios in *A.viridis* exposed to approximately a) 10 b) 20 c) 30 d) 40 e) 60 and f) 80 coracidia.


(a)
% Frequency
100
80
60
40
20
0
variance/mean=0.93
0 1 2 3 4 5 6
No. procercoids/copepod
(b)
% Frequency
100
80
60
40
20
0
variance/mean=1.01
0 1 2 3 4 5 6
No. procercoids/copepod
(c)
% Frequency
100
80
60
40
20
0
variance/mean=1.09
0 1 2 3 4 5 6
No. procercoids/copepod
(d)
% Frequency
100
80
60
40
20
0
variance/mean=1.04
0 1 2 3 4 5 6
No. procercoids/copepod
(e)
% Frequency
100
80
60
40
20
0
variance/mean=1.98
0 1 2 3 4 5 6
No. procercoids/copepod
(f)
% Frequency
100
80
60
40
20
0
variance/mean=1.27
0 1 2 3 4 5 6
No. procercoids/copepod

infection (Chi-Square, X^2=28.924, d.f.=5, P<0.001). Many of the copepods in the low density groups acquired infections, but the proportion acquiring infection decreased in the higher density groups (Figure 7.3(a)). Furthermore, the median intensity of infection was greatest at the lowest copepod density (Figure 7.3(b)) and similar at all other densities (Kruskal Wallis ANOVA, H=12.72, d.f.=5, P<0.05). However, there were no associated changes in the distribution of procercoid numbers in the various treatment groups, but rather a consistently random dispersion pattern (Figure 7.4(a-f)).

7.3.3 EXPOSURE OF *ACANTHOCYCLOPS VIRIDIS* TO *SCHISTOCEPHALUS SOLIDUS* CORACIDIA IN DIFFERENT INFECTION VOLUMES

Groups of 30 copepods were contained in volumes of 1, 2, 3, 4, 5, and 6ml of water for at least 4h before coracidia were administered. Each group received 30 drops of coracidia (mean=0.867 ± 0.133 per drop) and when all were either eaten or dead, the copepods were fed on protozoa and kept in conditions of constant temperature and photoperiod (see above).

Alteration of the infection volume and thus the overall concentration of coracidia, did not appear to alter the acquisition of *S.solidus* infection. Neither the prevalence (Chi-Square, X^2=8.485, P>0.05, N.S.) nor the intensity (Kruskal Wallis, H=4.67, d.f.=5, P>0.05, N.S.) varied with infection volume (Figure 7.5(a-b)). The distribution of procercoid numbers in the copepods was also independent of the infection volume, being random in each case (Figure 7.6(a-f)).

7.3.4 EXPOSURE OF *ACANTHOCYCLOPS VIRIDIS* TO *SCHISTOCEPHALUS SOLIDUS* CORACIDIA IN THE LIGHT AND IN THE DARK, FOLLOWING DIFFERENT PERIODS OF FOOD DEPRIVATION

Thirty copepods were selected at 4, 2 and 0h prior to exposure to coracidia and added to individual wells of a culture plate (so that hunger would vary across groups) and the volume in all wells was made up to 3ml. Each group was then exposed to 30 drops of coracidial suspension (mean=1.233 ± 0.202 coracidia per drop), the lids were replaced and the plates were left at 25^{o}C until no coracidia were apparent (as verified by examination with a high-power dissecting microscope). Following exposure, protozoa were added to the wells and the plates were transferred to a room with constant photoperiod and temperature (see above).

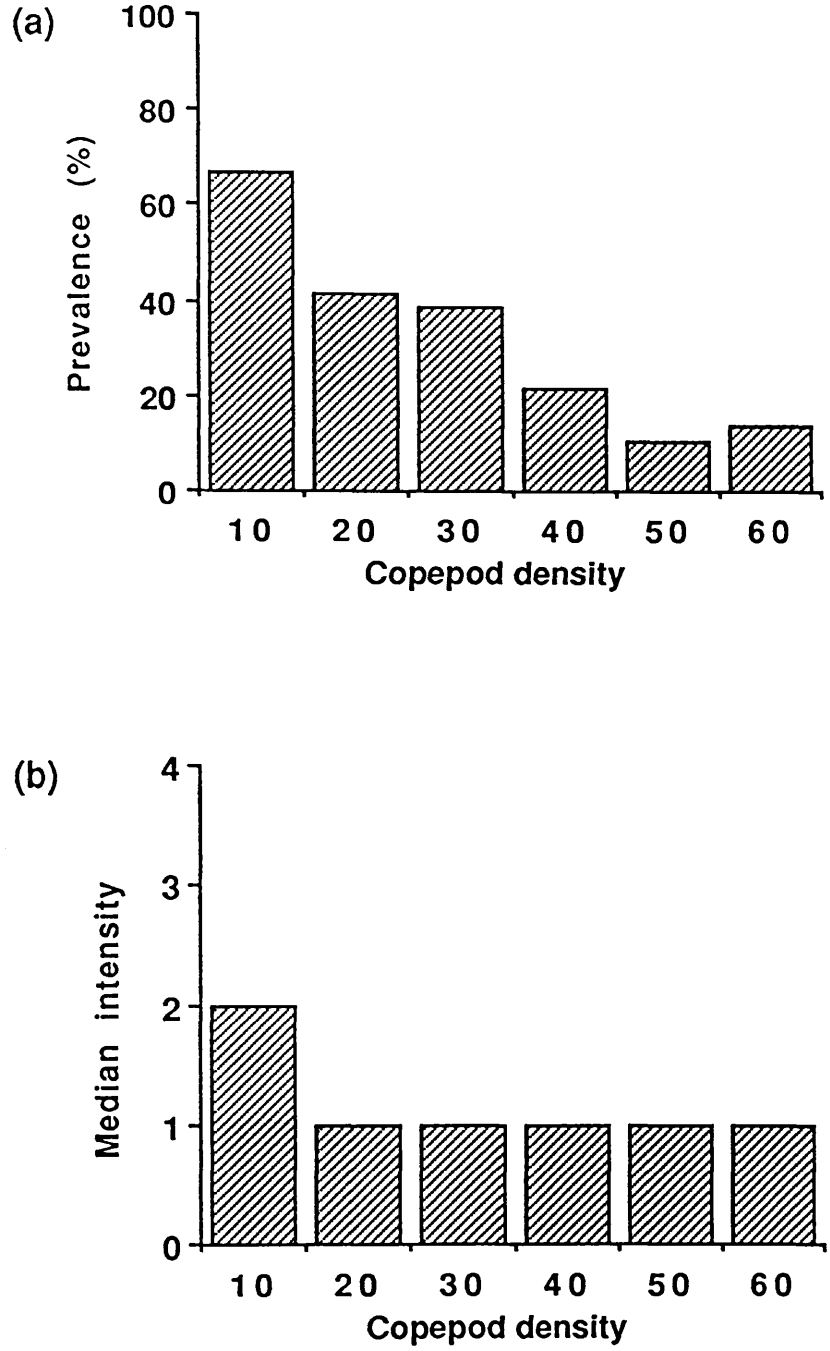
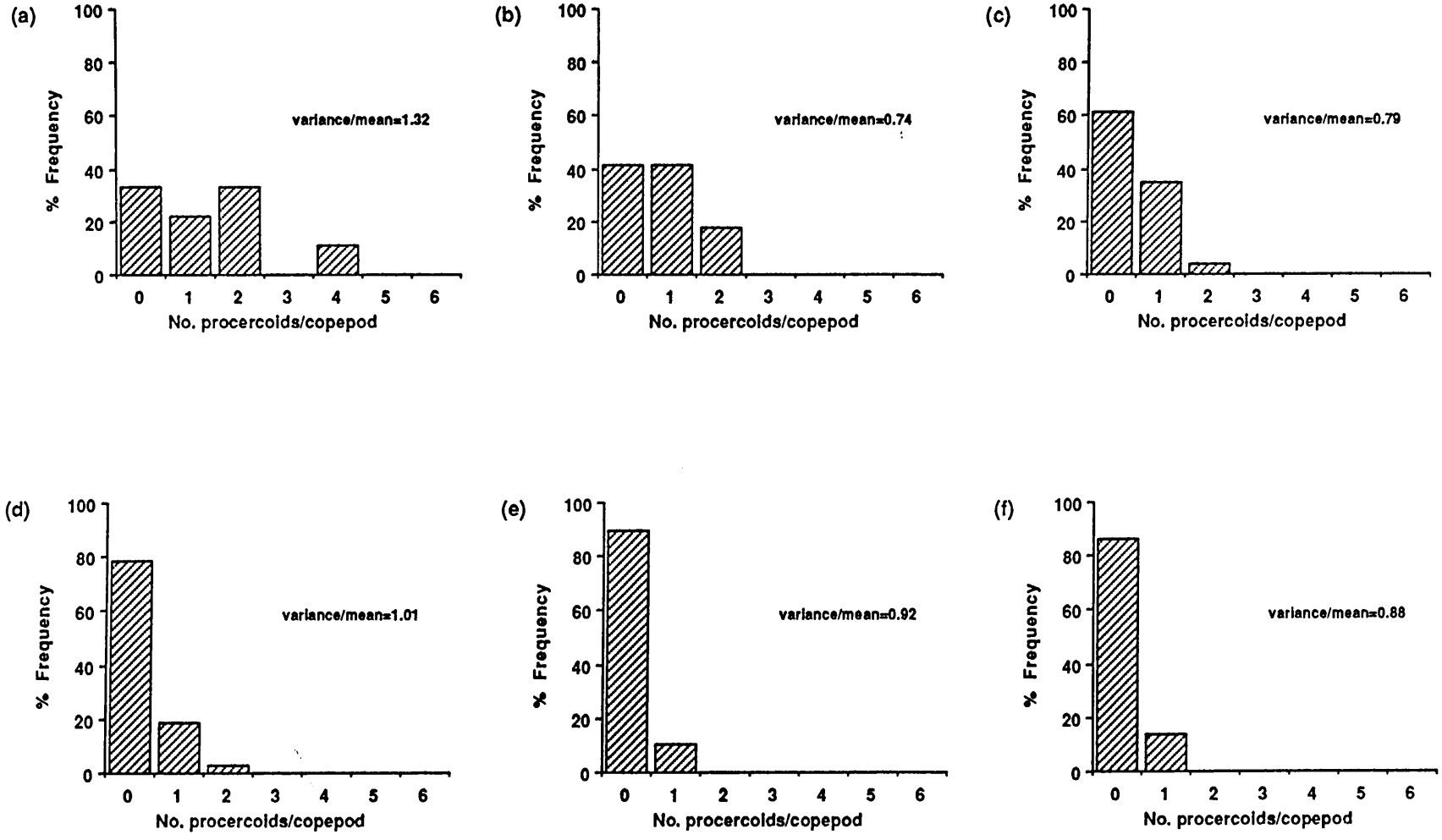


Figure 7.3: a) Prevalence and b) median intensity of *S.solidus* infection in *A.viridis* exposed to coracidia in densities of 10, 20, 30, 40, 50 and 60 copepods.

Figure 7.4: Frequency distributions of *S.solidus* procercoid numbers per host and corresponding variance-to-mean ratios in *A.viridis* exposed to coracidia in densities of a) 10 b) 20 c) 30 d) 40 e) 50 and f) 60 copepods.


(a)
% Frequency
100
80
60
40
20
0
variance/mean=1.32
0 1 2 3 4 5 6
No. procercoids/copepod
(b)
% Frequency
100
80
60
40
20
0
variance/mean=0.74
0 1 2 3 4 5 6
No. procercoids/copepod
(c)
% Frequency
100
80
60
40
20
0
variance/mean=0.79
0 1 2 3 4 5 6
No. procercoids/copepod
(d)
% Frequency
100
80
60
40
20
0
variance/mean=1.01
0 1 2 3 4 5 6
No. procercoids/copepod
(e)
% Frequency
100
80
60
40
20
0
variance/mean=0.92
0 1 2 3 4 5 6
No. procercoids/copepod
(f)
% Frequency
100
80
60
40
20
0
variance/mean=0.88
0 1 2 3 4 5 6
No. procercoids/copepod

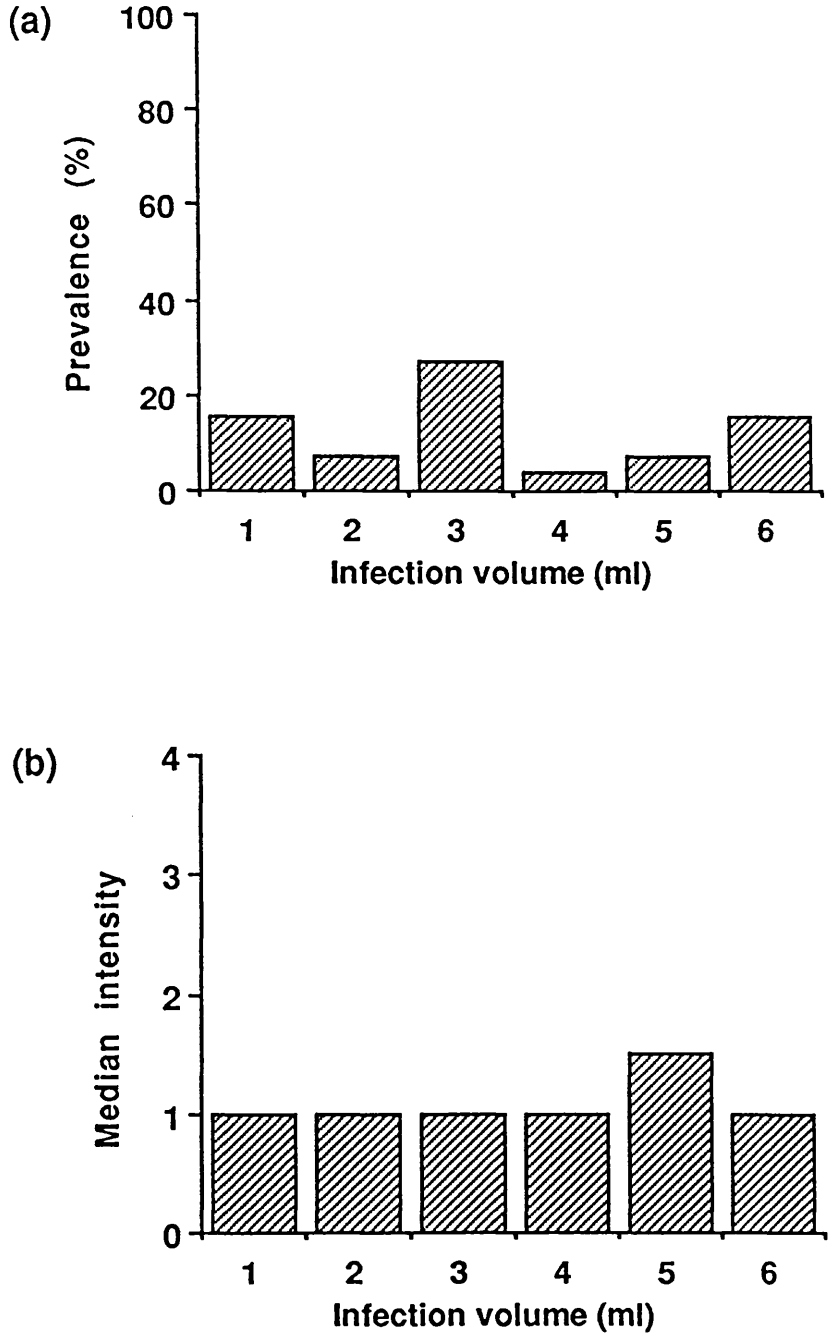
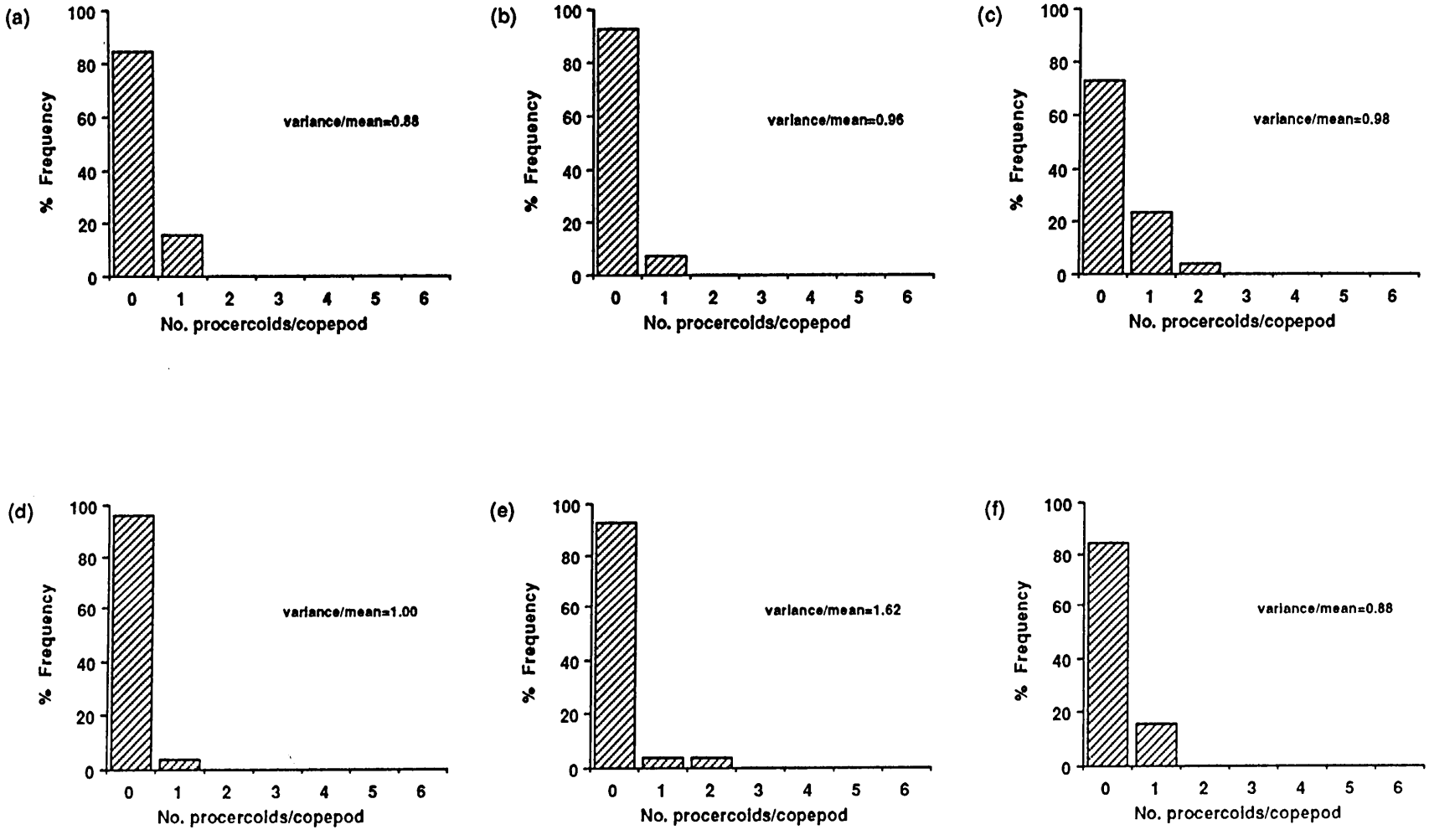


Figure 7.5: a) Prevalence and b) median intensity of *S.solidus* infection in *A.viridis* exposed to coracidia in 1, 2, 3, 4, 5 and 6ml.

Figure 7.6: Frequency distributions of *S.solidus* procercoid numbers per host and corresponding variance-to-mean ratios in *A.viridis* exposed to coracidia in a) 1 b) 2 c) 3 d) 4 e) 5 and f) 6ml.


(a)
% Frequency
100
80
60
40
20
0
variance/mean=0.88
0 1 2 3 4 5 6
No. procercoids/copepod
(b)
% Frequency
100
80
60
40
20
0
variance/mean=0.96
0 1 2 3 4 5 6
No. procercoids/copepod
(c)
% Frequency
100
80
60
40
20
0
variance/mean=0.98
0 1 2 3 4 5 6
No. procercoids/copepod
(d)
% Frequency
100
80
60
40
20
0
variance/mean=1.00
0 1 2 3 4 5 6
No. procercoids/copepod
(e)
% Frequency
100
80
60
40
20
0
variance/mean=1.62
0 1 2 3 4 5 6
No. procercoids/copepod
(f)
% Frequency
100
80
60
40
20
0
variance/mean=0.88
0 1 2 3 4 5 6
No. procercoids/copepod

When copepods in various states of hunger were exposed in the light to 30 drops of coracidial suspension, there appeared to be little effect on the prevalence of *S.solidus* infection (Chi-Square, X^2=2.523, d.f.=2, P>0.05, N.S.) and overall, only a small proportion of copepods (12.0-29.6%) were found to harbour procercoids (Figure 7.7(a)). Similarly, the median intensity of infection was low (1-2 procercoids per infected copepod, Figure 7.7(b)) and unaffected by the prior period of food deprivation (Kruskall Wallis ANOVA, H=4.19, d.f.=2, P>0.05, N.S.). The distribution of procercoids in *A.viridis* from each treatment group and the associated variance-to-mean ratios are given in Figure 7.8(a-c). There is a tendency for the parasite to be distributed in an almost random fashion after 0 and 2h food deprivation, but becomes clumped in copepods that have been deprived of food for 4h before exposure to coracidia.

Again 30 copepods were selected 4, 2 and 0h prior to exposure to coracidia, but this time added to individual wells of a blacked out culture plate to allow exposure to be carried out in the dark (to inhibit the visual capacity of the copepods). They were also exposed to thirty drops of coracidial suspension (mean=1.233 ± 0.202 coracidia per drop) and transferred to a controlled light and temperature room (see above).

The pattern is slightly different when the same treatments are carried out in the dark. Again the level of hunger had no impact on the subsequent prevalence of infection (Chi-Square, X^2=3.149, d.f.=2, P>0.05, N.S.) and the percentage of infected copepods (Figure 7.9(a)) was generally low (17.9-37.0%). Also, the median intensity of infection was low (Figure 7.9(b)) and varied little across groups (Kruskal Wallis ANOVA, H=2.00, d.f.=2, P>0.05, N.S.). In contrast to the situation in the light, there was a clumped distribution of procercoids in the group that had been subjected to coracidia when satiated and a random distribution of parasites following 2 and 4h food deprivation (Figure 7.10(a-c)).

7.3.5 EXPOSURE OF *ACANTHOCYCLOPS VIRIDIS* TO *SCHISTOCEPHALUS SOLIDUS* CORACIDIA FOR DIFFERENT PERIODS OF TIME

Thirty copepods were added to each well of a culture plate, the volume was made up to 3ml with water and the copepods were deprived of planktonic food in this way for at least 4h. Groups were exposed to 30 drops coracidial suspension (mean=1.3 ± 0.237 coracidia per drop) and after 15min, 30min, 1h, 2h, 4h and 5h the exposure period was terminated by draining the

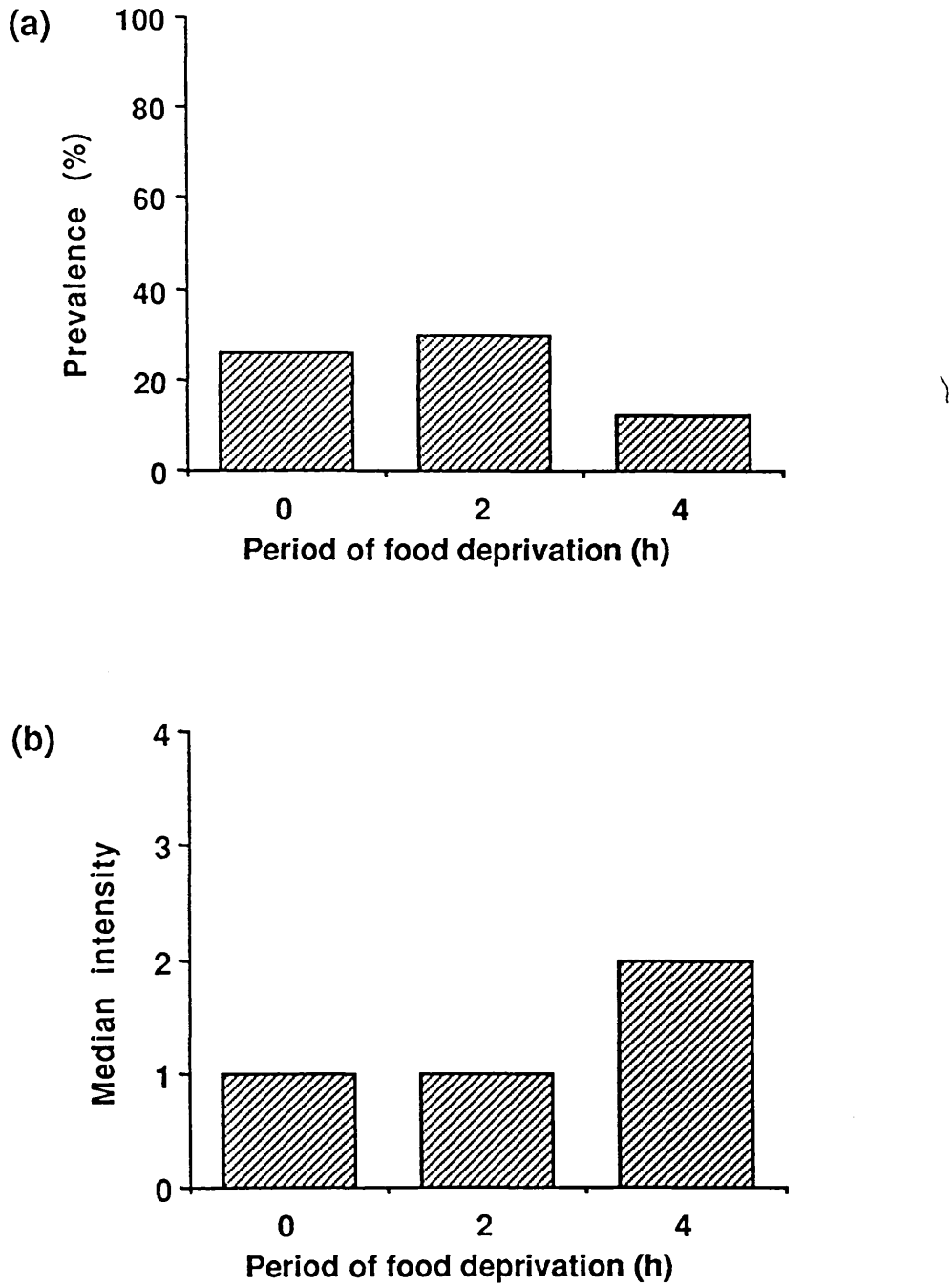
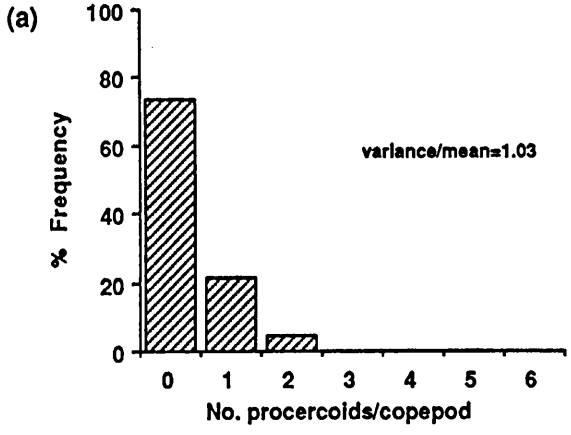
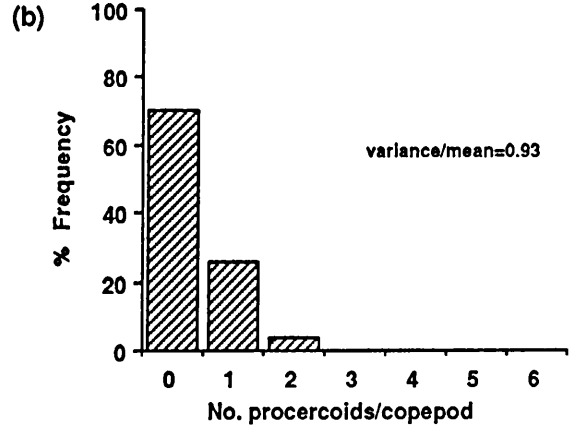
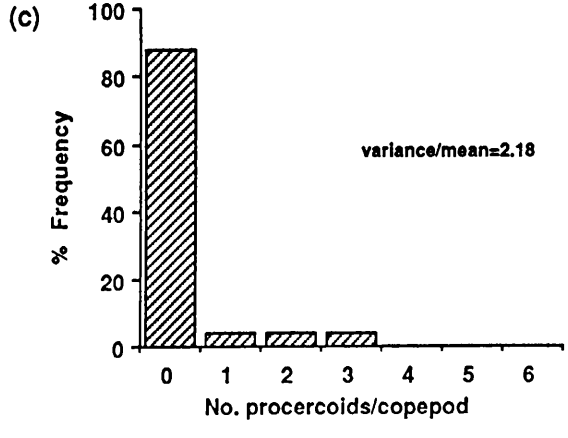


Figure 7.7: a) Prevalence and b) median intensity of *S.solidus* infection in *A.viridis* exposed to coracidia in the light following 0, 2 and 4h food deprivation.

Figure 7.8: Frequency distributions of *S.solidus* procercoid numbers per host and corresponding variance-to-mean ratios in *A.viridis* exposed to coracidia in the light following a) 0 b) 2 and c) 4h food deprivation.


(a)
100
80
60
40
20
0
% Frequency
variance/mean=1.03
0
1
2
3
4
5
6
No. procercoids/copepod


(b)
100
80
60
40
20
0
% Frequency
variance/mean=0.93
0
1
2
3
4
5
6
No. procercoids/copepod


(c)
100
80
60
40
20
0
% Frequency
variance/mean=2.18
0
1
2
3
4
5
6
No. procercoids/copepod

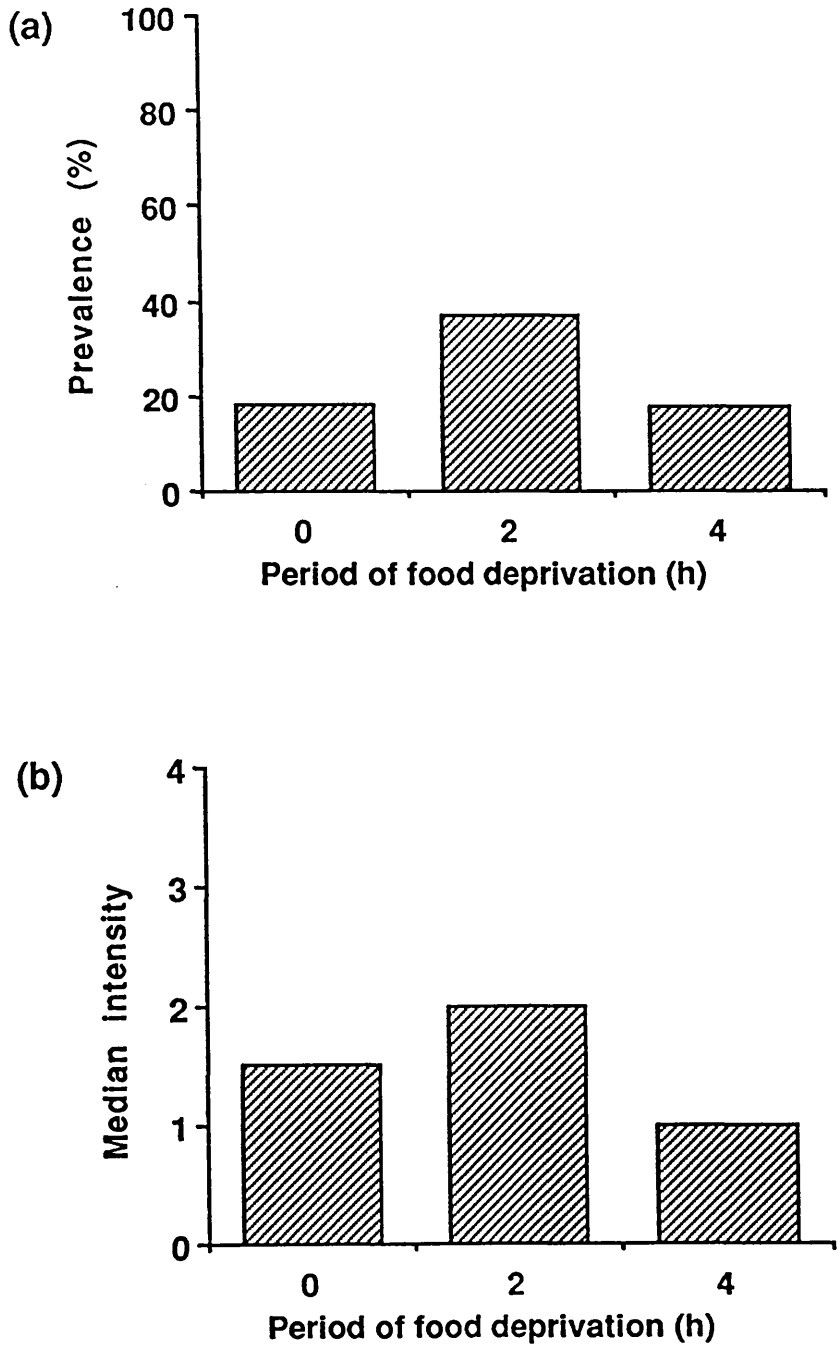
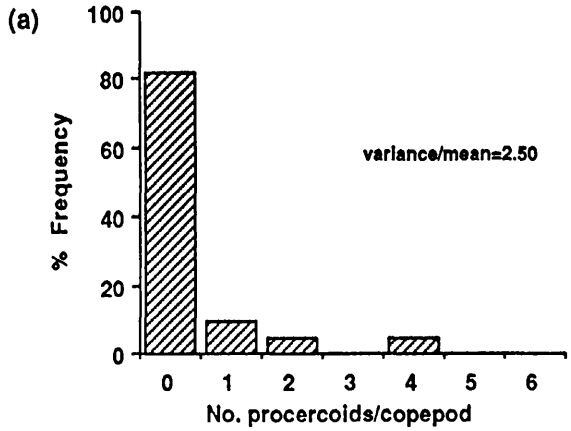
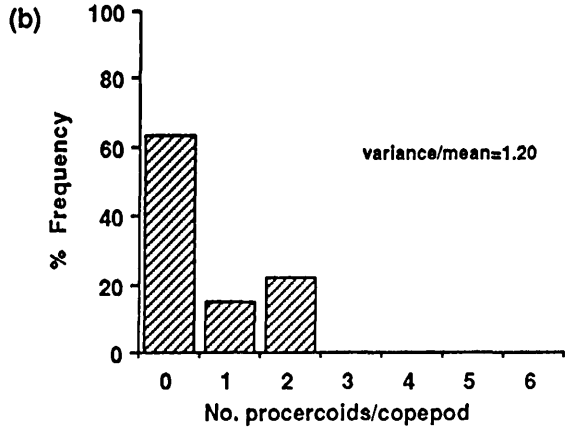
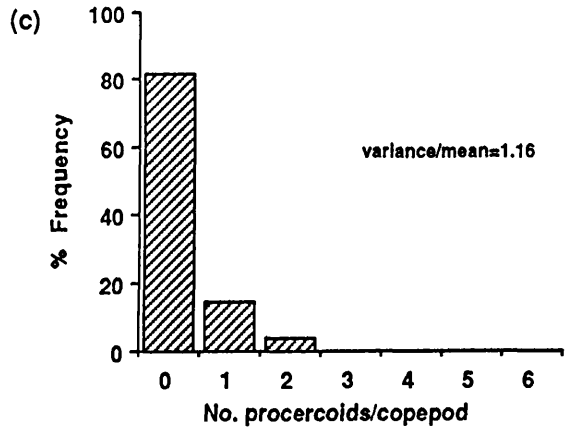


Figure 7.9: a) Prevalence and b) median intensity of *S.solidus* infection in *A.viridis* exposed to coracidia in the dark following 0, 2 and 4h food deprivation.

Figure 7.10: Frequency distributions of *S.solidus* procercoid numbers per host and corresponding variance-to-mean ratios in *A.viridis* exposed to coracidia in the dark following a) 0 b) 2 and c) 4h food deprivation.


(a)
100
80
60
40
20
0
% Frequency
variance/mean=2.50
0 1 2 3 4 5 6
No. procercoids/copepod


(b)
100
80
60
40
20
0
% Frequency
variance/mean=1.20
0 1 2 3 4 5 6
No. procercoids/copepod


(c)
100
80
60
40
20
0
% Frequency
variance/mean=1.16
0 1 2 3 4 5 6
No. procercoids/copepod

contents of the appropriate well in a 150μm mesh sieve. The copepods were washed and rinsed into their original wells with water. Once the final exposure time had lapsed all copepods were fed with protozoa and transferred to a constant photoperiod and temperature room (see above).

The duration of exposure to coracidia had no significant influence (Chi-Square, X^2=2.734, d.f.=5, P>0.05, N.S.) on the prevalence of *S.solidus* infection (Figure 7.11(a)). However, the median intensity was found to vary significantly with different periods of exposure (Kruskal Wallis ANOVA, H=12.05, d.f.=5, P<0.05), the maximum being found after 4h (Figure 7.11(b)). In addition, the distribution of procercoids in copepod groups was random, with exception of those exposed to coracidia for 4h, where a degree of over-dispersion was apparent (Figure 7.12(a-f)).

7.4 DISCUSSION

Modification of the relative density of coracidia to copepods had the most marked effects on the acquisition of *S.solidus* infection by *A.viridis*. Exposing groups of *A.viridis* to increased densities of *S.solidus* coracidia resulted in higher prevalences and intensities of infection and in addition, the total number of procercoids recovered rose from 3 to 40. It appears that acquisition of *S.solidus* infection is a function of the actual number of available coracidia, but since the proportion of coracidia that gave rise to procercoids was fairly constant and never maximal, there seems to be some limit on the ability of coracidia to encounter, to be ingested by and to infect *A.viridis*. The procercoids were randomly distributed in copepod groups. Anderson *et al.* (1978) found over-dispersion of *T.patialense* in zebra danios at very high cercarial densities, but at intermediate densities the dispersion pattern was random. However, Dupont and Gabrion (1987) exposed various cyclopoid copepod species to a fairly high density of *Bothriocephalus claviceps* (Pseudophyllidea) coracidia (15-20 per copepod) and the resultant distributions of procercoids in the copepods was random in four out of the five species. This potentially supports Mueller's notion (1959) that feeding on coracidia is the result of random collisions between copepods and coracidia and not the active pursuit of them as prey.

When a low density of copepods was exposed to coracidia (approximately 3 coracidia:1

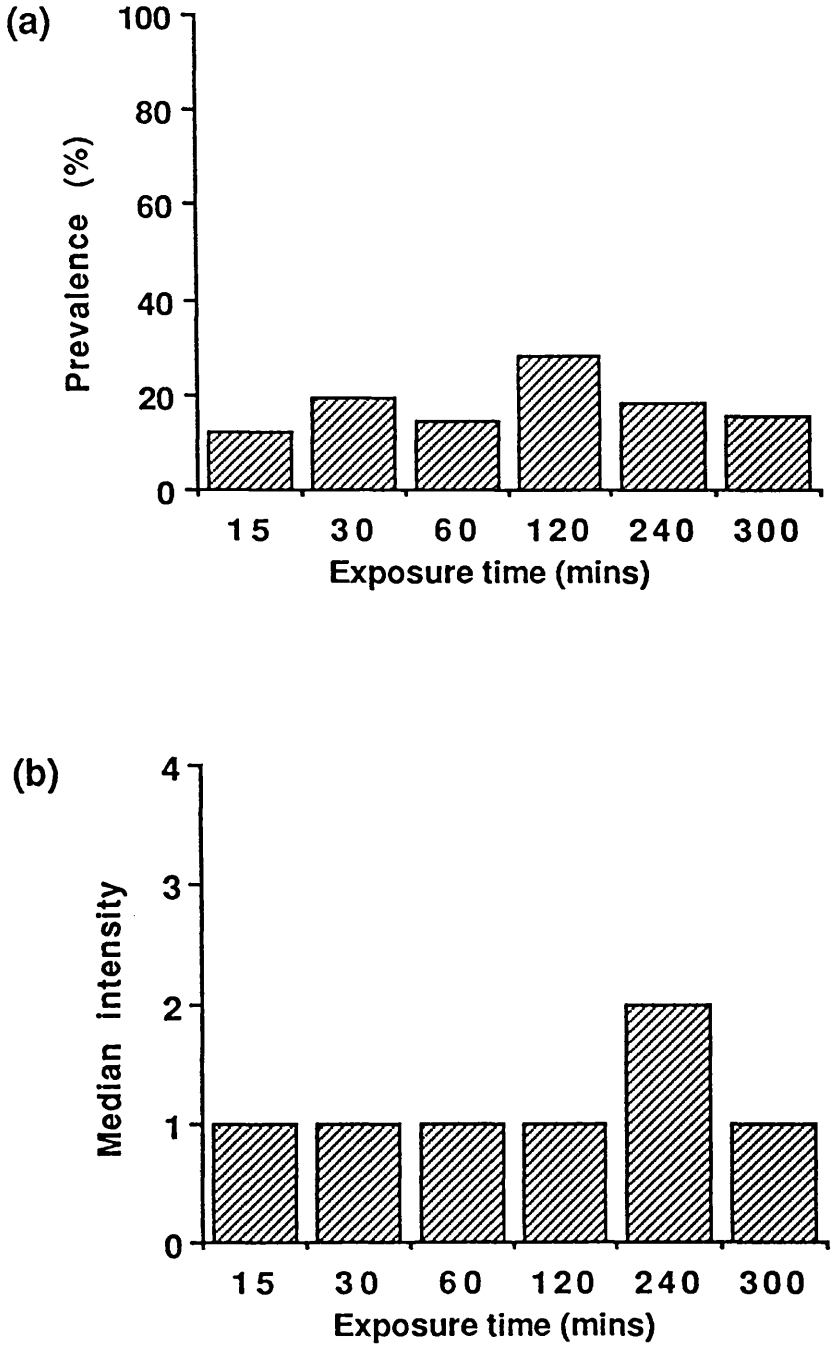
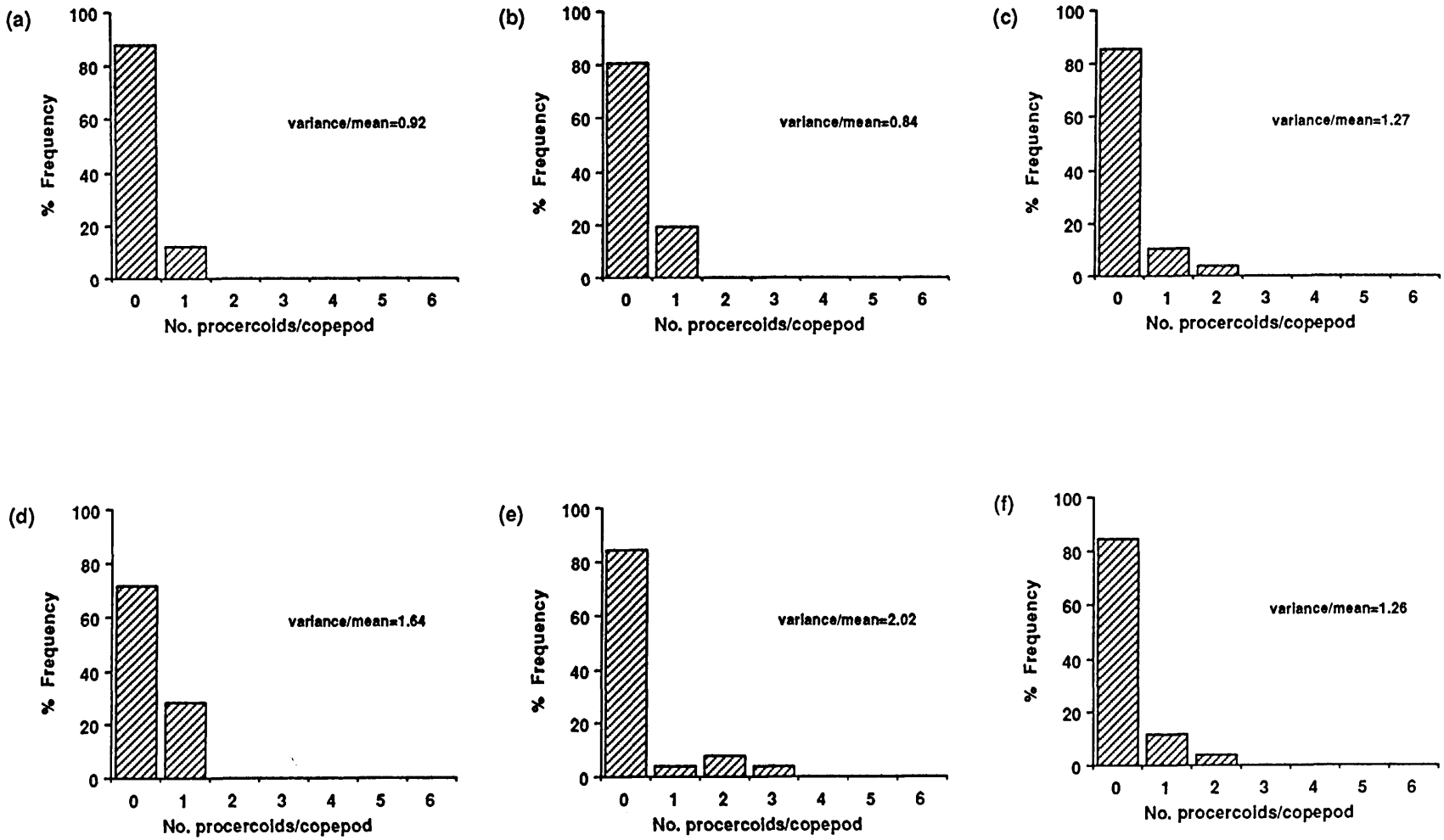


Figure 7.11: a) Prevalence and b) median intensity of *S.solidus* infection in *A.viridis* exposed to coracidia for 15, 30, 60, 120, 240 and 300min.

Figure 7.12: Frequency distributions of *S.solidus* procercoid numbers per host and corresponding variance-to-mean ratios in *A.viridis* exposed to coracidia for a) 15 b) 30 c) 60 d) 120 e) 240 and f) 300min.


(a)
% Frequency
100
80
60
40
20
0
variance/mean=0.92
0 1 2 3 4 5 6
No. procercoids/copepod
(b)
% Frequency
100
80
60
40
20
0
variance/mean=0.84
0 1 2 3 4 5 6
No. procercoids/copepod
(c)
% Frequency
100
80
60
40
20
0
variance/mean=1.27
0 1 2 3 4 5 6
No. procercoids/copepod
(d)
% Frequency
100
80
60
40
20
0
variance/mean=1.64
0 1 2 3 4 5 6
No. procercoids/copepod
(e)
% Frequency
100
80
60
40
20
0
variance/mean=2.02
0 1 2 3 4 5 6
No. procercoids/copepod
(f)
% Frequency
100
80
60
40
20
0
variance/mean=1.26
0 1 2 3 4 5 6
No. procercoids/copepod

copepod), the highest prevalence and intensity of infection were observed. An increase in the number of hosts and thus the lowering of relative density of coracidia to copepods caused a concomitant decrease in the levels of infection. This bears only some similarity to the controlled experimental infections carried out by Keymer (1982), where an exponential decline in the intensity of *H.diminuta* infection in flour beetles was apparent when the density of beetles was increased. However, in this study a reduction in the total number of infections was evident at high host densities and appeared to disagree with the assumption that the rate of infection acquisition is directly proportional to host density (Anderson & May 1979). Although the proportion of infected copepods did vary, the total number of procercoids recovered per treatment was quite low (4-13), relative to the concentration of coracidia, and did not seem to be correlated with host density. Under these conditions, perhaps only a fixed number of coracidia are ever encountered and infective and this could also explain the essentially random dispersion pattern. Thus, the apparent effects of increasing host density are to lessen the number of infected copepods and the number of procercoids per infected host, without influencing the overall return of procercoids.

In an attempt to manipulate the proximity of the coracidia to copepods, the infection volume was increased and decreased relative to that in the previous experiments. However, this had no impact on the infection levels or the distribution of procercoids. This implies that the spatial proximity of a coracidium to a copepod does not increase its chances of being ingested and becoming a procercoid, at least at the exposure density of approximately 1 coracidium per copepod.

Increasing the level of hunger prior to exposure to *S.solidus* coracidia did not appear to affect the acquisition of infection. It had been suspected that hungry copepods would feed more voraciously and perhaps eat a greater number of coracidia than satiated copepods, yet the prevalence and median intensity were similar irrespective of the treatment. These results may reflect the fact that the level of hunger was not properly manipulated, since algae could not be completely removed from experimental wells and may have been utilised as a food source (copepodites are omnivorous). Alternatively, the ingestion of *S.solidus* coracidia may have been independent of the foraging behaviour of the copepods, so that the level of hunger and

infection relied simply on chance encounters between coracidia and copepods. The fact that the same levels of infection resulted in the dark supports the view that copepods do not use visual cues to actively hunt coracidia like prey, but are more likely to passively contact and ingest them.

There was an effect of hunger on dispersion that varied according to whether the experiment was carried out in the light or in the dark. Over-dispersion of procercoid numbers was evident in those copepods that had been without food the longest, prior to exposure in the light. Minor initial heterogeneity in the levels of food in stomachs may have been accentuated after this period, resulting in heterogeneous degrees of hunger and variable host behaviour. Anderson *et al.* (1978) suggested that behavioural discrepancies between zebra danios acted as a mechanism for producing over-dispersion of *T.patialense* numbers. In the dark, over-dispersion was only apparent in the satiated copepods and may have resulted from another behavioural trait peculiar to them under these conditions.

Altering the exposure period had no effect on the subsequent prevalence of *S.solidus* infection, because the same proportion of copepods became infected after 15min exposure as after 5h. The median infection intensity was also found to vary little with the duration of exposure, except that after 4h exposure it was slightly higher. In addition, the distribution of parasite numbers per host was largely random except following 2 and 4h exposure. This is contrasted by other studies where the parasite burden (Keymer 1982) and the level of over-dispersion (Anderson *et al.* 1978) increased as a function of exposure time. However, by the use of a stochastic simulation model the latter authors revealed that exposure time would have no effect on the distribution of *T.patialense* in zebra danios if all hosts were equally susceptible. Furthermore, there was tendency for a random dispersion pattern to arise when zebra danios were subject to low cercarial densities of *T.patialense*. Possibly, the copepodites in this study were variably susceptible to *S.solidus* infection, but at the coracidial densities employed this had a limited effect except after quite long periods of exposure.

Overall, consistently less than 50% of the coracidia that were added established as procercoids, which cannot reflect the actual rate of contact between copepods and coracidia. It is proposed that not all coracidia consumed became procercoids and hence the prevalence

probably under-represents the ingestion rate. Sharp *et al.* (1990) found that although coracidia of *D.dendriticum* may be ingested by *Cyclops abysorrum* Sars and transform to oncospheres, they do not necessarily penetrate the intestine and their remnants can be detected in the copepod rectum and faeces. If these results were due to heterogeneous susceptibility of the copepods and the same process was operating in this case, then greater over-dispersion of procercoid numbers would have been expected (Anderson & Gordon 1982). As no coracidia were detected after treatments it is proposed that all available coracidia were consumed, but only a limited and generally random amount were ever able to transform to oncospheres, penetrate the intestine and develop as procercoids. If such a low level of parasite establishment is operating in the field, numerous infective stages and suitable copepod hosts would have to be present simultaneously so that encounters would be frequent and some would result in successful infections. The late summer period of maximum prevalence and intensity of *S.pungitii* in a Karelian lake (Sysoev 1987) perhaps represents just such a situation and corresponds quite closely to a period of acquisition of new infections in nine-spined sticklebacks *Pungitius pungitius*. Furthermore, the observed wave of *S.solidus* infection in sticklebacks from Inverleith pond (Chapter 3) may too reflect a period of maximum encounters and optimal levels of infections in copepods.

7.5 CONCLUSIONS

Manipulation of the relative density of *S.solidus* coracidia to *A.viridis* copepodites had the most dramatic impact on infection levels, whilst the proximity of the coracidia, exposure time and hunger have an almost negligible influence. Also the distribution of procercoids in copepods was usually random and is thought to reflect the random nature of encounters between copepods and infective stages. It is suggested that in this host-parasite relationship there is a limit to the number of coracidia that will successfully establish as procercoids. However, it is realised that without a thorough knowledge of the actual fate of ingested coracidia and replication of the treatments, interpretation of the results is somewhat restricted.

CHAPTER 8: GENERAL DISCUSSION

This dissertation has described the results of an investigation into the interactions between *Schistocephalus solidus* and its hosts. Field data were obtained on the impact and dynamics of infection of the plerocercoid stage in an annual population of three-spined sticklebacks. Experimental manipulations were carried out on the egg, coracidium, procercoid, plerocercoid and adult stages with an emphasis on viability and infectivity of life cycle stages, transmission between hosts and the epidemiology of infection. Data from these two approaches will now be collated to propose a scheme for the dynamics of *S.solidus* infection at Inverleith and the possible evolutionary consequences of infection for the parasite and its hosts are discussed.

Seasonal pattern of *Schistocephalus solidus* infection in sticklebacks from Inverleith pond, and the possible causes

There were two major trends in the pattern of *S.solidus* infection in the sticklebacks from Inverleith pond. In late summer and early autumn there was a sharp increase in the prevalence and intensity of infection; the initial increase in abundance of small-sized plerocercoids suggested that new infections were occurring at this time. An additional feature of the wave of infection was that the distribution of plerocercoid numbers became increasingly over-dispersed. During winter, a second major change in the levels of infection with *S.solidus* took place, as a large proportion of infected fish were lost. In order to explain such changes in the parasite population, it is necessary to reconstruct the possible events leading up to them.

The starting point for new infections in most cestode species is the production of viable eggs from the adult stage in the definitive host. The nature of the definitive host will determine the timing of egg production. Both the plerocercoid and adult stages of *Proteocephalus filicollis* (Cestoda: Pseudophyllidea) are located in the intestine of freshwater fish. The changes in temperature that the parasite experiences being in a poikilothermic host seem to be responsible for a seasonal pattern of maturation and egg production (Hopkins 1959). Gravid worms are found during the summer when the conditions are favourable for embryonation of eggs. By contrast, *S.solidus* matures in the intestine of an avian piscivore and the associated increase in temperature is sufficient to stimulate maturation and egg production (Smyth 1946). Each of these processes occurs irrespective of the suitability of external conditions for

transmission and could potentially result in the waste of infective stages. However, Kennedy (1983) suggests that, for cestodes, the aquatic environment is less hazardous than a terrestrial environment, where there is high mortality of larval stages (e.g. due to dessication or temperature extremes) and hence transmission is jeopardised. The short patent period of *S.solidus*, which may last from a few days (Chapter 6) up to two weeks (McCaig & Hopkins 1963) compared with as much as 18 months in *Hymenolepis diminuta* (Cyclophyllidea) may be in response to the favourable environment. Also, eggs produced from the cyclophyllidean cestodes are usually embryonated and this limits the influence of exogenous factors on their viability and infectivity. On the other hand eggs of *S.solidus*, and of other Pseudophyllidea, are unembryonated and have a requirement for elevated temperatures to allow them to develop. It is likely that at Inverleith pond appreciable levels of embryonation are not found until temperature rises in summer; in conjunction with increased sunlight, this probably culminates in a period of synchronised hatching of *S.solidus* eggs. Within this wave of hatching there are likely to be bursts of hatching arising from the temporal variability in development that has been detected in eggs derived from the same adult worm and between adult worms (Chapter 6). This would result in a patchy temporal distribution of the coracidial stage. Assuming eggs from adult *S.solidus* will be released in bird faeces over different parts of the pond, there is also probably patchiness in the spatial distribution of coracidia.

The uptake of coracidia by copepods at Inverleith pond may be higher than detected in the laboratory because host species will vary in their susceptibility to *S.solidus*. Dupont & Gabrion (1987) found that 5 species of copepod differed markedly in their susceptibility to *Bothriocephalus claviceps* (Pseudophyllidea) when exposed to the same levels of coracidia. However, regardless of the high experimental susceptibility of *Eudiaptomus gracilis* to *Schistocephalus pungitii* that has been demonstrated (Dubinina 1966, cited in Sysoev 1985), this copepod did not appear to act as a natural intermediate host (Sysoev 1985). So in actual fact experimental observations may just as easily over rather than underestimate natural levels of infection of copepods. Indeed, if coracidia are patchily distributed, have a limited life span and are not necessarily 100% infective to copepods, then there may be very low natural levels of infection, as observed by Sysoev (1985) for *S.pungitii*. Nevertheless, in excess of 50% of

sticklebacks from Inverleith pond were infected in November 1988. The higher trophic level of the stickleback and the small size of copepods may ensure that a great many copepods will be consumed, allowing some infections to establish although only a small proportion of copepods may be infected. Even then, consuming an infected copepod does not guarantee that a stickleback will become infected. There are indications that as little as 30% of sticklebacks given copepods with single procercoids will develop infections with plerocercoids (personal observation). A further mechanism by which transmission to the stickleback host may be facilitated is by parasite manipulation of the copepods behaviour in such a way as to increase its vulnerability to predation. *Acanthocyclops viridis* is a benthic copepod and infection with an a visibly mature procercoid promotes a significantly greater use of surface water (Tierney, Huntingford & Crompton in prep.) which may increase the likelihood of predation. Furthermore, selective predation by sticklebacks of *Cyclops scutifer* harbouring infective procercoids of *S.solidus* has been observed (Urdal & Jakobsen pers. comm.).

As cyclopoid copepods are present all year round, it is thought that the seasonally restricted wave of infection is largely a product of environmental regulation of coracidial release. Many fish (>40% at the peak of prevalence) were uninfected by *S.solidus* and must either have been inherently insusceptible (e.g. immunologically, physiologically) or have avoided ingesting infected copepods. Exposure of sticklebacks to infected copepods could have been limited if they exploited feeding areas that were free from *S.solidus* infection or did not include copepods as part of their diet, suggested by Lozano (1991) as means of preventing infection.

Associated with the wave of infection was an increase in over-dispersion of *S.solidus* numbers in sticklebacks. This may have been the result of over-dispersion of procercoids in the copepod intermediate host, but the laboratory studies with *A.viridis* (Chapter 7) indicate that this is unlikely to be the case, because few copepods harboured more than one procercoid. Also, Dupont and Gabrion (1987) found over-dispersion of procercoid numbers in only 1 out of 5 species of copepod exposed to *Bothriocephalus claviceps* (Cestoda: Pseudophyllidea) coracidia and even then the level of over-dispersion was minimal. Therefore, the over-dispersion of plerocercoid numbers may reflect heterogeneous host susceptibility, a clumped

distribution of infected copepods or simply a series of overlapping random exposures of sticklebacks to infection.

Whatever the reason for the over-dispersion, it raises the possibility of regulation of the parasite population through the death of the most heavily infected sticklebacks (Anderson & Gordon 1982) and this may be the case during the winter of 1988. Those fish harbouring the highest parasite burdens in terms of the numbers of plerocercoids, the size of the plerocercoids and hence the relative weight of the plerocercoids, were lost. Together with some large uninfected counterparts these heavily infected fish may have disappeared from the pond as result of predation by *Larus ridibundus*. The capture of infected fish may have been enhanced by inferior predator avoidance behaviour in sticklebacks harbouring infective plerocercoids (Chapter 4). Hence, there appeared to be not only a reduction in the plerocercoid population, but regulation of the parasite population via the death of heavily infected hosts.

With the loss of these heavily infected sticklebacks over winter, the composition of the parasite population changed markedly. Uninfective (<50mg) plerocercoids were more abundant and the behaviour of infected sticklebacks may have changed accordingly. Plerocercoids in this size range appear to enhance the anti-predator behaviour of sticklebacks (Chapter 4) and such increased vigilance may explain the lowered food intake and greater reliance on plant matter by infected sticklebacks during the winter. Body condition was very low in all sticklebacks at this time and was unaffected by the presence of *S.solidus*.

When temperatures increased in spring and sticklebacks resumed growth, plerocercoid growth was also accelerated, particularly in one-worm infections. The exploitation of host nutrients by the parasite seemed to have serious consequences for the reproductive potential of male and female sticklebacks. Few infected sticklebacks developed to maturity and those that did may have been unable to reproduce. Hepatosomatic indices of infected mature females were low. Since this is the major buffering organ in the production of ripe ovaries (Allen & Wootton 1982), such infected females may not have been capable of spawning or at least their overall fecundity may have been depressed. Those infected males that did mature were in good condition and had well developed secondary sexual characteristics. Nevertheless, it is not clear whether they would have all been able to defend a territory, build a nest and attract a mate. Any

effects on the reproduction of infected fish may have been heightened by their reduced food intake (relative to uninfected fish) at the peak of breeding.

The final growth period of plerocercoids occurred in the spring and summer and resulted in larger plerocercoids than at any other time during the survey. As the life span of the host sticklebacks is 12-18 months, most of these worms would have been lost as the cohort gradually died out. There is evidence from the few 1+ sticklebacks from 1988 that some infected adults manage to survive until winter and so there is a possibility some large plerocercoids may be transmitted to the definitive host.

Coevolution in host-parasite relationships

Reciprocal genetic change in response to an interaction between two species (i.e. coevolution) perhaps features in the relationships between *S.solidus* and its hosts at Inverleith pond. It was once a common view in parasitology that host-parasite interactions should evolve towards mutualism, but theory and some empirical evidence have shown that parasites need not necessarily evolve to be harmless to their hosts (May & Anderson 1983). The path of coevolution (i.e whether it will tend toward mutualism or not) will depend on the relationship between pathogenicity and transmissibility of the parasite and the possible costs to the host of evolving resistance (May & Anderson 1983). Extending this approach, Ewald (1987) proposed that a consideration of the costs and benefits in host-parasite relationships, at different points on the continuum between mutualism and severe parasitism would enable predictions to made of the direction in which evolution should proceed. He suggested that in relationships where transmission is via predation, there should be evolution towards severe parasitism in the prey hosts and towards benign parasitism in the predator hosts. This fits rather well with the *S.solidus* life history where there are severe consequences of parasitism in the stickleback and possibly in the copepod, but no apparent detrimental effects on the avian definitive host. A number of other mechanisms and outcomes of coevolution have been proposed (Thompson 1989), but Toft & Karter (1990) stress that there is still a fundamental need for more empirical work on host-parasite associations (particularly helminths and other macroparasites) to elucidate the processes of host-parasite coevolution.

Of course not all of the outcomes of a host-parasite interaction will have a selective

advantage for one or other species. In fact some will be purely by-products of infection with no adaptive significance. With this framework in mind the rest of this chapter comprises an entirely speculative evaluation of the points in the life history of *S.solidus* where coevolution may be operable and of those where purely pathological consequences are involved.

If, as suggested, the chances of copepods acquiring infection are low then the parasite may be expected to evolve first toward increased egg quality (i.e. an increased ability of eggs to develop and hatch) and second toward low specificity and high infectivity at the coracidial stage. Furthermore, an ability of the procercoid to alter the vulnerability of copepods to predation by sticklebacks would be selectively advantageous. It is possible that parallel evolutionary change to avoid infection would also take place in copepod population e.g via lowered susceptibility.

There appear to be several ways by which selection could operate on the plerocercoid stage to improve its transmissibility. Those parasites that enhanced the anti-predator behaviour of sticklebacks until they were infective and then suppressed the host's anti-predator response would be favoured. Depression of host body condition by the plerocercoid may play a part in the latter effect. At Inverleith pond where the levels of infection are rather low, selection pressure for such behavioural alteration is likely to be strong. In contrast, in the population studied by Arme & Owen (1967), where the prevalence was 100%, there would probably be less scope for the evolution of altered behaviour, because any fish captured by a bird would harbour a parasite. Nevertheless, individual parasites with the ability to reduce stickleback responsiveness would still have a selective advantage. Sticklebacks that breed late and thus produce fry later in the year could reduce the chances of their fry picking up infected copepods during the autumn wave of infection and thereby increase the chances that their offspring will survive the winter. Furthermore, being produced late in the season the offspring would also be likely to produce fry late in the following season. However, such an adaptation may be countered in sticklebacks that became lightly infected in autumn, because their potential for breeding may be reduced.

It is difficult to imagine the selective advantage for the parasite of reducing the body condition by uptake of host nutrients and suppressing the reproductive potential of the host if

they subsequently die without transmission. However, one might argue that if the parasite has infected a high quality stickleback whose body condition is not so drastically reduced then the stickleback may survive longer than average and the parasite be eventually transmitted. Similarly, it is hard to envisage how reduced food intake and altered diet structure could be a parasite adaptation. Higher food intake would be expected in a stickleback harbouring such a nutritionally demanding parasite. Perhaps it is actually an adaptation by the stickleback to reduce the chances of further infection and to limit the growth and hence pathological effects of the parasite. However, it its more plausible that the two effects of parasitism outlined above are purely pathological consequences of infection parasite with no adaptive significance.

Infection with the adult stage of *S.solidus* is probably inconsequential for the avian host in terms of pathology, because the levels of infection in Inverleith pond are low and the adult stage of the parasite absorbs no nutrients, does no damage to the intestinal mucosa and is short-lived. In fact it may be to a potential definitive hosts advantage to select infected rather than uninfected sticklebacks, because there is probably a reduction in the cost of catching them, but without any fitness cost associated with infection.

It is clear from this study, that the epidemiology and impact of a parasite on its hosts will be influenced by both the parasite and host species. In addition, other characteristics of the host-parasite interaction will be important, such as the developmental stage of the parasite or degree of host maturity. Furthermore, the consequences of an interaction may extend from the individual level to the population as and whole and indeed there may be long term evolutionary changes as a result.

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