The Epidemiology and Population Ecology of *Argulus* spp. Infections in UK Stillwater Trout Fisheries

A thesis presented for the degree of
Doctor of Philosophy to the University of Stirling

By

Nicholas Giles Hutton Taylor

Institute of Aquaculture
University of Stirling
Stirling
Scotland

October 2004

DECLARATION

I hereby declare that this thesis has been composed by myself and is a result of my own investigations. It is neither been accepted, nor submitted for any other degrees. All sources of information have been duly acknowledged.

.....

N. G. H. Taylor

Dedicated in memory of Ash

ACKNOWLEDGEMENTS

Firstly many thanks to all of the fisheries that took part in the project, with out their help the study would not have been possible. Special thanks go Tim Small, Jimmy & Matt, Stuart Balmain and Randle Sparrow for allowing the continual invasion of their fisheries. Thank you to the study sponsors: The Environment Agency (EA) and especially Chris Williams, the Association of Stillwater Game Fisheries Managers (ASGFM), the Salmon & Trout Association (S&TA), the British Trout Farmers Restocking Association (BTFRA) and the Association of Scottish Stillwater fisheries (ASSF).

Thank you to Stuart Balmain & family, Jason Lowe & Richard Jones and Bill, Faye & Buster for all their help, cooking expertise and friendship throughout the study. Thanks to Rob & Jo, Anne & Les and Kerry for board, lodgings and good company during the field trips, and again to Rob for leaving his computer desk to increase 'sampling' efficiency.

Thanks to everyone I worked with at the Institute of Aquaculture, but especially to Jimmy Turnbull for putting up with all my stupid questions, James Bron for his challenging thoughts, great lunches and dinners, Astrid Holzer for her lively spirit and Andy Shinn for just being a bloody nice bloke and supporting me throughout. Well done (and big thanks) to Chris Sommerville and Rod Wootten for supervising and putting up with me, taking my abuse, translating my thesis into English and successfully getting me through the Ph.D. – a definite challenge!

Finally and most importantly thanks Mum, Anna and Barney (my dog) for your unwavering support and encouragement during the project, I couldn't have got there without you!

CONTENTS

LIST OF TABLES

Chapter 3

Chapter 4

LIST OF FIGURES

Chapter 3

Chapter 4

ABSTRACT

Argulus spp. are branchiuran crustaceans, the majority of which are ectoparasites of fish. In the UK three freshwater species have been recorded: *A. foliaceus*, *A. japonicus* and *A. coregoni*. *Argulus* spp. have been reported to cause mortality in stillwater trout fisheries, but little data is currently available as to the problems they cause or ways to control them. This study was carried out within the broad aims of the sponsors, which were as follows:

a) To review of the current perception of the extent and severity of *Argulus* spp. infections in UK Stillwater trout fisheries, and identification of the methods currently employed for controlling these infections.

b) To review and increase the knowledge of the biology and ecology of *Argulus* spp. in relation to these systems.

c) To assess the prospects for novel control and management strategies to reduce economic loss.

A cross-sectional, questionnaire-based study of 69 randomly selected stillwater trout fisheries showed that *Argulus* spp. infections may cause economic loss, through a reduction in the number of anglers attending a fishery. *Argulus* spp. are perceived to reduce the feeding and therefore catchability of trout. This, in combination with the resulting reduction in condition and aesthetic appeal of fish is believed to reduce angler numbers. In year 2000, 29% of stillwater trout fisheries in the UK suffered a problem infection. *Argulus* spp. were widely distributed throughout the UK, although problem infections appeared more common in central and southern England and Wales. *A. foliaceus* was the most common species being found in all but one of the infected study waters. The cross-sectional study identified three factors associated with problem *Argulus* spp. infections. Presence of an algal bloom

/ high turbidity, and slow stock turnovers were both associated with an increased risk of a problem infections, and a drop in water level was associated with a reduced risk of a problem infection.

A longitudinal study of the population ecology of *A. foliaceus* in five trout fisheries of varying management intensity was conducted to identify correlations between risk factors and increase our knowledge of the population dynamics of *A. foliaceus*. The study also investigated the effects of temperature and identified key points in the life-cycle of *Argulus* spp. around which interventions could be targeted. Low water clarity and high temperatures were significantly correlated with a high abundance of *A. foliaceus*. Low water clarity was also associated with reduced stock turnover and would suggest that high numbers of *A. foliaceus* alone may not affect the catch.

The abundance of *A. foliaceus* is greatest towards the end of summer and drops to low levels over winter. The first and second cohorts of the season hatch, in May and June respectively, from eggs that have over-wintered. These cohorts became adult and started laying eggs in July and August. The over-wintering of hatched stages of *A. foliaceus* is dependent on a slow winter stock turnover of fish and the presence of reservoir hosts. If these stages over-winter they lay eggs at the end of April that hatch in July. A cohort then hatches every month until September, giving a total of 5 cohorts hatching in a year. The cohorts hatching in July and August lay eggs that over-winter, and the September cohort over-winters as hatched stages. Field studies also elucidated valuable information on the egg laying habits of *A. foliaceus*, determining that egg laying occurred between April and September,

before stopping over the winter month. Depth of egg laying was found to increase as the season progressed, and was found to occur deeper in clear water than turbid water.

Laboratory experiments were used to study the effects of illuminated & darkened conditions, different host species and temperature on the infection success and survival of the parasite. Infection success was high under all conditions. Subsequent survival was similar in all of the experiments, but the likelihood of the parasite reaching adult size was greatest at temperatures of 20°C. Experiments also showed that the metanauplii of *A. foliaceus* could not survive without a host for more than 3 days.

This project has successfully identified the problem posed by *Argulus* spp. infections to UK stillwater trout fisheries and determined the extent and severity of such problem infections. Several risk factors associated with problem infections were identified and studies were carried out in relation to the parasites population ecology. This work has greatly increased our understanding of the factors controlling *A. foliaceus* populations, and has led to the development of a series of management recommendations and opened avenues for further research.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

The argulidae are branchiuran crustaceans, the majority of which are ectoparasites of fish, there are 12 recorded genera in the family comprised of 196 species (Anon, 2001). Argulids are found distributed throughout much of the world (Fryer, 1968, Kabata, 1970, Post, 1987), however, only members of the genus *Argulus* are known to occur in the UK. Worldwide, 143 *Argulus* spp. have been described (Anon, 2001), although it is likely that many of these are synonymous. In the UK three freshwater species have been recorded: *A. foliaceus* (L.), *A. japonicus* (Thiele) and *A. coregoni* (Thorell). These species are also found throughout much of Europe and many other parts of the world (Post, 1987). A marine species, *A. arcassonensis* (Cuénot), has also been recorded around the UK coast (Rushton-Mellor, 1992), but little information is available on this species.

Argulus spp. have been reported to cause problems in fisheries and fish farms throughout the world, including the UK. Many of the early records of *Argulus* spp. in Europe refer to them as a problem in carp farming, with much of the German literature referring to the 'Carp Louse' (Herter 1927, Kollatsch 1959, Schluter 1978 and Stammer 1959). In recent years, however, *Argulus* spp. have been reported as causing problems in UK stillwater trout fisheries, where it is known to cause mortality (Knight 1996, Northcott, Lyndon & Campbell 1997, Gault, Kilpatrick & Stewart, 2002 & personal communications with representatives of the trout industry). Interestingly, the first known record of *A. foliaceus* by Baldner 1666 (cited by Wilson 1902) describes it as predominantly affecting trout. This chapter aims to review the available literature on the three freshwater UK species of

Argulus, and identify areas requiring further study in order to understand the mechanisms behind problem infections in stillwater trout fisheries.

1.2 Stillwater Trout Fisheries

Trout fisheries can be regarded as managed, recreational, sport fisheries. These waters are generally stocked with rainbow (*Oncorynchus mykiss*) and brown trout (*Salmo trutta*), for which anglers pay to fish. Trout are bought in from fish farms, the number, frequency and size of fish stocked varying between waters. Several different suppliers can supply a single fishery in a year year. In some cases fish are bought in at a small size, and are grown on in cages within the fishery. UK trout fisheries can be broadly divided into two types, (i) commercial fisheries that are intensively managed with high numbers of anglers, and (ii) syndicate or club waters, which often receive little management and are lightly fished. Trout fishing in the UK is an important rural industry. The fishing rights to trout fisheries in England and Wales (including running water) are estimated to be worth around £500 million (Environment Agency 2001), however, stillwater sport fisheries often have very narrow profit margins, making as little as £100 per annum profit (Knight 1996). Such narrow profit margins mean that the effects of *Argulus* spp. or other pathogens are potentially devastating to a business or syndicate water. In addition to the economic effects on an individual fishery there may be significant effects on related businesses. Angling makes a substantial contribution to the economy of rural Britain, supporting between 5000-6000 full time equivalent jobs through the supply, retail and independent sectors (Countryside Alliance 2002). There are

approximately 430,000 trout anglers in England and Wales alone, spending around £300 million on game fishing and contributing £6 million to the Environment Agency through rod licence fees (Simpson & Mawle 2001, Environment Agency 2001). The vast majority of trout waters rely on supply by the trout farming industry and hauliers to obtain their stock. In year 2000, 121 farms in England and Wales produced 3427 tonnes of trout for restocking, with an additional 843 tonnes being produced in Scotland (Dunn 2002). These figures demonstrate that the failure of a fishery can have considerable effects along the economic chain. Despite this little work has been published on the control or prevention of disease within these systems.

1.3 The Parasite

1.3.1 Development & Morphology

Argulus spp. are semi-transparent, with a pale green-brown cryptic colouration. They are broadly ovoid in shape, dorso-ventrally flattened and convex dorsally (Figure 1.1a). *A. foliaceus* and *A. japonicus* are of similar size, the adults being around 6-8mm in length (Shafir & Van As 1986, Rushton-Mellor & Boxshall 1994). The adult of *A. coregoni* is larger, reaching up to 12mm in length (Gurney 1948). There appears to be some confusion over the number of developmental stages between hatching to adulthood in the three UK species of *Argulus*. In *A. coregoni*, both Stammer (1959) and Shimura (1981) describe 9 stages. Stammer (1959) does, however, disagree with Tokioka (1936) regarding the number of developmental stages undergone by *A. japonicus*, suggesting 9 stages, whereas Tokioka (1936) describes only 7 stages. Rushton-Mellor and Boxshall (1994) cast doubt on

Stammer's observations, suggesting there is inadequate detail in his conclusions. The most debate, however, occurs in the case of *A.foliaceus*, with Rushton-Mellor & Boxshall (1994) describing 11 stages until adulthood, Stammer (1959) describing 9, Clark (1902; as cited by Rushton-Mellor & Boxshall 1994) 6 and Claus (1875) only 5. Of all these descriptions, those by Rushton-Mellor & Boxshall (1994) for *A. foliaceus*, Tokioka (1936) for *A. japonicus* and Shimura's (1981) for *A. coregoni* appear to be the most detailed.

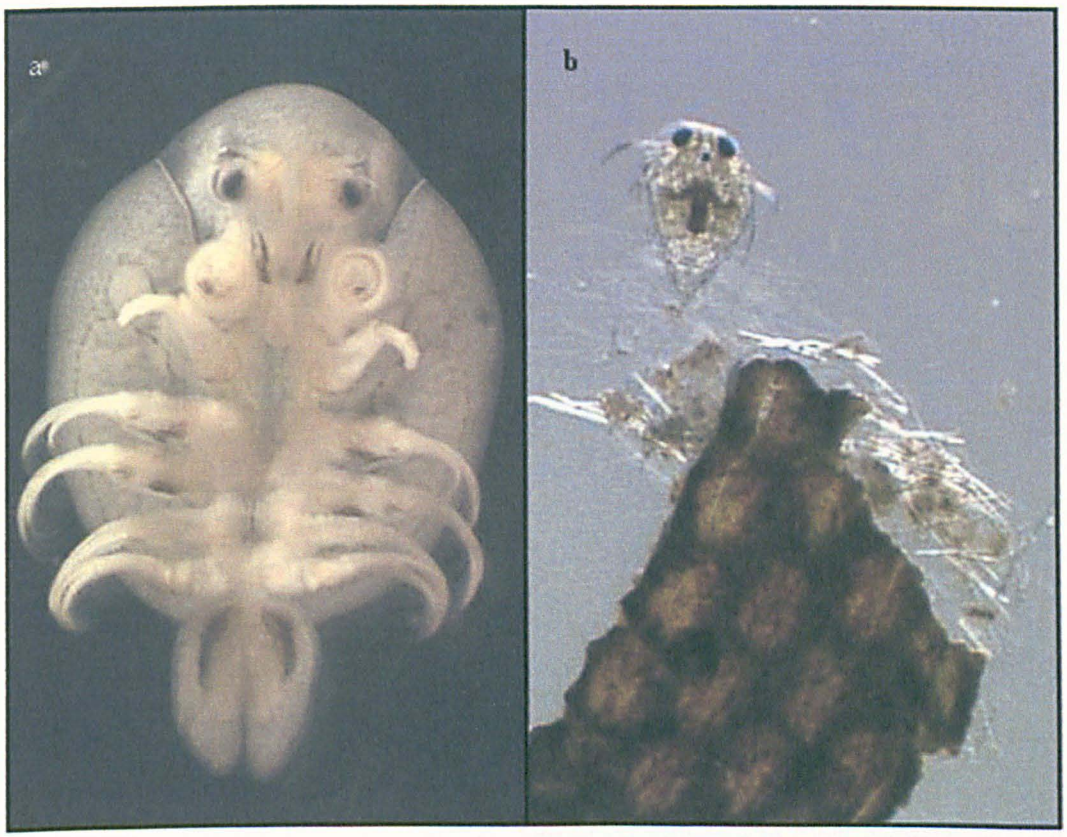


Figure 1.1 a) Ventral view of an adult male specimen of *A. foliaceus*.

b) *A. foliaceus* metanauplius freshly hatched from an egg string.

It is well established that in *A. foliaceus, A. japonicus* and *A. coregoni,* the parasite hatches out at a relatively advanced stage known as a "metanauplius" (Figure 1.1b), which is recorded by some authors as a copepodid (Tokioka 1936, Shafir & Van As

1986, Rahman 1995). Not all species of *Argulus* hatch as a metanauplius, some, for example *A. africanus,* hatch at an even more developed form that resembles the adult parasite (Fryer 1968). Rushton-Mellor & Boxshall (1994) describe the metanauplius of *A. foliaceus* as sub-oval in shape, transparent, and with two highly visible black compound eyes. This stage is approximately 0.7-0.8mm in length in the three UK species (Tokioka 1936, Shimura 1981, Rushton-Mellor & Boxshall 1994, Lutsch & Avenant-Oldewage 1995). The metanauplius of all three species are extremely similar in morphology, and it is not until the parasite reaches the mid-developmental stages (moult 5) that they can be accurately speciated by their gross morphology (Shimura 1981, Rushton-Mellor & Boxshall 1994).

A pre-oral spine and mouth tube are present in the metanauplius, demonstrating that, although this stage does not resemble the adult, it is a feeding stage (Tokioka 1936, Shimura 1981, Rushton-Mellor and Boxshall 1994, Lutsch & Avenant-Oldewage 1995). The first antenna is hooked as in the adult and probably aids in host attachment along with the 1st maxilla. The characteristic features of the metanauplius are the antennal exopod and mandibular palp which are the main locomotory organs. The thoracic appendages are all relatively underdeveloped at this stage (Tokioka 1936, Shimura 1981, Rushton-Mellor & Boxshall 1994, Lutsch & Avenant-Oldewage 1995).

All three UK species begin to resemble the adult after their first moult, assuming an ovoid shape. The antennal exopod and mandibular palp degenerate and the thoracic appendages develop. In developmental stages 1-5, in the case of *A. foliaceus* and *A. coregoni*, and stages 1-4 in *A. japonicus*, the first maxilla is not a developed sucker

as in the adult, but a strong hook for attachment. It is not until the subsequent moult (stage 6 in *A. foliaceus* and stage 5 in *A. japonicus*) that the hook is completely modified and the sucker is fully formed. At this point, genital sockets and pegs become clearly visible in males, on the coxa of the 3rd limb and the basis of the 4th limb, respectively. The parasite continues developing through a further 5 stages (in *A. foliaceus*), with each stage showing subtle morphological differences, and increases in size until sexual maturity is reached. *Argulus* spp. continue moulting and growing even when adult (Gurney 1948), in contrast to sea lice and other copepods which do not undergo further moults once adult (Gurney 1948).

Argulus spp. reproduce sexually and male and female parasites mate on or off the host (Kimura, 1970). During copulation, Martin (1932) suggests the genital pegs and sockets lock together, trapping the females 4th thoracic appendages between the 3rd and 4th of the male, thus preventing escape. Gurney (1948) described copulation to occur in a similar fashion. Martin (1932) suggested that sperm transfer was through a single median opening on the ventral side of the last thoracic segment; this was confirmed by Avenant-Oldewage & Swanepoel (1993). The adult female has a single median ovary cut off from the haemocoel, which runs the entire length of the thorax. The abdomen of the female holds a spermatheca, into which the male transfers sperm during copulation (Avenant-Oldewage & Swanepoel, 1993). Although several matings maybe observed, it is thought that one is sufficient to fertilise all the eggs that a female will ever produce (Kollatsch, 1959). The sperm are then used to fertilise eggs individually upon laying (Martin 1932, Avanent-Oldewage & Swanepoel 1993). Melanophores are found covering the testes in males and the ovary in females, possibly to protect them from UV light which has

the potential to damage genetic material, or could perhaps cover what are likely to be otherwise reflective areas of the parasite to make them less conspicuous to predators (Poly, 1998).

1.3.2 Life Cycle

Most *Argulus* spp. lay their eggs off the host on a suitable surface (Kimura 1970, Shimura & Egusa 1980), in rows that are attached by a gelatinous material that hardens on contact with water (Martin 1932, Bower-Shore 1940, Shafir & Van As 1986 and Rahman 1995). Eggs of *A. foliaceus* and *A. japonicus* are laid in batches comprising 2-4 rows, with each batch containing up to 400 eggs (Bower-Shore 1940, Kimura 1970, Shafir & Van As 1986). *A. coregoni* lays its eggs in mats that can contain up to 900 eggs (Hakalahti, Hakkinen & Valtonen, 2004). According to Kimura (1970) *A. japonicus* can lay between 1 and 10 egg batches following a negative binomial distribution, i.e. most parasites only lay once with very few laying 10 times. The number of eggs laid on each occasion was shown to be variable (Kimura, 1970). The eggs of all three UK species are mainly laid at night in the bottom to middle layers of ponds (Kimura, 1970, Shimura & Egusa, 1980, Mikheev, Pasternak, Valtonen & Lankinen 2001). A greater proportion of *A. foliaceus* eggs laid in deeper water are viable compared with those laid in shallow water (Mikheev *et al.*, 2001). The egg laying habits of argulids are discussed further in chapter 3.

When laid, individual eggs are ovoid, white to pale yellow in colour and measure approximately 0.2mm x 0.3mm. Within 24 hours their colour changes to a deeper

yellow to light brown (Bower-Shore 1940, Shafir & Van As 1986, Rahman 1995). Shafir and Van As (1986) describe three subsequent developmental stages in eggs of *A. japonicus*; the first is the development of eye spots, the second is the development of the thoracic appendages and the third stage includes the movement of the embryo at approximately 24-48 hours before hatching. The rate at which these stages occur is dependent on temperature. Rahman (1995) carried out more detailed observations of the stages in the eggs of *A. foliaceus*, and described nine stages in total. At 23°C Rahman (1995) noted that the initial colour change occurred after only 3 hours. Stages 2 –7 took approximately 12 days. Eye pigmentation and the appearance of appendages occurred at stage 8 (15-16 days after laying). Stage 9 is the hatching stage and occurred at around day 17 post laying at 23°C. Metanaupli exit the egg through a longitudinal split on its dorsal surface.

Hatching times of *Argulus* spp. eggs appear to vary between species and with temperature. Bower-Shore (1940) states that the eggs of *A. foliaceus* take 30-35 days to hatch at an undisclosed temperature. Shafir and Van As (1986) found that the eggs of *A. japonicus* hatched in an unsynchronised fashion both within the egg string and between batches. However, Pasternak, Mikheev and Valtonen (2000) found overwintered *A. foliaceus* eggs showed synchronised hatching. Eggs from the subsequent generation of parasites also showed synchronised hatching in a fish farm environment but asynchronous hatching in a lake. It was hypothesised that synchronicity may increase with host density, suggesting that in waters where hosts are scarce extended periods of recruitment would be beneficial, as the availability of a host will be unpredictable. It is difficult to see how this might be controlled. Hakalahti *et al.*, (2004) found hatching between and within batches of *A. coregoni*

eggs was asynchronous and took up to 200 days from start to finish in an individual batch. Below 8-10°C, eggs of the three UK species do not appear to develop, and this may be a mechanism to allow eggs to over-winter until more favourable conditions in spring and summer (Macheen 1940, Hindle 1948, Stammer 1959, Shimura 1981 and Shafir & Van As, 1986, Hakalahti, *et. al.*, 2004). Pasternak *et. al.* (2000) found that eggs of *A. foliaceus* that had over-wintered had a much-reduced hatching success. Mikheev *et al.*, (2001) found that eggs of *A. coregoni* can remain viable under over-winter conditions for up to 2 years. Further work is required to understand the mechanisms causing the observed variability in hatching times.

Shafir and Van As (1986) found that at 15°C eggs of *A. japonicus* took 61 days to hatch, which is in close agreement with Tokioka (1936), who found them to take 60 days at the same temperature. Kimura (1970) showed through laboratory studies that the relationship between the hatching time of *A. japonicus* eggs and temperature was exponential and could be described by the function $\log_{10}T=2.3-0.046\theta$ for temperatures of 16-29°C, where T = Development time in days of the egg from laying to hatching, and θ = Temperature. Hatching success was between 66.7-96.5% at temperatures between 14.4 to 30^0C, but Kimura (1970) suggested that scraping eggs from their surface of attachment for the purpose of the experiments, reduced hatching success by 30%. In a subsequent study where eggs were left attached to a surface, a 95.2-100% hatch success was observed. Hatching success of *A. japonicus* was shown by Shafir and Oldewage (1992) to be greatest at 25°C.

Shafir and Van As's (1986) study on the laying, development and hatching of *A. japonicus* eggs produced an interesting finding that may warrant further investigation. They found greater hatching success, and faster egg development, in 'control tanks' than in trial tanks. It was hypothesised that the greater degree of temperature fluctuation observed in the control tanks (2°C in control tanks c.f. 0.5°C in trial tanks), was responsible for the increase in hatch success and rate of egg development. If this is true it could lead to high burden of *Argulus* spp. small still waters, as they are prone to large diurnal temperature fluctuations. Bai (1981) showed that in *A. siamensis* both photoperiod and light intensity were important in determining hatching rate. This study suggested that long photoperiods of high light intensity reduced egg development time, although it is not clear whether light intensity or photoperiod was responsible for the effect as both variables were adjusted at the same time. Mikheev *et al.* (2001) found that eggs of *A. coregoni* buried in sediment had reduced hatch success and took longer to hatch. This was attributed to light intensity as they found that by increasing light intensity the rate of hatching increased, as did the hatch success. Temperature is also important in controlling the rate at which the hatched stages of the parasite develop, with increases in temperature increasing the rate of development. This relationship is discuss in detail in chapters 3 and 4.

At the level of the individual waterbody, the population biology and ecology of *Argulus* spp. throughout a year is also correlated to temperature. In lake systems in temperate climates, numbers of argulids tend to be low in winter (Kimura 1970, Shimura & Egusa 1983b, Shafir & Oldewage 1992, Pasternak *et al.* 2000, Gault *et al.* 2002), with laboratory experiments suggesting that most lice die off in the cold

conditions (Kimura, 1970) and after periods of reproduction (Pasternak *et al.* 2000). As already noted, eggs appear to lie dormant at <10°C, allowing them to survive the colder months unhatched until spring. A rise in temperature in spring causes the majority of eggs to hatch over a short period. This hatch of metanaupli makes up the bulk of the lice population at this time (Shimura 1983b, Shafir & Oldewage 1992, Pasternak *et al.* 2000, Gault *et al.* 2002). Populations build until they peak in the summer with high temperatures, after which numbers decline once again with the advent of winter, due to mortality following reproduction and the effects of the cold temperatures (Bower-Shore, 1940, Kimura, 1970, Shimura, 1983b, Shafir & Oldewage, 1992, Pasternak *et al.* 2000, Gault *et al.* 2002). The relationship between temperature and the life cycle and population dynamics of the parasite is discussed in greater detail in chapters 3 and 4.

1.3.3 Attachment, Feeding & Pathology

Argulus spp. attach to their host by means of hooks in the case of juveniles (pre-moult 5) and suckers in the more advanced stages (post-moult 5). The hooks and suckers used for attachment cause abrasions to the skin, fins and gills of the fish, and leave the attachment site haemorrhagic and erythmic. The most serious pathology however, is probably caused by the feeding behaviour of the parasite. (Prabhavathy & Sreenivasen 1976, Post 1987, Rahman 1996). Although Bower-Shore (1940) found blood in the intestine of the parasite, to date it has not been proved that *Argulus* spp. feed predominantly on blood. *Argulus* spp. feed through a mouth tube containing mandibles. These may act as cutting structures during feeding (Fryer 1968), suggesting the parasite is not an obligate blood feeder.

During feeding a pre-oral stylet repeatedly punctures the skin of the feeding site (Bower-Shore 1940 and Kabata 1970). Shimura and Inoue (1984) provided some experimental evidence that suggests an anti-coagulant is delivered by this stylet to increase blood flow to the feeding site. The feeding activity of large numbers of argulids is associated with skin abrasions, severe haemorrhaging and ulcerative lesions. The damage caused by *Argulus* spp. can lead to secondary bacterial, fungal, parasitic and viral infections (Bower-Shore 1940, Pfeil-Putzien 1978, Pfeil-Putzien & Baath, 1978, Shimura, Inoue, Kudo & Egusa 1983, Ahne, 1985, Singhal, Jeet & Davies 1990, Rahman 1996, Molnar & Szekely 1998 and Moravec, Vidal-Martinez & Aguirre-Macedo 1999). The parasite can also act as a vector for various species of nematode (Molnar & Szekely 1998, Moravec, Vidal-Martinez & Aguirre-Macedo, 1999). *Argulus* spp. do not normally cause a problem in natural environments as a single or a few parasites are unlikely to cause significant damage or lead to mortalities except when infecting very small or weakened fish (Fryer 1968). In artificial or manipulated systems, however, populations of argulids can grow to the point of causing mass mortalities (Wilson 1902, Fryer 1968, Menzees, Ramos, Pereira & Moreira da Silva 1990, Northcott *et al.*, 1997).

In some circumstances *Argulus* spp. infections may lead to host mortality. In laboratory experiments, Rahman (1996) found that 83.3% of 1.5-2cm carp (*Cyprinus carpio*) were killed within 24 hours when infected with 20-30 metanauplii. Stammer (1959) found that in tank trials 175-200 lice would kill carp of 36cm held at 21°C within 2 weeks of infection. Jafri and Ahmed (1994) report a mortality rate of 30-40, 15-25cm carp per day with infections of 70-100 lice, on an Indian fish farm. Menzees *et al.*, (1990) Northcott *et al.*, (1997) and Gault *et. al.*

(2002) described cases of massive mortalities in still waters where lice numbered up to 1500 lice per fish. Figure 1.2. demonstrates the level of infection that can occur in a fishery. The mortality caused by *Argulus* spp. can also have a significant effect on wild fish populations. After studying *A. canadensis* in a community of three different species of stickleback, Poulin and Fitzgerald (1987) hypothesised that *A. canadensis* could actually play a role in structuring a fish community as mortalities in certain species were highly significant. This appeared to be dependent on the behaviour of the particular species of fish.

Figure 1.2. Two, 1kg rainbow trout each carrying over 1000 *A. foliaceus*.

Clinical signs of infection include red blotches, lesions, scale loss (Prabhavathy 1976 and Hoffman 1977) and heavy mucus secretions (Post 1987). Many reports note a reduced growth rate, and loss of physical condition that leaves fish weak and emaciated, and thus stressed and susceptible to secondary infection (Chen 1933, Prabhavathy 1976, Shimura, Inoue, Kudo & Egusa 1983, Poulin & Fitzgerald 1987, Singal *et al,* 1990, Jafri & Ahmed 1994 and Rahman 1996). This is followed by a period of sluggish aimless swimming before a loss of equilibrium, which is

normally shortly followed by death (Prabhavathy 1976, Hoffman 1977, Post 1987, Knight 1996, Jafri & Ahmed 1994, Rahman 1996 and Northcott *et al,* 1997). Singal *et al,* (1990) found that over an eight week period the mean weight increase of carp infected with *Argulus* spp. was 0.24% compared with 2.6% in uninfected fish, there was also a highly significant reduction in the condition factor in infected fish. These effects can affect production in farm ponds (Jafri & Ahmed 1994), but can also reduce the aesthetic appeal to anglers of fish in a sports fishery (personal communications with fishery owners). Heavy infections may also cause noticeable behavioural changes, which develop as the severity of the outbreak increases. Knight (1996) describes this process from a fishery owner's perspective. At an early stage of infection, fish in lakes are reported to jump, flash and swim erratically, possibly in an attempt to rid themselves of lice. Following this, feeding is reduced and the fish become uncatchable by anglers. As infection levels rise, fish have been observed to swim in tight shoals, possibly a parasite avoidance mechanism (Poulin & Fitzgerald 1998d).

1.3.4 Host Finding & Behaviour

To infect a host, a parasite must first come into contact with it, either by a random chance encounter or by active searching. Poulin and Fitzgerald (1987) demonstrated a positive correlation between fish density and the percentage of an *A. Canadensis* population attached to fish. This finding is not surprising, as, by increasing the number of hosts, an increase in the rate of contact between host and parasite and therefore the chance of a random encounter would therefore be expected. There is also substantial evidence that *Argulus* spp. actively seek their

hosts. Argulids are able to detect chemical cues in the mucus of fish and become activated in its presence (Galarowicz & Cochran 1991, Mikheev *et al.*, 1998, 2000), attaching more readily to a surface coated in mucus than one without (Kaestner 1980, as reported by Galarowicz & Cochran 1991). Argulids also appears to be stimulated by light. In a highly reflective environment *A. foliaceus* swim 4.4 times faster than in darkness, and are attracted towards reflective objects such as knives (Mikheev *et al.*, 1998). Bai's (1981) and Rahman's (1995) observations concur with these findings as they note metanaupli to be photopositive, congregating in well illuminated sites within an aquarium. However, in highly reflective environments, low infection rates were observed, suggesting the parasites became disorientated (Mikheev *et al.*1998). In aquaria that were illuminated but had no reflection, infection success was much greater (Wilson 1902, Mikheev *et al.*1998). At night other senses may replace vision, with rheosensitive bristles at the anterior edge of the cephalon possibly being the most important sense organ (Madsen, 1964). Mikheev *et al.* (1998) and Mikheev *et al.* (2000) found hydromechanical disturbance (turbulence) was an important factor in the stimulation of *A. foliaceus*, and showed that a fish must be within 6cm of the parasite for an infection to be attempted. They also found that male *A. foliaceus* can attach to a host significantly faster (1.6 times) than females, however, Poulin and Fitzgerald (1989b) showed infection success of male and female *A. canadensis* to be similar. Mikheev *et al.* (2000) studied the behaviour of *A. foliaceus* under light and dark conditions and compared it with that of the host. Their results suggest that the parasite adopts two different strategies that have evolved to coincide with host behaviour. During the day, when most fish are active, *A. foliaceus* acts as an ambush predator, suspended in the water column and waiting for a fish to swim past. This "sit and wait" strategy

could also make the parasite less conspicuous to any potential predators and at the same time save energy. At night, however, when fish tend to be more sedentary, *A. foliaceus* adopts an active searching behaviour in order to find a host. In darkened conditions the parasite was shown to swim 4.7 times faster, and use up 25% more energy than in illuminated conditions.

Fish that are already infected have an increased chance of further infection compared with uninfected fish (Poulin & Fitzgerald, 1989a). There are several factors that could explain this, such as an attraction to chemical cues given off by either the host fish as a distress response, or by attached parasites. Another explanation that corresponds to the findings of Mikheev *et al.* (1998) is that infected fish are likely to swim in an erratic fashion, causing reflected flashes of light that may attract the parasite. Poulin and Fitzgerald's (1989s) findings, however, disagree with those of Buckley and Morrice (1976) who found no evidence to suggest that infected fish were more prone to infection than uninfected fish. This may be due to differences in the species of *Argulus* and host studied by the two sets of authors.

Argulids appear to show preference for certain sites on the fish. Infection tends to occur from beneath or to the side of fish, with the lateral regions being the most popular sites for initial attachment (Mikheev *et al.* 1998). Once attached, lice move to a site of preference (Thomas 1961). In the cases of *A. foliaceus* and *A. japonicus* this appears to be the head and the base of the caudal fin, followed by the dorsal region and lower flanks (Bower-Shore 1940, Basal, Lucky & Dyk 1969, Buckley & Morrice 1976, Schluter 1978). Schluter (1978) found that mature parasites tend to congregate at the anterior end of the fish, whereas more juvenile parasites were

found in the caudal region. A study on salmonid fish by Shimura (1983b) suggested that *A.coregoni* have different sites of preference on the fish to *A. foliaceus* and *A. japonicus*. The head, opercular, flank and caudal regions appeared to remain largely free from adult *A. coregoni,* with the adult parasites located mainly on or around the pectoral and pelvic fins. No significant difference was observed between numbers infecting either side of the fish. Juvenile *A. coregoni* under 1.5mm were, however, found all over the fish, with 96% on the body and fins of the fish, compared with 4% in the oral and gill cavities. As these parasites developed they moved from the body of the fish to the areas around the pectoral and pelvic fins. Differences in site preference by *A. coregoni* compared with *A. foliaceus* and *A. japonicus,* may be due to the host fish species, and the fast flowing habitat that they inhabit. It could be that the locations on and around the hosts fins provide some shelter from the strong hydrodynamic forces that are likely to be encountered by *A. coregoni* in a riverine habitats.

1.3.5 Host Specificity

Argulus spp. are obligate parasites and must therefore be able to locate and attach to a host rapidly in order to survive, however, the chance of finding a host can be further increased by having a wide variety of potential hosts. Fryer (1968) suggests that some African species of *Argulus* are more selective than others, but this may be due to a lack of other suitable hosts in their location. In general *Argulus* spp. appear to have a wide host tolerance and they have been recorded on most species of fish found in the UK (Bower-Shore 1940, Fryer 1968 and Kabata 1970), including the

eel (*Anguilla anguilla*) (Evans & Matthews 2000). Some species of *Argulus* have even been recorded on frogs and tadpoles (Wilson 1902).

Although *Argulus* spp. have a wide host tolerance, some fish appear to be more susceptible than others (Kimura 1970, Buckley & Morrice 1976, Mishra 1991), although this is by no means well defined. Gurney (1948) found no evidence of particular host selection, but Bower-Shore (1940) states that highly coloured fish are less susceptible than duller fish and uses the example that brown trout were often thought to be infected more readily than the brighter rainbow trout. Kimura (1970) found the number of *A. japonicus* on a variety of ornamental species was greater with increased caudal fin length. This is possibly as fish with elaborate finnage are sluggish in their behaviour and have a larger area for settlement, making them more susceptible to infection. LaMarre and Cochran (1992) attempted to determine whether host preference could be due to evolutionary factors by comparing selection by *A. japonicus* on goldfish (which they suggest is their natural evolutionary host) and fathead minnows. The study showed no significant differences in attachment/detachment on the two hosts, suggesting evolutionary routes do not play a role in host selection. Mikheev, Valtonen and Rintamati-Kinnunen (1998) studied host finding in *A. foliaceus* and found perch to be selected over roach in a darkened aquarium, Pasternak *et al.* 2000 also found this to be the case in the wild. A subsequent study by Mikheev *et al.*, (2000) showed this selection to be due to both fish behaviour and colouration. The high infection levels observed in perch in darkened conditions corresponded with their relatively sedentary behaviour compared with roach (Rutilus rutilius). In light conditions perch were more active and were infected less frequently than in the dark. Roach were more active but

were infected more often in the light than the dark, suggesting their bright, reflective colouration could be an important factor. This conflicts with the views of Bower-Shore (1940). More evidence that fish behaviour plays an important role host selection comes from the work of Poulin & Fitzgerald (1988), who observed that the location of a fish in the water column affects its chance of being infected. Fish living lower in the water column were more likely to become infected than those located further up the water column. Clearly, at present there is a great deal of debate over the occurrence of host preference and the mechanisms behind this. To date no work has been published on the immune response of different fish species to infection by *Argulus* spp., and the role this may have on infection levels between host species.

1.3.6 Epidemiology and Distribution

Two of the *Argulus* species found in the UK can be regarded by the literature as native; these are *A. foliaceus* and *A. coregoni*. *A. foliaceus* is a widely recorded species that is regarded as charecteristic of mesotrophic and eutrophic lakes (Gurney, 1948, Campbell, 1971, Okland, 1985), however, *A. foliaceus* can also tolerate salinities of up to 8-12ppt at temperatures up to 25°C (Moller, 1977). Pasternak *et al.* (2000) also noted that *A. foliaceus* can reach epizootic proportions in brackish waters. *A. coregoni*, is regarded as typical of rivers, streams and cool oligotrophic lakes with a large flow (Gurney 1948, Rushton-Mellor 1992, Campbell 1971 and Okland 1985), although mixed populations of *A. foliaceus* and *A. coregoni* have been noted in Finland (Mikeev *et al.* 2001). The third species of fish louse found in the UK, *A. japonicus* is apparently a non-native species probably

introduced from the Far East through the ornamental trade (Rushton-Mellor 1992, Northcott; Lyndon & Campbell 1997). *A. japonicus* is recorded throughout Europe, Africa, North America and the Far East. At present there are limited records of *A. japonicus* in the UK, but it is possible that it has been misidentified as *A. foliaceus* due to difficulty in differentiating the two species, especially females. Little is known about the rate of spread, tolerance to the UK climate or the virulence of *A. japonicus* in comparison with *A. foliaceus,* but Rushton-Mellor (1992) suggested *A. japonicus* was confined mainly to the South of England. The distribution of *Argulus* spp. is covered in more detail in chapter 2, however little is currently known about the distribution of the three species in UK trout fisheries, nor the extent and severity of the infections caused in this type of environment.

1.3.7 Prevention and Control.

At present there are no chemicals or chemotheraputants that are licensed for use in the UK that are effective against *Argulus* spp. (Bark, 2000). Much of the older literature describes the use of various organophosphate treatments and other pesticides. The use of chemicals such as benzene hexachloride, DDT, DFDT, Diptrex, Diflubenzuron, Dylox, Gammexane, Lysol, potassium permanganate, pyrethrine and their appropriate doses have been described by Hindle (1948), Stammer (1959), Hoffman (1977), Hoffman (1980), Singhal, Jeet & Davies (1986), Post (1987), Knight (1996) Tonguthai (1997) and Williams (1997). Of these treatments diptrex appears to be referenced most commonly for the treatment of *Argulus* spp.. Tonguthai (1997) for example, suggests spraying diptrex over the entire water surface at a dose of 0.2-0.3ppm, but states that this has no effect on the

eggs so therefore 2-3 treatments must be applied in a season. Although many of these chemotheraputants appear to have some effect, the potential damage to the environment and human health has not been assessed and thus no licences have been granted in the UK. Simple treatments such as changes in salinity have also been described (Chen 1933, Singhal *et al,*. 1986), but few of these measures are likely to be practical in a fishery, and many of them involve changing the salinity levels beyond the tolerances of many species of fish before they will have an effect on lice. Raune, McCarthy and Reilly (1995) suggest the development of a vaccine may be possible in the future, but at present there appears to be little progress toward such a measure. They did, however, demonstrate that although there was no mucosal antibody response to *A. foliaceus* antigens, there was a significant humoral response in rainbow trout, and they also observed cross-reactivity with sea lice antigens. Studies by Hakalahti, Bandilla & Valtonen (2003) would, however, suggest that this response had no protective effect, as they observed no difference in infection levels by *A. coregoni* on rainbow trout that had been exposed to a prior infection, compared with a naive control group.

Little is known about how *Argulus* spp. are introduced into a fishery or farm, but some 'grey literature' suggests trying to prevent their introduction by avoiding the import of fish, weed or boats from potentially infected sources, and also by stopping anglers using live bait, and by ensuring the drying or disinfection of tackle, footwear and equipment prior to and after fishing (Northcott & Walker 1996, Northcott & Walker 1997, Northcott 1997, Northcott 1998, Anon 1998 and Bark 2000). Tonguthai (1997) suggests quarantining fish before stocking if possible. Hoffman (1977) suggests that covering pond inlets with 3.2mm mesh could prevent

the introduction of adult lice, although this may not be practical due to filters becoming clogged with particulate matter. Bark (2000) also suggests filtering incoming water, or where possible using bore hole water as a supply.

If the parasite is established in a fishery, Hoffman (1977) suggested increasing flow through the lake, fertilising to darken the water, refraining from stocking until after the 'spring hatch' and removing potential spawning substrates such as rocks, weed and wood. In small lakes it may be possible to add an artificial spawning substrate that could be removed and dried to reduce egg numbers (Bauer 1970, Hoffman 1977, Gault *et al.*, 2002). The efficacy of many of these strategies appear to be at best speculative, and in some cases unfounded. However, Gault *et al.* (2002) provided some evidence to suggest that populations of *A. foliaceus* in stillwater trout fisheries can be controlled using artifical egg laying substrates in the form of floating PVC boards. Fifty, $1m^2$ opaque corrugated plastic boards, suspended horizontally 6mm beneath the water surface were placed around the edge of a 12.9 hectare reservoir. Boards were removed and replaced every 2 weeks. The year after this intervention was introduced there was a 146-fold reduction in egg laying, and the prevalence and intensity of the *Argulus* spp. infection was reduced 9-fold and 6-fold, respectively. The impact of this measure over a longer time period is unknown but, Hakalahti, Pasternak & Valtonen (2004) suggest a similar method for controlling *A. coregoni* in trout farms. Wilson (1902) and Bark (2000) suggests that introduction of sticklebacks, small dace and roach could be an effective form of biological control. Although there is evidence that these species and several others will predate upon *Argulus* spp. (Wilson 1902, Bowershore 1940, Thomas 1961, Bauer 1970, Kimura 1970 and observations by the author) there is no evidence to

suggest that this is an effective control method, and it could in fact exacerbate the problem as all of these species can themselves be infected by *Argulus* spp..

Should a heavy infestation by *Argulus* spp. occur the only courses of action likely to be effective at present are to have a fallow period to allow the parasite to die out naturally (Tonguthai 1997), or to drain the lake. Drying or frost should kill the exposed eggs (Chen 1933, Stammer 1959, Northcott *et al.* 1997 and Anon 1998), but as an extra safeguard, lime can be applied to the lakebed to change the pH to at least 9.8. The lake should be left for several days before being refilled to allow the pH to return to normal before restocking (Chen 1933, Stammer 1959, Bauer 1970, Jafri & Ahmed 1994 and Tonguthai 1997). Values for the dosage of lime to be applied vary considerably, but Chen (1933) gives the lowest value of around 92kg per hectare. These methods may, however, not be entirely practical, depending on the scale of the fishery or the potential loss of earnings due to closure of the fishery whilst the intervention is conducted.

1.4 Conclusions and Study Aims

Although there is an extensive literature available on argulids, much of it lacks detail and is speculative. There is little information on the biology of the parasite in trout fisheries and a lack of quantitative data on the mechanisms generating problem infections. Prior to this study the problems associated with the parasite in trout fisheries had not been identified, and it was not known whether *Argulus* spp. were always responsible for the perceived problem. Of the available literature much is concerned with the morphology and development of the parasite. There are also

many records on the pathology caused by *Argulus* spp., but they contain little detail and it is difficult to determine whether the parasite is truly responsible for the clinical signs associated with infections, or the massive mortalities that have been observed in fisheries and farms. Although many observational studies have been carried out on the behaviour, ecology and population dynamics of *Argulus* spp., there is a lack of quantitative data, even for *A. foliaceus*. This means there is a poor understanding of the mechanisms resulting in problem causing infections. At present there is lack of control measures and management strategies against outbreaks of argulids in the UK. Prevention through improved management practices is obviously more favourable to the use of chemical treatments, and probably most practical option when dealing with a large open water system, but to do this such strategies require a better understanding of the parasite.

In depth studies into the unknown aspects of the population ecology and epidemiology of the parasite in the UK may lead to the identification key points in the parasites life-cycle, and risk factors associated with epidemics. Although it is probably not possible to totally eradicate a population of Argulids from a fishery, it may be possible by manipulating risk factors, to keep population numbers at a non-problem causing level. Surprisingly, prior to this project, little hard data has been available as to the nature of the problem caused by *Argulus* spp. to stillwater trout fisheries, its distribution or the proportion of fisheries affected. In addition, the lack of effective management strategies or licensed chemical treatments has raised concerns within the Environment Agency of England and Wales, that illegal pesticides are being used to control the parasite. With this information in mind a three-year research study was designed with the following objectives:

a) Review the current perception of the extent and severity of *Argulus* spp. infections in UK Stillwater trout fisheries, and identify methods currently employed for controlling these infections.

b) Review and increase knowledge of the biology and ecology of *Argulus* spp. in relation to these systems.

c) Assess the prospects for novel control and management strategies to reduce economic loss.

CHAPTER 2: CROSS-SECTIONAL STUDY OF UK TROUT FISHERIES

2.1 Introduction

There is a limited but significant amount of literature on the impact of *Argulus* spp. at the individual host level, but very little information on the impact of *Argulus* spp. at the host population level. Of the literature that is available, most refers to impact in fish farms rather than fisheries. In farms, *Argulus* spp. infections can reduce growth rate (Singal *et al.*, 1990), condition factor (Rahman, 1996), cause scale loss and lesions (Menezes *et al.*, 1990), make fish susceptible to secondary fungal and bacterial infections (Bower-Shore 1940, Pfeil-Putzien 1978, Shimura, Inoue, Kudo & Egusa 1983, Singhal, Jeet & Davies 1990, Rahman 1996, Molnar & Szekely 1998, Moravec, Vidal-Martinez & Aguirre-Macedo 1999) and, in extreme cases, lead to significant mortality (Stammer 1959, Menzees *et al.*, 1990 and Knight 1996, Rahman 1996, Northcott *et al.*, 1997).

Argulus spp. is widely distributed in rivers and lakes throughout the UK, however at present little is known of the impact that the three UK species of *Argulus* spp. may have in fisheries. The Environment Agency (E.A.) of England and Wales have received reports that many stillwater trout fisheries suffer problem infections, however, no attempt has been made to quantify or establish the exact nature of the perceived problem (personal communications with E.A. representatives). Gault *et al.* 2002 and Northcott *et al.* (1997) described a series of signs attributed to *Argulus* spp. exhibited by trout in infected fisheries. In the early stages of infection there was a noticeable increase in the frequency of fish jumping and a reduction in feeding. As the infection progressed secondary fungal and bacterial infections developed, and fish

may exhibit increased shoaling behaviour. If the infection continued, large-scale mortalities were observed. Knight (1996) provided a similar account from a fishery owner's perspective.

Fisheries are highly variable both in their physical nature (e.g. size, depth, water source) and the way in which they are managed (e.g. size and strain of fish, frequency of stocking, closed seasons). Due to this level of variability, there is an apparent lack of effective, practical or legal control measures for *Argulus* spp. infections. Gault *et al.* (2002) suggested control may be possible by providing artificial substrates on which the parasite lays its eggs and which can subsequently be removed. This has been shown to be effective in small fish farm ponds (Bauer, 1970), however, although the study of Gault *et al.* (2002) suggested this method may have some efficacy in a fishery environment, further large-scale trials are required before firm conclusions can be drawn. Draining and drying a fishery and applying lime at a dose of 92kg per hectare, or having a fallow period, may be effective in reducing or eradicating the parasite (Chen, 1933). However, many fisheries are very large systems, making fallowing or draining impossible. In addition many fishery owners do not own the lake, but only the rights to run their business on it, thus they may not be able to obtain permission to drain or fallow. Finally, even if a fishery owner can implement one of these interventions, the cost is often prohibitive, both in terms of applying the intervention and in the loss of revenue whilst the fishery is closed. There is thus an obvious need for the development of effective management strategies to control *Argulus* spp. populations in these systems.

An initial step in the development of practical and effective management strategies is the identification of factors that are associated with infected waters but not uninfected waters, i.e. 'Risk Factors'. Risk factors are identified by comparing a population of cases, i.e. individuals that have a particular disease or health related state, with a relevant control population in which the disease is not present, and then establishing the level of exposure to a risk factor in each group (Thrusfield, 2003). The impact of a factors can also be assessed by exposing a population to a hypothesised risk factor, following it through time and comparing the occurrence of the disease in that population, compared to a population that has not been exposed to the factor of interest.

Risk factors can be identified either through experimental/clinical trials or observational studies. In the early stages of an investigation when little is known about a disease or health related state, observational studies are commonly used to generate hypotheses and establish association between possible factors and the outcome, i.e. clinical disease. Clinical trials are generally used to test a particular hypothesis, drug or control strategy under tightly controlled conditions and therefore are commonly used after initial hypotheses have been developed through observational studies. Due to a lack of information on *Argulus* spp. infections, this study used observational studies to identify potential risk factors through statistical association, and generate hypotheses about ways in which they may be associated with a problem infection.

Observational studies can either be conducted at the level of the individual or at the level of the population, the latter being known as an ecological study. The three most

common types of observational study are: case-control, cohort and cross-sectional. Case-control studies normally require that representative populations of infected (case) and uninfected (control) individuals or sites can be identified prior to the survey. They are normally retrospective in nature and have the advantage of allowing the matching of certain factors (Thrusfield, 2003) e.g. comparison of lake age and size. This type of study is particularly useful when an outcome is rare as the number of cases is selected and not left to chance, and was used by Jarp *et al.* (1993) to identify risk factors for infection by *Aeromonas salmonicida* in Norwegian salmon hatcheries. The study successfully identified three risk factors at the hatchery level after matching case and control hatcheries by region. These were migration of anadromous fish into the water supply, sharing of personnel between farm sites, and a high number of infected farms in close proximity to the hatchery of interest. Clearly the first two of these can be managed relatively easily. The third cannot be managed but provides useful information on the spread of the disease.

Cohort studies generally assume populations can be identified that have and have not been exposed to a risk factor. These are normally prospective studies where the groups are followed through time and the proportion of cases occurring in each group compared (Thrusfield, 2003). This type of study is useful if a risk factor is rare, but limits the number of factors that can be studied to one as groups are defined based on a factor of interest. Cohort studies are generally expensive, time consuming and losing subjects to follow up (i.e. sites drop out of the survey or go out of business) can be a major constraint, they do, however, allow a temporal relationship between a disease and risk factor to be established. Vagsholm and Djupvik (1998a, 1998b & 1999) used cohort studies to successfully identify a series of risk factors for the

occurrence of skin lesions, spinal deformations and abdominal adhesions in farmed Atlantic salmon. The study followed slaughter groups (batches) of fish over time. Within the aquatic environment it is difficult to conduct a fish level survey without an efficient method of tagging that allows individuals to be followed through time. It is also be difficult to follow sufficient numbers of farms or fisheries over time to obtain risk factors at this level through a cohort study.

The two types of study detailed above make the assumption that either suitable case and control populations, or that groups exposed to a factor can be identified. Unfortunately in the case of *Argulus* spp. infections there is no suitable information from which such populations could be identified, as fishery owners are under no obligation to report infections and government bodies such as the Environment Agency and CEFAS are not required to screen for them. This also means that there is no definition of a 'problem *Argulus*' infection on which to base a case. The third type of observational study is a cross-sectional study, which makes few assumptions about the disease status of the population of interest. A random population of individuals or sites is selected, and then screened for the disease and then surveyed to determine the level of exposure to risk factors in the diseased and non-diseased fractions of the population. No prior knowledge on disease status is therefore required (Thrusfield, 2003). This type of study allows large numbers of potential risk factors to be screened and is useful when little information is available on a disease within a population. Cross-sectional studies also have the advantage of allowing the prevalence of a disease within a population to be established. Several cross-sectional studies have been conducted to identify risk factors in various aquatic systems. Jarp *et al.*, (1995) identified risk factors for infectious pancreatic necrosis in salmon farms,

Bebak, Baumgarten and Smith (1997) studied bacterial gill disease in batches of hatchery reared rainbow trout, and Leung, Tran and Fast (2000) identified farm level risk factors for disease occurrence in Asian shrimp culture. All of these studies have been conducted at the farm or batch level, and there appears to be no published work on the identification of risk factors for disease within fisheries.

Cross-sectional (and other observational) studies cannot establish a causal relationship between a risk factor and an outcome but fulfil at least the first two of nine epidemiological tenets of causality set out by Hill (1965), who argues that for a factor to have a causal association with a disease the following must be established:

1. **Strength of association** – the stronger the statistical association between a factor and an outcome the greater the likelihood that it will be causal.
2. **Biological plausibility** – can a biologically plausible hypothesis be generated on how a factor may be acting?
3. **Temporal relationship** – exposure to the factor must occur before the occurrence of the disease.
4. **Biological gradient** (dose response) – the greater or more intense the exposure to a factor the greater the likelihood of developing the disease.
5. **Consistency with other knowledge** – are the results consistent with other studies, or could the study be replicated to produce consistent results.
6. **Biological coherence** – is a cause and effect hypothesis consistent with the life-cycle and biology of the disease?
7. **Specificity** (supportive but not a necessary tenet) – is the risk factor specific to the type of disease of interest.

8. **Experimental evidence** – clinical/laboratory based studies exposing groups to the disease and factor of interest.
9. **Analogy** – similarity to other known cause and effect associations.

In this study the unit of interest is the fishery or site, and risk factors are those associated with infected fisheries as opposed to infected fish. Due to the current lack of information on the impact or extent of *Argulus* spp. infections in UK stillwater trout fisheries, it was decided to conduct an ecological cross-sectional study with the aim of determining the following:

a) The perceived problem associated with *Argulus* spp. infections.
b) The extent and severity of the problem, i.e. distribution and prevalence.
c) Management strategies that were being employed.
d) Factors associated with problem infections around which hypotheses could be generated and further research targeted.

2.2 Materials and Methods

An ecological, cross-sectional study of UK stillwater trout fisheries was conducted from May to December 2001. The survey was retrospective with data collected referring to the situation in year 2000. This approach was taken as it would not have been possible to survey a sufficient number of fisheries during summer 2001 when problem infections were likely to occur. The sampling period was deemed to be long enough to allow sufficient survey data to be collected but short enough to reduce any recall bias that can be an inherent problem in retrospective surveys (Powell 2000, Thrusfield 2003). All data was collected by the author in the form of site based interviews and sampling.

2.2.1 Study Site Selection and Survey Approach

Trout fisheries in the UK fall into two main categories: commercial (day ticket) fisheries and syndicate (club) waters. It is relatively easy to obtain lists of commercial fisheries as the majority advertise their business allowing fishing magazines to produce comprehensive lists. Syndicate waters are more difficult to identify as many are only advertised through word of mouth. To obtain a representative list of all the fishery types in the UK, a list of commercial fisheries (The Trout Masters Guide) was amalgamated with the membership records of the largest association representing UK trout fisheries – the Association of Stillwater Game Fisheries Managers (ASGFM).

The list was comprised of 406 fisheries distributed throughout the UK. The Trout Masters Guide had stratified the list in to 11 geographic regions. To ensure fisheries from throughout the UK were selected, 30% of the waters from each region were randomly selected. This was done by numbering each fishery in each region and using a random number generator to select the necessary number of sites. A total of 123 waters were selected, which was felt to be a realistic number of sites to survey given the time and resources available. Some fisheries comprise more than one lake, which are often managed in different ways. At sites where more than one lake was present, one was selected at random so as to reduce the interview time and the bias that could occur if interviews lasted for a different time between sites. Lakes within a site were selected by arranging them in size order, and numbering them 1 to n. A dice was then used to select the study lake.

A standardised letter (Appendix 1) stating the purpose of the project and the funding bodies involved was sent to all selected sites. To increase response rates the letter highlighted the fact that all data obtained would be treated as confidential and would not be released without the written permission of the owner. The letter also highlighted the fact that it was equally important for fisheries that had not experienced *Argulus* spp. infections to take part in the project. Fisheries were then contacted by telephone to request their participation. All fisheries that agreed to take part in the project were visited. An interview was conducted with the fishery owner using a standardized questionnaire (2.2.2 and Appendix 2) and water samples were collected in an attempt to characterize lakes by their water chemistry. Where possible fish were examined to check for the presence of *Argulus* spp.. If *Argulus* spp. were found specimens were collected and preserved in 70% ethanol and identified to species in

the laboratory using the key of Fryer (1982). At sites where *Argulus* spp. had been noted but not found at the time of the visit, a vial of alcohol and a self addressed envelope were left so that the fishery owner could forward any specimens subsequently found. Of the 123 sites initially contacted 77 agreed to take part in the study and were visited. Due to the low number of sites surveyed in each study region, prevalence estimates for each region would have been relatively meaningless. Instead, regions were amalgamated into northern, central and southern areas prior to the survey, by combining geographically the 4 most northerly, 4 central and 3 southern regions (as the latter contained the most sites). This gave similar numbers of sites between regions: North = 21, Central = 25 and Southern = 23.

To further determine the distribution of *Argulus* spp. in the UK, an E.A. database containing records of *Argulus* spp. in stillwaters (including non-trout waters) in England and Wales was screened and locations of the occurrence of *Argulus* spp. noted. The database was based on the findings of over four years of fish health checks (Section 30: Salmon & Freshwater Fisheries Act, 1975) conducted by the E.A. that are required before fish can be moved between waters. Unfortunately the movement of trout from a fish farm to a fishery is not included under this legislation, so the majority of these data refers to the movement of other fish species between lakes and rivers. These data, along with confirmed records of *Argulus* spp. obtained as part of the cross-sectional survey and from the literature, were used to plot distribution maps.

2.2.2 Questionnaire Design and Validation

"A questionnaire is a standardised, structured instrument which is administered in a standard way to a desired sample of a population of interest" (Anon, 2003).

A questionnaire should be clear, concise, non-leading, only contain necessary questions and be aimed at the level of the target population. Questions within a questionnaire fall into two categories, open and closed (Pallant, 2001). Open questions allow the respondent to answer how they want, and do not require any prior knowledge on the part of the surveyor of the answers that may occur. The disadvantage of open questions is that they are difficult to analyse, and although it may be possible to categorize the answers post-survey, this may add a degree of bias (Pallant, 2001). Closed questions provide the respondent with set answers. They are easy to complete and analyse but assume that the surveyor knows all the potential answers that are likely to be obtained to a question and may reduce the range of information that will be gained. A combination of open and closed questions is a good option in novel areas of research (Pallant, 2001). The majority of information can be gained through closed questions, but the inclusion of some open questions allows responses that the questionnaire designer had not anticipated and thus extra information to be obtained. The design of questionnaires has been reviewed by Oppenheim (1992).

The survey questionnaire was designed using a combination of open and closed questions. Information that was deemed important to the survey was identified through literature searches on the biology and ecology of *Argulus* spp. and the

management of fisheries, and from industry representatives. This information was turned into a series of questions that were phrased in a simple, non-scientific way. Questions were then arranged in a logical, structured fashion. Questions relating to commercially sensitive information were placed towards the end of the questionnaire to ensure some information was gained even if the fishery owner refused to continue with the interview. Where possible, cross validation questions were incorporated into the questionnaire to ensure that the data being given was accurate, e.g. How many fish were stocked in year 2000? How many times did you stock in year 2000? On average how many fish were stocked per stocking? The latter two questions allow the consistancy of the first to be checked. Sites which provided inconsistent answers to these questions were removed from the survey. The questionnaire was kept as short and a simple as possible so that interest by the respondent was maintained throughout and equal effort was put into the entire questionnaire. Open-ended questions were generally reserved for data where no information was available in the literature or elsewhere, e.g. what a fishery owner perceived to be the problems associated with *Argulus* spp. infections.

Once drafted, the questionnaire was discussed by the project review panel comprised of a mixture of scientists and fishery managers to ensure the questions were in line with the project aims, were unambiguous and ethically sound. The questionnaire was tested at 9 fisheries that had not been selected in the random site selection, but had contacted the author with an interest in participating in the study. This process identified problems with the questionnaire such as ambiguity in the phrasing of the questions, and highlighted unexpected answers, allowing the questionnaire to be

adjusted accordingly. These completed test questionnaires were not used in any subsequent analyses.

The final questionnaire comprised three sections:

1. History and occurrence of *Argulus* spp. infections and other fish health problems.
2. Site details and lake characteristics.
3. Fishery management.

These topics allowed the problems associated with *Argulus* spp. infections to be determined, the timing and occurrence of problem infections to be established, the identification of susceptible lake types and a comparison of management practices.

2.2.3 Interview Procedure

Questionnaires can be delivered by post, telephone interview or face-to-face interview. Postal questionnaires are inexpensive and a large population can be targeted. However, response rates are generally low and only a limited amount of information can be gained. Telephone interviews have a higher response rate and allow more questions to be asked, but it can be difficult to validate the responses given. Face to face interviews are the most expensive and time consuming form of survey, but have the highest response rates, allow the most information to be gained and make it easier to validate results through visiting the subject/site of interest. For the purpose of the survey, face-to-face interviews were thought to be the most appropriate option due to the high response rate and the amount of data that could be

collected. The site visit also allowed additional data to be collected, i.e. water and parasite samples. All interviews were conducted by the author. Using this approach meant the interviewer had to be careful not draw conclusions during the survey as it may have biased the way in which subsequent interviews were conducted.

At the start of each interview the purpose of the project and the confidential nature of the information was re-iterated. Participants were also told that they were not obliged to answer any questions and that they were free to terminate the interview at any time. At the end of the interview, participants were thanked for their time and co-operation. Each interview lasted approximately an hour and a half, and was conducted in an informal manner so as to put the participant at ease.

2.2.4 Data Collation and Analysis

A database was created in Epi-info 2000 (Centre for Disease Control (CDC)) to collate all the survey data. Where possible tick boxes and drop down menus of answers were included to reduce errors and time spent entering data. Macros were also written so that the database would automatically calculate variables such as lake volume and rate of stock turnover from the survey data. This further reduced processing time and improved accuracy. Table 2.1 shows the variables determined automatically and the formulae used to calculate them. All descriptive statistics and graphs were produced in Excel 2000, Epi-Info 2002 or Sigma Plot.

Table 2.1 Variables calculated automatically in Epi-info 2000 database and the formulae used to calculate them.

Variable	Formula
Rods per hectare per annum	Total no. rods / site size
Lake volume	Lake surface area x average depth
Kg of fish per hectare	Standing stock x mean fish weight / surface area
Fish per hectare	Standing stock / surface area
Kg of fish per unit volume	Standing stock x mean fish weight / lake volume
Fish per unit volume	Standing stock / lake volume
Time taken to replace entire stock of trout in lake (weeks)	52 / (Total no. fish stocked in the year / standing stock)
Total feed added per hectare per annum	Total feed added / surface area

An Excel spreadsheet was used to collate longitude and latitude of both study sites and sites identified as having been infected with *Argulus* spp. from E.A. records. The longitude and latitude was available for the E.A. data and was determined for the study sites by locating sites on Streetmap (www.streetmap.co.uk) through their postcodes, which Streetmap automatically converts. This spreadsheet was saved as a .dbf file, which could be read by Epi-info's G.I.S. program Epi-map. Using this program, sites were then plotted over a *.shp file of the UK. E.A. data only referred to waters in England and Wales. Similar information was not available for Scotland.

Risk factors were identified based on whether they were statistically associated with the dichotomous outcome "Presence of problem *Argulus* spp. infection in year 2000 = Yes/No". This outcome is a subjective opinion of the interviewer, and was chosen over presence of the parasite as this would not necessarily have represented a problem

to a fishery. **A case was defined as an infection that was perceived to be sufficient to reduce feeding and aesthetic appeal of fish sufficiently to reduce angler numbers.** This definition of a problem infection was established through asking the fishery owners what they perceived the problems associated with *Argulus* spp. to be, and whether they had suffered these problems in year 2000.

Cases were defined after the problem had been established, i.e. retrospective to the survey but prior to the examination of the survey data, so that no pre-judgements were made. A infection was defined as a case if the fishery owner answered yes to all the following signs, identified through literature searches and discussions with industry representatives:

- Presence of the parasite in 2000 (based on descriptions by the fishery owner and where possible the screening of fish).
- Abnormally high levels of jumping by fish
- Reduction in feeding (based on catch records) from other years.
- Aesthetic appeal of fish reduced (reduced condition and presence of lesions & secondary infection).
- Anglers reporting the presence of *Argulus* spp.

Questions on the above, and other signs, were asked before the fishery owner was asked what they perceived the problem associated with *Argulus* spp. to be, to avoid biasing their responses.

It was not possible to determine the sensitivity or specificity of this method of screening as the presence of a 'problem' infection was a perceived issue and it is therefore possible that these signs were not actually caused by the parasite.

Unfortunately, as infections by *Argulus* spp. and associated information are not normally reported to government organizations or veterinarians, no retrospective information on infection status of sites was available, making it impossible to define a case in any other way. Due to the obvious nature of the parasite and the simplicity of the clinical signs used it was felt the results were sufficiently accurate. Fishery owners were also asked about additional signs not associated with *Argulus* spp. infections, and unlikely to be observed in cases of an *Argulus* spp. problem, in order to identify owners who might have been giving the answers they perceived the interviewer wanted to hear. As a result, three fishery owners answering yes to all of the signs presented to them were removed from the survey.

2.2.5 Identification of Risk Factors

Logistic regression is a statistical technique commonly used in the identification of risk factors in epidemiological studies (Jarp *et al.*, 1993, Motulsky, 1995, Tu, 1996, Bebak *et al.*, 1997, Leung *et al.*, 2000, Thrusfield, 2003). It allows the assessment of the strength of association between factors and a dichotomous outcome (e.g. present/absent, true/false, yes/no) through statistical models. In studies of sufficient size, it can be used to predict the probability of a site suffering a problem infection (Tu, 1996), although this was not an aim of the current study.

In logistic regression models, the effect a variable has on the outcome is expressed as a point estimate that can be converted into an odds ratio (O.R.) by taking its natural logarithm (ln) where:

$$\text{Odds } O.R. = \frac{\text{The odds of a disease occurring in the exposed group}}{\text{The odds of a disease occurring in the unexposed group}} \text{ are}$$

defined as p/(1-p), where p = the probability of the outcome occurring.

Odds ratios give an assessment of how a factor influences the odds of the outcome occurring:

OR<1 then the factor is preventative

OR=1 then the factor has no effect

OR>1 then the factor leads to increased risk.

An O.R. of 2 would mean that the odds of the outcome occurring in a group exposed to that factor are twice that of a group not exposed to that factor. In the case of a continuous variable such as age, it would mean that the odds double for every unit increase in the factor (i.e. for every one year increase in age, the odds of the outcome occurring double). The further the O.R. lies from one the greater the impact of the risk factor on the outcome.

Logistic regression will handle dichotomous, categorical (e.g. red, blue yellow or young, old, very old) and continuous data (e.g depth, age), and makes far fewer assumptions than linear regression. It does, however, assume the following (Moltusky 1995, Garson 2002):

- Subjects are representative of the population from which they are drawn and are independent of one another.
- That no more than 1 explanatory variable is entered into a model per 5 cases observed.
- All relevant variables are included in the model.

- All irrelevant variables are excluded from the model.
- Low error in the measurement of explanatory variables.
- A linear relationship between the logit of continuous explanatory variables and dependent variable.
- Models are additive and interactions between variables are accounted for, i.e. specific interaction terms between variables are examined to account for factors that have a multiplicative effect upon one another.
- Explanatory variables do not exhibit multicollinearity, i.e. there is not a strong linear relationship between them.

Building multivariable models allows for the adjustment of confounding variables allowing real associations to be identified. Confounding occurs when an extraneous variable is related to an independent variable of interest, but not the outcome variable. This makes it difficult to establish which factor is responsible for variations in the outcome. A commonly cited example of this is the effect of smoking and drinking on the occurrence of lung cancer. Smoking is associated with drinking, making it appear that drinking is associated with lung cancer, when in fact smoking is the causal agent. These factors are therefore said to be confounding and if left unadjusted, biased point estimates of the strength of association between a variable and the outcome will occur.

In the present study, due to the large number of potential risk factors and relatively small sample size it was necessary to screen the data set in order to reject some variables. Using the case definition: problem *Argulus* spp. infection in year 2000 = yes/no as a dichotomous outcome, potential risk factors were first screened

individually through univariable logistic regression in SPSS v.11 and then incorporated into multivariable models. The aim of this analysis was to identify the factors that had the strongest statistical association with the outcome so they could be researched further.

Continuous factors were first screened to make sure assumptions of a linear relationship between the logit of the explanatory variable and dependant were met. This was done by taking the natural log of the explanatory variable and incorporating the cross product of this and the untransformed data as an interaction term within the model. A significant interaction means that the relationship is non-linear (Garson 2002). Such variables were then split at their median and incorporated as dichotomous variables (i.e. high/low) in the subsequent analysis. Continuous data that did meet the assumptions of linearity were included in further analysis as both a dichotomous and continuous variable. All factors identified as having a P-value ≤ 0.25 through the univariable analysis were retained and used to build multivariable logistic regression models. There are several different approaches to building these models, two of the most commonly used methods are forward stepwise and backward stepwise (Moltusky 1995, Leung *et al.*, 2000). The forward stepwise procedure can be considered as a bottom up approach. Each factor of interest is analysed individually, with the factor explaining the greatest amount of variation in the dataset (highest Cox & Snell R^2 value) being retained as the first variable in the model. Each of the remaining factors are then regressed individually with this factor, with the factor that most increases the Cox & Snell R^2 value being retained. This procedure is continued to the point when factors entered into the model do not show a statistically significant association when $p>0.05$, or there is no significant reduction in the –

2loglikelihood statistic. The backward stepwise procedure can be considered as a top down approach in which all factors studied are included in the initial model. Factors that make the least significant change to the model are removed individually until all the variables remaining in the model have a statistically significant association with the outcome at $p \leq 0.05$ (through examination of the Wald statistic) or until a significant increase in the –2loglikelihood statistic occurs. These procedures often produce the same results, however, differences can occur (Moltusky, 1995). As the purpose of this analysis was to establish the most strongly associated factors it was deemed important to use both methods and to compare the results should differences occur.

Models were built manually in SPSS v.11, and validated using the automated option available in the package. Factors included in the models were examined for multicollinearity through correlation in SPSS. Factors with R-values >0.7 were not included in the same model. Interactions between variables were also examined through the incorporation of interaction terms in the models. Any interactions significant at $P \leq 0.05$ were retained in the model. Outlying data points were identified and removed through plots of the standardised residuals, delta-beta's, leverage statistics and Cook's distance according to Garson (2002) and SPSS v.11 help files. The overall fit of the models was determined by a significant reduction in the –2loglikelihood statistic and an increase in the Cox & Snell R^2 statistic. Models were also examined to determine the proportion of cases and controls in the dataset that were correctly predicted.

2.2.6 Water Chemistry

Water samples were taken at each site visited between the start of June and the end of August 2001 in an attempt to characterise susceptible lakes by their water chemistry. At each site, five samples were collected in clean 500ml plastic bottles from the lake margins and at locations around the lake at a depth of 30cm. Each sample was analysed on site for the following parameters: alkalinity, conductivity, pH, total dissolved solids (TDS), hardness and nitrate. Conductivity, TDS and pH were measured using a HACH SensION 156 pH/conductivity meter. Alkalinity, hardness and nitrate were measured using a HACH Drel/2400 portable water quality laboratory following the protocols documented in the accompanying manual. Five 20ml water samples from each site were sent in sealed tubes to the Environment Agency's national laboratory service in Wales. These samples were analysed by mass spectrometry to determine the total phosphorus content of the water. A mean value was calculated for each parameter at each site. At sites where a boat was available, a water clarity reading was taken in the centre of the lake using a Secchi disk. This was lowered into the water tied to a rope marked at 30cm intervals until it disappeared from sight. At this point the depth that it had reached was taken by counting the number of rope marks that had been submerged. In lakes where the Secchi disk was still visible on reaching the lakebed a reading of bottom was recorded.

2.3 Results

2.3 Cross-sectional Study

2.3.1. Study Response and Perceived Problem

Of 123 sites contacted, 77 (63%) agreed to take part in the survey. Of those 77 sites interviewed, 69 (90%) gave consistent answers to the questions and were deemed to have provided reliable data suitable for further analysis. The following results relate only to these 69 sites. Of the site owners interviewed, 61% had heard of *Argulus* spp. and were aware of some of the problems they caused. The presence of *Argulus* spp. had been noted at least once during the history of the fishery at 42% of sites; with 30% of sites stating it had been present in year 2000. Only 6% of sites interviewed perceived fish mortality as the biggest problem associated with *Argulus* spp. infections, but 33% of fisheries perceived the biggest problem to be that fish stopped feeding. This inappetance reduced catchability and affected the condition/aesthetic appeal of the fish, which showed reduced weight and scale loss together with the presence of the parasite. These two factors were perceived to lead to an economic loss through a reduction in the number of anglers attending the fishery. Based on the definition that the infection was sufficient to reduce feeding and the aesthetic appeal of trout to the point that angler numbers were reduced, 25% of the study waters had suffered 'Problem *Argulus* spp.' infections in 2000. At some fisheries, cases did not occur in the study lake but did occur in another lake present at the site in 2000. This meant that an additional 4% of sites had suffered a problem infection in year 2000, resulting in a total of 29% of sites having suffered a problem infection.

All infected sites stated that numbers of *Argulus* spp. peaked between July and August, and this corresponded to what should have been their busiest time of year. When asked in which months of the year *Argulus* spp. were present the response was highly varied, ranging from year round to during July and August only. The number of *Argulus* spp. noted per fish at the peak of infection was also highly variable ranging from single figures to hundreds. This suggests that either there is a high degree of variation in the accuracy of the counts between fisheries, or it is not necessarily the total number of the parasite present per fish that determines whether the infection becomes a problem. Of the 20 sites found to have suffered problem infections in year 2000, 17 were able to provide specimens of *Argulus* spp. for speciation. Specimens from all but one of the fisheries were identified as *A. foliaceus,* the other was identified as *A. coregoni.*

2.3.2. Distribution of *Argulus* spp.

The study found *Argulus* spp. to be widely distributed throughout central and southern England, and southern Wales. The study did not detect *Argulus* spp. in northern England or Scotland, however, contact with sites outside the survey did confirm its presence in these areas. Environment Agency data sets dating back to 1996 also confirm *Argulus* spp. species to be widely distributed throughout England and southern Wales. This data also appears to confirm that *Argulus* spp. is less common in the north of England (although there is no readily available information on the number of waterbodies within these regions and therefore the population at risk). The distribution of *Argulus* spp. as determined by this survey, confirmed reports and Environment Agency records are summarized in Figure 2.3.1. Information on the

distribution of *A. japonicus* from Environment Agency records is included on the map as little is currently known about this introduced species. These data suggest that *A. japonicus* has a similar distribution to other *Argulus* species. Environment Agency data was also plotted separately for each year to see if there was any indication whether *Argulus* spp. has become more widespread throughout England and Wales in recent years. The data showed *Argulus* spp. to be widely distributed since 1996, suggesting it has not spread further in recent years, and therefore separate annual maps are not presented here.

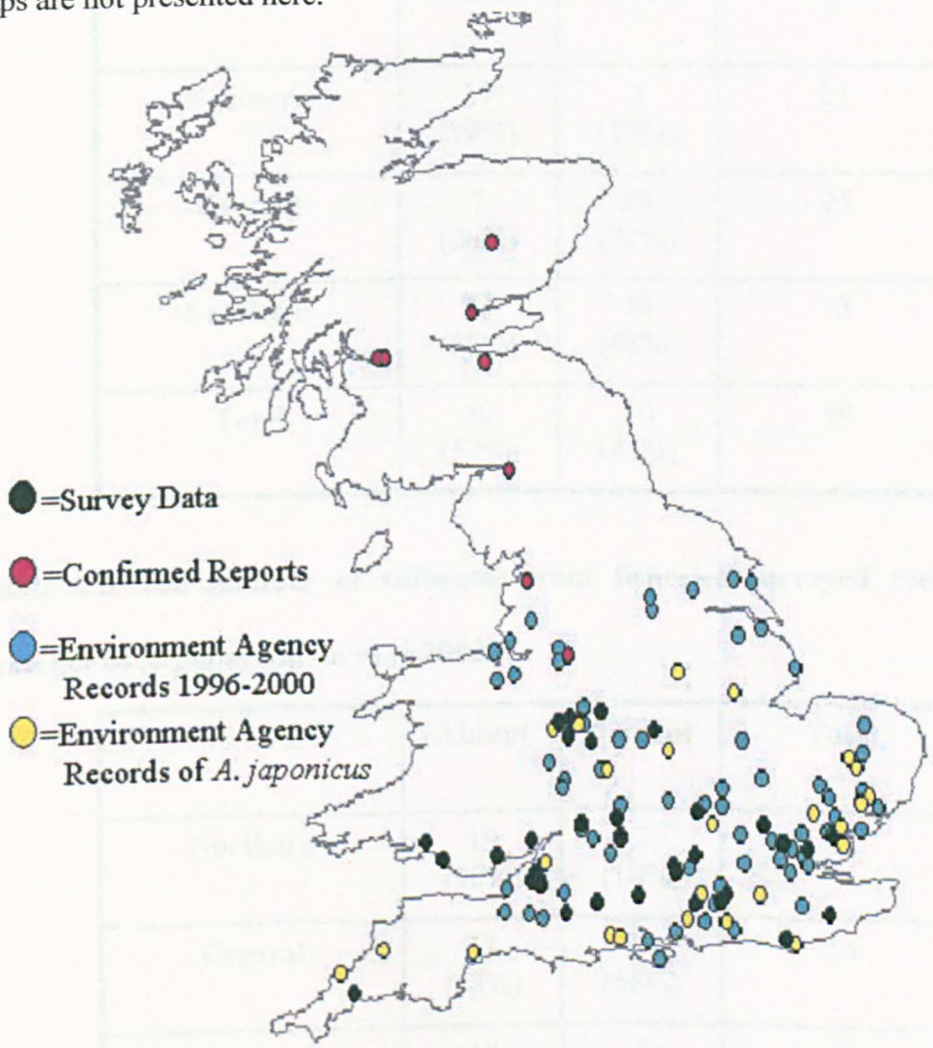


Figure 2.3.1. The distribution of *Argulus* spp. in UK stillwaters from cross-sectional survey data, confirmed reports and Environment Agency records from 1996 to 2000.

Tables 2.2, 2.3 & 2.4 show that, in terms of historical data, the presence of *Argulus* spp. in 2000 and problem infections in 2000, *Argulus* spp. is least prevalent in the northern region. *Argulus* spp. is most common in the central regions, with infected sites outnumbering uninfected sites.

Table 2.2. The number of stillwater trout fisheries surveyed by region, noting the presence of *Argulus* spp. at any time during their history.

	Absent	Present	Total
Northern	19 (90%)	2 (10%)	21
Central	7 (28%)	18 (72%)	25
Southern	13 (57%)	10 (43%)	23
Total	39 (57%)	30 (43%)	69

Table 2.3 The number of stillwater trout fisheries surveyed recording the presence of *Argulus* spp. in year 2000.

	Absent	Present	Total
Northern	19 (90%)	2 (10%)	21
Central	11 (44%)	14 (56%)	25
Southern	18 (78%)	5 (22%)	23
Total	48 (70%)	21 (30%)	69

Table 2.4 The number of stillwater trout fisheries surveyed suffering 'Problem *Argulus* spp.' infections in year 2000.

	Absent	Present	Total
Northern	21 (100%)	0 (0%)	21
Central	12 (48%)	13 (52%)	25
Southern	19 (83%)	4 (17%)	23
Total	52 (75%)	17 (25%)	69

2.3.3. Perception of Fish Species Susceptible to Infection by *Argulus* spp.:

Of the infected survey sites with more than one fish species present, nine considered that trout were the most susceptible to infection by *Argulus* spp.. Pike (*Esox lucius*) and sticklebacks (*Gasterosteus aculeatus*) were each perceived as being most susceptible by two sites. Two sites also stated that apart from trout, carp and bream were most susceptible to infection. Three sites did not state whether certain species were more susceptible than others. All the infected sites stated that trout appeared to suffer the most from infection by *Argulus* spp.. The majority (11) of infected sites considered that all sizes of trout were equally susceptible to infection. Of the remaining infected sites, four stated that large fish were more susceptible, two, smaller fish and two did not know. Six sites considered freshly stocked and over-wintered fish to be equally susceptible to infection. Five sites perceived over-wintered fish as more susceptible, one fresh stocked and seven did not know.

2.3.4. Current attempts at the control of *Argulus* spp.:

All the infected sites surveyed stated the need for an effective, legal method of controlling *Argulus* spp. infections. The most commonly used treatment was Diptrex 80. Five sites admitted to having used this compound and it is perceived as being very effective against the hatched stages of the parasite. The standard dose used was 750g per acre, applied up to four times a year. Two of the fisheries questioned, netted fish from their lakes for treatment. One treated with Diptrex 80, which appeared to be effective against the parasite. The other site treated with potassium permanganate, which although it killed the parasite, also killed most of the fish. Two sites attempted to manage *Argulus* spp. through draining, drying and applying lime to the lakebed at a dose of 1500kg per hectare. This was deemed to be effective at reducing the numbers of *Argulus* spp. in the subsequent year, but it was necessary to repeat the treatment at least every other year to prevent the numbers building up again. The site that had removed fish and treated with Diptrex 80 also reduced its stocking density and introduced a policy of stocking small batches of fish regularly (trickle stocking) as opposed to stocking large batches of fish occasionally, which was perceived to improve the situation. Three sites that had suffered problem *Argulus* spp. infections in the past stated that the intensity of infection had been reduced in their fisheries since the introduction of weed treatments such as Regalone and Clarosan. The site owners were not sure whether the chemical was toxic to the lice or whether it was the removal of weed that was the important factor.

2.3.5. Determination of Risk Factors

Initial examination of the data set showed there to be substantial differences in the lake types and management between regions. It was deemed important to incorporate this variation into the data analysis. Strong confounding was observed between region and several factors. The Northern region was associated with larger waters, the practice of catch and release and an absence of coarse fish species. However, due to a lack of cases in the Northern region, logistic regression was unable to adjust for the confounding effects of these variables when incorporated as a fixed effect within the models. A possible way of overcoming this is to incorporate region as a random effect within a hierarchical model, allowing the causes of regional variations to be identified and adjusted for (Rashbash *et al.*, 2002). Data is thus also analysed taking region as opposed to fishery as a sampling unit, however insufficient regions were available in this study to conduct this form of analysis. After consideration, data from the northern region was removed from all subsequent analyses as it was felt its influence might lead to misleading results. This led to a final sample size of 48, 17 of which were cases.

Using the cut-off $p \leq 0.25$, univariable analysis reduced the number of potential candidate variables to 17 (Table 2.5). One of these factors, presence of crayfish, was rejected from subsequent analysis as its potential importance had only become apparent during the survey and it had therefore only been recorded during the latter stages of the survey. Turbidity was also removed from any further analysis as this was a single reading taken from each site in 2001, and was not considered to be reliable enough for inclusion in the subsequent analysis.

Table 2.5. Risk factors identified for the presence of problem *Argulus* spp. infections, through logistic regression analysis of cross-sectional study data.

Variable	Cox & Snell R^2	Crude Odds Ratio	P-Value
Region: Central vs South	0.127	5.146	0.016
Average depth of lake: shallow/deep	0.068	0.911	0.106
Fishery flooded in 2000 merging with another water body? Yes/No	0.045	0.353	0.139
Flow of water through the fishery during the summer of 2000? Yes/No	0.054	0.364	0.106
Lake exposed to wind? Yes/No	0.143	2.560	0.143
Turbidity <1m No/Yes	0.237	0.05	0.009
Presence of an algal bloom in 2000? Yes/No	0.150	6.462	0.011
Crayfish present in 2000? Yes/No	0.144	5.625	0.094
Did the lake water level drop by >30cm in summer 2000? Yes/No	0.077	0.260	0.066
Were other species of salmonid present in 2000? Yes/No	0.036	0.390	0.202
Coarse fish other than pike present in 2000? Yes/No	0.077	3.843	0.066
Coarse fish including pike present in 2000? Yes/No	0.083	6.545	0.089
Average weight of trout per acre of fishery (kg) in 2000? (Continuous)	0.030	1.002	0.230
Time taken to replace entire stock of trout in 2000? (Continuous)	0.120	1.091	0.055
Time taken to replace entire stock of trout in 2000? (Fast vs. Slow)	0.217	9.800	0.002
Total No. rods per acre in 2000	0.054	0.998	0.175
Was weed removed during summer 2000? Yes/No	0.034	2.250	0.207

Of the 15 remaining candidate variables two were found to show multicollinearity and could not be included in the logistic regression models together. These were time taken to replace entire stock of trout in 2000, and total number of rods per acre in 2000, which were strongly correlated (R= 0.78, p=0.012). In this case only the most statistically significant factor, time taken to replace entire stock of trout in 2000, was included in the multivariable analysis. The 14 remaining candidate variables were analysed for further investigation through multivariable logistic regression to identify the factors most strongly associated with problem infections. With only 17 cases in the data set a maximum of three factors can reliably be detected through these models (Moltusky, 1995).

Both forward and backward stepwise procedures produced the same models, identifying three factors strongly associated with the outcome after adjusting for the effects of confounding. The factors identified were: the presence of an algal bloom in 2000 (Yes/ No), time taken to remove and replace entire stock of trout in 2000 [Stock turnover (Fast / Slow)] and whether the lake water level dropped by over 30cm in the summer of 2000 (Yes / No). No significant interactions were found between any of the variables in the model.

The output from the final model is presented in table 2.6 and shows that the association between all three of the factors and the outcome is statistically significant ($P<0.05$). According to the model, slow residence times and the presence of an algal bloom both increase the odds of suffering a problem *Argulus* spp. infection. Slow residence times (>10.4 weeks) appear to increase the odds of suffering a problem infection by 38.2 times, and the presence of an algal bloom by 13.7 times. A drop in

the lake water level of over 30cm in the summer of 2000 appears to have had a preventative effect reducing the odds of suffering a problem infection by 38.5 times (inverse of the odds ratio given due to the direction in which the variable was coded = 1/0.026).

Table 2.6 Results from multivariable logistic regression of cross-sectional study data against the outcome 'problem *Argulus*' in year 2000 =Yes/No.

Risk Factor	*B*	S.E.	Odds Ratio	95% C.I.	P-value
Residence Time (Fast vs. Slow)	3.643	1.223	38.218	3.474 - 420.438	0.003
Drop in Water Level (True vs. False)	-3.666	1.320	0.0260	0.002 – 0.340	0.005
Algal Bloom (Yes vs. No)	2.614	1.175	13.655	3.474 – 420.438	0.026
Constant	-6.856	2.093	0.001	-	0.001

Goodness of fit statistics for the model (table 2.7) fit the data well. The -2 log likelihood goodness of fit statistic is low (suggesting a good fit), and decreased significantly at each step in a forward stepwise model (Chi-square test, $p<0.05$). A Cox & Snell R2 value of 0.454 suggests the model explains just over 45% of the variation observed in the data set. Using these three variables to model was able to correctly predict 83.3% of cases and controls observed in the study.

Table 2.7 Goodness of fit statistics for the final multivariable logistic regression model resulting from the 2001 cross-sectional study.

-2 loglikelihood	Cox & Snell R2	Percentage of data set predicted correctly
33.312	0.454	83.3

Although the model appears to fit the data well, when all three factors are included, confidence intervals around the point estimates are very wide. Cross-tabulation between these factors and the outcome (table 2.8) revealed that due to the relatively small sample size, zero counts occurred in some of the cells, which probably explains the width of the confidence intervals. Because of the width, care should be taken in how the model is used, since it would not be a reliable model for predicting the occurrence of problem infections.

Table 2.8 Cross-tabulation of risk factors identified through multivariable logistic regression of problem *Argulus* spp. infections.

Residence Time	Drop in Water Level	Algal Bloom	Problem *Argulus* spp.	
			False	True
Fast	False	False	11	0
		True	4	3
	True	False	3	0
		True	3	0
Slow	False	False	1	3
		True	1	8
	True	False	3	0
		True	5	3

The analysis shows there to be a high degree of confounding between all the variables included in the models. Comparison of the unadjusted odds ratios (from the univariable analysis) with the adjusted odds ratios of each factor shows that after adjustment the influence of all the factors increased (as odds ratios move further from

one). This adjustment must be interpreted with care as the small sample size and zero cell counts are likely to lead to unrealistically inflated point estimates. However, it is clear that all these factors have a strong statistical association with the occurrence of problem *Argulus* spp. infections.

2.3.6 Water Chemistry

Logistic regression analysis revealed no significant correlations between the occurrence of problem *Argulus* spp. infections in 2000 and any of the water chemistry parameters collected in the summer of 2001. Table 2.9 shows the range of water chemistry parameters in lakes in which *Argulus* spp. were found.

Table 2.9. Range of water chemistry parameters in lakes in which *Argulus* spp. were found.

	Minimum	Maximum
pH	**7**	**8**
Total Phosphorous (mg/L)	**1.12**	**3.14**
Nitrate (mg/L)	**0.1**	**5.9**
Alkalinity (mg/L)	**21**	**226**
Conductivity (µS/cm)	**0.18**	**0.84**
TDS (mg/L)	**0.09**	**0.76**
Hardness (mg/L)	**47**	**269**

2.4 Discussion

This study was designed to determine the problem posed to UK stillwater trout fisheries by *Argulus* spp. infections, establish the extent of the problem and identify risk factors associated with problem infections. The study has established the proportion of fisheries affected, identified current management strategies that are being employed and elucidated the distribution of *Argulus* spp. species in the UK. Several risk factors have been identified around which hypotheses can be generated and further research targeted.

2.4.1 The Perceived Problem and Prevalence of Infection

The survey response rate was high, suggesting that the issue was deemed to be important by fishery owners. The survey also showed awareness of the problems associated with *Argulus* spp. infections to be high. Although mortality associated with *Argulus* spp. does occur, the main problem perceived to be associated with *Argulus* spp. is that fish become difficult to catch and lose aesthetic appeal. This results in a reduction in the number of anglers leading to an economic loss to fisheries. The perceived problem is a complex issue and it seems likely that the problem is not solely caused by the presence of *Argulus* spp., but by a combination of factors. Data from the cross-sectional study supports the hypothesis that the presence of *Argulus* spp. does not necessarily cause a problem, as 42% of fisheries knew that *Argulus* spp. was present in their fishery, but only 29% developed a problem in year 2000.

Based on the random selection of survey sites it can be assumed that the proportion of infected survey sites is representative of the total UK population of stillwater trout fisheries suffering a problem infection in year 2000. Interpretation of survey data must be conducted with caution, as surveys are often open to response bias. In the case of this survey, it was not clear in which direction (if any) a response bias was acting. It is possible that prevalence is over-estimated as sites suffering a problem may have been more likely to participate in the survey. However, due to the stigma attached to *Argulus* spp. infections it may be that infected sites were actually less willing to participate, owing to concern that their details might become public.

2.4.2. Distribution of *Argulus* spp.

Although the sample size was relatively small, results from the cross-sectional study suggest that problem *Argulus* spp. infections are more common in central and southern England and Wales than in the north of England and Scotland. The Environment Agency data set also appears to support this observation, however, there is little data available on the situation in Scotland. Rushton-Mellor (1992) described *Argulus* spp. as being restricted to areas south of the central highlands of Scotland and Campbell (1971) reported *A. foliaceus* as being widely distributed in rivers, canals and lochs on a variety of salmonids and sticklebacks south of the highlands. At the time of publication *A. coregoni* had only been recorded in the River Clyde in Scotland and does not subsequently appear to have been recorded elsewhere in Scotland. More recently Northcott *et al.* (1997) described an epizootic of *A. foliaceus* in Perthshire. This is the most northerly record in the literature of *Argulus* spp. in the UK, however, a confirmed case of *A. foliaceus* in Aberdeenshire was reported to the author in 2003.

In some areas of the UK records of *Argulus* spp. are sparse, however, there are no readily available records of the number of lakes in those areas so it is difficult to know the actual prevalence of the parasite. Differences in prevalence were noted during the survey between northern and southern UK. The survey identified a lack of coarse fish and larger stillwaters in the northern UK compared with other regions. These factors were not shown to have statistically significant association with in *Argulus* spp. infections as there were too few cases in the northern regions for meaningful comparison. The distribution data suggests that *Argulus* spp. has been widely distributed throughout central and southern England and Wales since at least 1996, but has not become widespread in the north in recent years. Insufficient data is available to demonstrate any possible spread of *Argulus* spp. in Scotland. When comparing the prevalence of problem infections they were found to be higher in central compared with southern England and Wales. This is perhaps surprising as a milder climate in the south might be expected to exacerbate the problem by allowing extra generations of the parasite to occur in a season. Logistic regression models built using region as an outcome (southern vs. central) showed that the only factor on which data was collected that held a significant association with regional differences was the practice of catch and release. This was more common in central regions and explained 13.3% (Cox & Snell R^2) of the variability observed between the regions, it was not, however, found to be associated with problem *Argulus* spp. infections. It was not possible to identify any other factors causing regional variations. One hypothesis is that there is a greater abundance of spring fed chalk waters in the southern region, which may result in cooler summer water temperatures. This is an important factor in determining the developmental rate of *Argulus* spp. (Kimura,1970, Schluter, 1979, Shafir & Van As,1986) and could determine whether an infection will lead to a

problem. Other factors that were not investigated, such as lake altitude, may also be important.

Only *A. foliaceus* was detected in sampled sites, with the exception of one site in which *A. coregoni* was found. This suggests *A. foliaceus* to be the predominant species in UK stillwater trout fisheries. *A. japonicus* is thought to be a fairly recent introduction to the UK (Rushton-Mellor, 1992) which may explain why it is not as common as *A. foliaceus*. *A. coregoni* is regarded as a native species however stillwater trout fisheries may not be a suitable habitat for the species. Gurney (1948), Campbell (1971) and Okland (1985) concluded that *A. coregoni* favours cool, fast flowing waters or large lakes compared with *A. foliaceus* which is more common in warm, shallow, eutrophic still waters. These charecteristics are in keeping with the survey site in which *A. coregoni* was found. It was one of the largest sites surveyed that had noted a problem infection, being 75 acres in size; averaging 4m in depth and reported as remaining very clear year round. It is also possible that *A. coregoni* (and *A. japonicus*) are more prevalent in trout fisheries than suggested by the survey, but do not reach a level where they cause a problem and are therefore unlikely to be noted or reported. Environment Agency data suggests that *A. japonicus* has a similar pattern of distribution to *A. foliaceus*, and supports the records of Rushton-Mellor (1992). It is possible that *A. japonicus* is more abundant than present data indicates because of difficulties in differentiating it from *A. foliaceus* (Rushton-Mellor & Boxshall, 1994). At present there appear to be no published records of *A. japonicus* in Scotland.

2.4.3. Susceptible Fish Species

Responses to the survey suggested trout were the fish species most susceptible to *Argulus* spp.. However, it is unlikely that a fishery owner would observe other fish species in these lakes to the same extent as trout. The cross-sectional survey did not identify the presence of coarse fish as a significant factor in the occurrence of problem infections, however, it was not possible to collect detailed information in the quantity or time of introduction of these species. This is an important area for further study as alternative hosts could play an important role in the life-cycle of the parasite, acting as a potential route of introduction to the site or as a reservoir of infection and contributing to the development of problem infections. Most *Argulus* spp. are able to live on a wide range of hosts (Bower-Shore 1940, Fryer 1968, Kabata 1970), and there is some evidence that alternate hosts play a key role in the life-cycle of *A. coregoni*, as the host preference of this species changes from roach (*Rutilus rutilus*) in the first half of its life span to brown trout in the second half (Mikheev, Pasternak & Valtonen, 2004). High numbers of alternate hosts may allow the parasite population to persist in unfavourable conditions, or allow parasite numbers to build more rapidly leading to an epizootic (Haydon *et al.*, 2002). The survey showed that currently fishery managers base their stocking calculations purely on numbers of trout, with no adjustment for the overall density of fish including other species. This may be an important factor in determining the level of infection by *Argulus* spp..

The cross-sectional study produced no evidence to suggest that brown trout were more susceptible than rainbow trout, which was suggested to be the case by Bower-shore (1940). There was also no evidence to suggest that susceptibility was related to

fish size. Survey data did suggest that over-wintered fish were more susceptible, and this may be because these fish had been exposed to the parasite for longer.

2.4.4. Current methods of control

Treatment and management of *Argulus* spp. was often considered to be difficult in fisheries for one or more of the following reasons: size of water body, inability to control incoming/outgoing water supply or lack of ownership, i.e. fishing rights leased from lake owners. The organophosphate Diptrex 80 was perceived to be the most effective form of treatment, but was recognised as having no effect on the eggs of *Argulus* spp.. This was normally made into a solution (of an undisclosed dose), added to the lake water at different locations around the lake and allowed to mix naturally. There was some suggestion that resistance to this treatment had built up in certain waters, with some fishery owners stating that they had gone from treating once a year to 4-5 times. There also appeared to be a lack of knowledge as to the best time to treat, with most fishery owners waiting until a problem infection was evident before treating. The banning of organophosphates throughout Europe now means that fishery owners are unable to obtain these chemicals, and this may be the reason for the recent perceived increase in problem *Argulus* spp. infections.

In lakes in which it was possible, draining and liming was perceived as being effective. It was felt by fishery owners that this form of control needed repeating every other year and was deemed to be expensive in terms of cost and time, with the lake being unfishable for a substantial period of time after treatment. Few site managers felt fallow periods (removal of all fish from the lake) were feasible as it

would: a) be difficult to remove all fish, and b) mean the fishery was closed for a long period. Some fisheries observed that weed treatments had reduced their *Argulus* spp. problem. If this is the case it could be that: a) weed treatments are toxic to *Argulus* spp. and b) removal of weed changes the habitat in a way that is detrimental to *Argulus* spp. populations. Analysis of the cross-sectional survey did not identify high weed cover as a risk factor. Weed cover could only be assessed in a subjective manner by asking the fishery owner what proportion of the lakebed was covered in weed during the summer of year 2000, it would, however, be worthy of further study, as fish sheltering amongst the weed are likely to be relative sedentary and possibly more susceptible to infection. High weed cover could also reduce predation upon the parasite by fish. Tolonen *et al.*, (2003) hypothesised that swimming and crawling invertebrates sheltered amongst aquatic macrophytes to reduce predation.

There was great interest in the use of PVC boards to collect eggs, as suggested by Gault *et al.* (2000), however, at the time of the survey none of the fishery owners were using them. There were also concerns that boards would impede angler's casts and reduce the aesthetic appeal of the fishery. None of the measures described by the fishery owners were perceived by them to be totally effective. Due to the high proportion of fisheries suffering from *Argulus* spp. infections and the current lack of licensed treatments or practical control measures, it is clear that an effective management strategy is required.

2.4.5. Water chemistry

No associations were found between water chemistry and problem infections, suggesting *Argulus* spp. to be versatile in terms of their tolerance to water type. The worldwide distribution of *A. foliaceus* in a variety of climates and conditions also suggests this is the case. It was not possible discriminate between case and control sites using the water chemistry data due to small sample sizes and the time period of a year between the problem occurring and the samples being taken.

2.4.6. Risk factors

Univariable analysis of the cross-sectional data set identified 17 potential candidate variables. These were analysed through multivariable logistic regression in order to identify the factors that were most strongly associated with problem *Argulus* spp. infections, around which further research could be targeted. The logistic regression models identified three factors; the presence of an algal bloom in 2000, rate of stock turnover and whether the lake water level dropped by over 30cm in the summer of 2000. Inflated point estimates and broad confidence intervals associated with the small sample size mean that the model has limited predictive power, however, it did show that the higher the number of the above factors present in a fishery the greater the risk of a problem *Argulus* spp. infection. It must be noted that, although these risk factors are associated with the outcome (presence of "problem" *Argulus* spp. infection), they do not necessarily have any direct causal effect upon it. Under Hill's (1965) tenets of causality, this study meets the first two: statistical significance and strength of association, and the generation of biologically plausible hypotheses for the

action of these. However, without further information causality cannot be inferred and it is not known whether removing or manipulating a risk factor will have any effect on the outcome. Many hypotheses can be generated about how associations between risk factors and the outcome variable occur and these are discussed below for each risk factor.

Algal Blooms: The cross-sectional survey showed that algal blooms were associated with an increased risk of suffering "problem" *Argulus* spp. infections. Univariable analysis on turbidity readings taken during the cross-sectional study suggested that it is water clarity that is the important issue and not necessarily an algal bloom *per se.* The turbidity data was not included in the final logistic regression analysis as this measurement was only taken at one time point in the survey and was therefore felt not to be truly representative.

Algal blooms may not directly affect a population of *Argulus* spp., but stress the fish reducing their feeding rate and therefore making them less likely to be caught. Trout are predominantly visual feeders and reduction in water clarity has been shown to reduce their feeding success (Rowe 1984, Barrett, Grossman & Rossenfield, 1992 and Stuart-Smith, Richardson & White, 2004). An additional effect of this may be to reduce predation upon the parasite. Predation on *Argulus* spp. by trout has been observed by the author in the laboratory, and also noted in the field by examination of trout stomach contents. Predation by other fish species has also been recorded in the literature (Wilson 1902, Thomas 1961). Mikheev *et al.* (2000) noted that under light conditions *Argulus* spp. tend to remain relatively motionless in the water column

acting as an ambush predator, i.e. waiting for a host to swim past. It is possible that this behaviour makes the parasite prone to being predated upon by trout.

On the other hand heavy infections of *Argulus* spp. may lead to reduced water clarity. Infection may cause fish to rub themselves on the lakebed to remove the parasite, thus churning up the sediment and releasing nutrients, which may cause clouding of the water or algal blooms. This behaviour was observed by a fishery owner during the study and is recorded by Shimura (1983). There is some evidence from the literature to suggest water clarity directly influences the population dynamics of *Argulus* spp. as Gurney (1948) suggests *A. foliaceus* to be most abundant in warm, shallow, eutrophic lakes; presumably with low water clarity. Further investigation is require to establish a temporal association between water clarity and infections by *Argulus* spp.

Stock Turnover: The survey data suggests that the shorter the period it takes to remove and replace the entire stock of trout in the lake, the lower the risk of suffering a problem *Argulus* spp. infection. This is biologically plausible as, every time a fish is removed, *Argulus* spp. are also removed thus reducing the parasite population. It is also possible that *Argulus* spp. infections could be responsible for increasing the residence time of fish, as they are perceived as making fish more difficult to catch. Further investigation is required to establish cause or effect.

It became apparent during the survey that a greater proportion of syndicate/club waters suffered problems from *Argulus* spp. than commercial waters. The survey showed such waters are stocked with large batches of fish on a few occasions during the year as opposed to "trickle stocking" of small numbers of fish throughout the year.

This form of stocking and low fishing pressure means that fish remain in a lake for a long time allowing them to acquire heavy infections. In addition to this, many clubs have a closed season from October to the end of March, which could allow significant numbers of the hatched parasite to over-winter, as their abundance would not reduced through fishing. Results of the population study (chapter 3) suggest this to occur in waters with a slow winter stock turnover. The survey showed that these sites stock a large batch of fish in March, ready for the start of the season. This is one month earlier than Gault *et al.*, (2002), and the results of the population study (chapter 3) suggest that hatching of *A. foliaceus* occurs. This would mean a high density of hosts would be present in the fishery as the parasite hatches, possibly increasing infection success.

Drop in summer water level >30cm: A 30cm drop in the summer water level of a lake was found to be a preventative factor for problem *Argulus* spp. infections. This finding conflicts with the findings of Shafir and Van As (1986) and Gault (2002) who found infection levels peaked as water levels dropped and temperatures rose. It is possible that the inclusion of this factor is a chance occurrence as with so many factors being screened, and a cut-off of $p \leq 0.05$, 1 in 20 observations are likely to occur by random chance. Some evidence was found for this through re-examination of the data set, which revealed that all but one of the waters in which the water level dropped were large public water reservoirs that were drawn down during the summer months. Few of these reservoirs suffered problem infections. However, a drop in water level could expose *Argulus* spp. eggs to drying thus making them non-viable. Gault *et al.* (2002) observed high levels of egg laying by *A. foliaceus* on boards floating on the surface of fisheries, and Bauer (1959) suggests there is a preference for

egg laying in shallow water. This would imply that A. foliaceus lays its eggs in the lake margins or on structures near the water surface. If this is the case then a drop in water level could desiccate the eggs, reducing subsequent recruitment. On the other hand, Kimura (1970), Shimura and Egusa (1980), and Mikheev *et al.* (2001) found the majority of eggs of *A. japonicus* and *A. coregoni* were laid towards the bottom of raceways and ponds in trout farms and under laboratory conditions. These results may be attributed to species differences, however, further investigation is required into the egg laying habits of *Argulus* spp. in fisheries, to establish whether there is a biological association between a drop in summer water level and problem infections.

2.4.7 Summary

This study has greatly increased our knowledge of *Argulus* spp. infections in UK stillwater trout fisheries, providing useful data that will aid in the management of problem infections. Prior to this study there was little data on the impact or extent of *Argulus* spp. infections in the UK. The use of a cross-sectional study proved a useful method of establishing the problems caused by *Argulus* spp. infections, the proportion of sites affected, the distribution of different species in UK trout fisheries, and constraints in the management of the parasite. The study has established that the problem perceived to be associated with *Argulus* spp. infections is one of economic loss through reductions in the number of anglers attending a fishery. About one third of the fisheries surveyed had suffered a problem in year 2000, caused, in all but one case, by *A. foliaceus*. The results suggest that, in the majority of cases, both *A. coregoni* and *A. japonicus* are not responsible for the problems observed in stillwater trout fisheries. Small sample sizes and lack of prior knowledge about the status of the

problem limited the amount of analysis that could be conducted on the data set; however, three biologically plausible risk factors (i.e. presence of an algal bloom, rate of stock turnover and drop in water level) have been identified through logistic regression analysis. Hypotheses have been generated to explain how they may interact with a population of *Argulus* spp. to cause a problem infection. These now require further investigation and testing to establish whether a true causal relationship is present.

CHAPTER 3: POPULATION ECOLOGY

3.1 Introduction

Studies on the population dynamics of organisms in the field can reveal factors responsible for causing population fluctuations or heterogeneities amongst them. Wilson *et al.* (2001) review factors at the host level responsible for causing heterogeneities in parasite populations. These factors include host sex, age, size and strain. Bron (pers. comm.) observed variability in the number of sea lice (*Lepeophtheirus salmonis*) infecting farmed salmon (*Salmo salar*), showing early maturing fish in a population to be more susceptible to infection. Perkins *et al.* (2003) found sexually mature, male yellow-necked mice (*Apodemus flavicollis*) of high biomass to be responsible for 80% of the transmission of tick-borne encephalitis to a population. Such findings have obvious importance in preventing the spread of infection, by perhaps allowing targeting of susceptible individuals by methods such as vaccination or culling.

External factors can also be responsible for population fluctuations. Begon, Mortimer and Thompson (1996) reviewed the work conducted on the life-cycle and dynamics of the Colorado potato beetle (*Leptinotorsa decemlineata*), andshowed how data on immigration (including recruitment) and emigration (including mortality) from each stage of the animal's life-cycle can be used to identify key-factors responsible for driving population fluctuations. This work ruled out rainfall, frost and predation as

significant factors, finding adult emigration to be the most important driving factor. Several studies on populations of fish parasites have determined the importance of temperature in controlling population dynamics. Temperature controls egg hatching, and the rate of development of *Argulus* spp., with high temperatures increasing abundance (Shimura, 1983, Shafir & Oldewage, 1986, Pasternak *et al.* 2000, Gault *et al.* 2002, Hakalahti & Valtonen, 2003). A similar relationship with temperature is also observed in the ciliate protozoa *Icthyophthirius multifiliis* (Bauer, 1970). Anderson (1974) however, found high temperatures reduced survival of the cestode, *Caryophyllaeus laticeps* in populations of bream (*Abramis brama*), due to an increased immune response by the host in warmer water (Kennedy, 1971). Ritchie, Mordue, Pike & Rae's (1993) population study of *Lepeophtheirus salmonis* in salmon farms in Scotland found low temperatures increased recruitment by increasing the parasite's egg production, however Tucker, Sommerville & Wootten (2000) showed infection success and subsequent survival to be greater at higher temperatures. A knowledge of the effects of environmental factors, such as temperature, on parasite populations allows prediction of future events, which is important when implementing control strategies. By understanding the timing of recruitment, egg laying and changes in the mortality rate, it may be possible to target interventions or modify management practices at specific time points in the year in order to reduce the parasite burden in a cost-effective way. For example if an intervention was targeted at the first cohort of the year, before the parasite commenced egg laying, then recruitment would be reduced for the entire year.

Bron, Sommerville, Wootten & Rae (1993) provide an example of the successful development of effective, targeted management strategies through studies on population ecology. Their study found that salmon farms holding only one year class of fish and allowing a fallow period between batches of fish, had reduced levels of infection by *Lepeophtheirus salmonis* than farms holding more than one year class. Implementation of this management strategy was shown to reduce the need for chemical treatments, thus providing both environmental and economic benefits. In another example Hudson, Dobson & Newborn (1998) used population studies to develop models that would predict population crashes in red grouse (*Lagopus lagopus scoticus*) due to helminth parasites. They demonstrated that effective control could be achieved by treating the birds with anthelmintics only in years when models predicted high mortality, as opposed to treating on an annual basis.

The population dynamics of *Argulus* spp., including *A. foliaceus*, *A. japonicus* and *A.coregoni* appear to follow a generally similar pattern in countries with temperate climates. Some variability in the parasite population dynamics can be observed between species, but variability observed within species by different authors is probably due to climatic differences. *Argulus* spp. have a direct life-cycle, but leave their host to lay their eggs on a firm substrate. Several studies have shown that *Argulus* spp. will lay their eggs on an artificial substrate that can subsequently be removed, this is useful for monitoring egg laying activity but has also been suggested as a method of control (Bauer, 1970, Shimura & Egusa 1980, Gault *et al.* 2002).

In central Finland Pasternak *et al.* (2000) found low numbers of *A. foliaceus* to over winter as adults and eggs. No hatching of the parasite was observed until the end of May when the water temperature had risen above 10°C. Egg laying was not observed until the parasite was greater than 4mm in length and this peaked in mid August when the parasites abundance also peaked. These authors suggest hatching of eggs occurs over an extended period and that the population dynamics of the parasite is determined by host abundance. Hakalahti & Valtonen (2003) found a similar pattern in *A. coregoni* in Finland. Again eggs did not hatch until the water temperature rose above 10°C and the majority of recruitment was observed in early summer, but the exact timing of recruitment varies between years. The authors suggest temperature and light intensity to be the main factors controlling this variation. Development from hatching until adult was reported to take *ca* 600 degree-days and egg laying was first observed between June and July. As in the case of *A. foliaceus*, few hatched stages of *A. coregoni* were found to over-winter on fish. Under these conditions they suggest one full generation occurs in a year and that juveniles hatching after August died before reaching maturity. Shimura (1983) found two full generations in a year in Japan, which Hakalahti & Valtonen (2003) suggest is due to a longer summer than in Finland. Shafir & Oldewage (1986) studied the population dynamics of *A. japonicus* in a reservoir in South Africa. They observed new cohorts to emerge each month over the six-month summer period when the abundance of the parasite was highest. No recruitment was observed over the winter months and did not occur until the water temperature rose above 11.5°C. Female specimens were found year round, and the authors hypothesised that the parasite has a life span of up to one year.

In the UK the population ecology of *Argulus* spp. has received little study. Both Bower-Shore (1940) and Gault *et al.* (2002) describe the population dynamics of *A. foliaceus* in the UK to be correlated with temperature, peaking in summer and being low over the winter months. Moller (1978) and Schluter (1979) both showed development of *A. foliaceus* to be temperature dependent with the rate of development increasing with rising temperature. Gault *et al.* (2002) found parasite numbers to be low over the winter months with no recruitment or egg laying activity occurring. Recruitment began in spring once the water temperature rose above 10°C, from eggs that had over-wintered. Egg laying began in May probably from over-wintered hatched stages of the parasite, peaked in June and stopped in October. They suggest two complete generations occur in a year, but also found the dynamics to differ between years in one fishery. At present there is no information on variability in the population dynamics of *Argulus* spp. between waters in the UK, and if present how this may occur.

The cross-sectional study detailed in chapter 2 identified several factors associated with "problem" *Argulus* spp. infections. Although the study determined that these factors were associated with such infections, it could not establish whether they were causal or an effect of the infection. One step in establishing a causal association is to establish a temporal link because, for a factor to affect a population, a change in it must occur prior to a change in the outcome of interest. Currently there is little detailed information on the population dynamics and life cycle of *A. foliaceus* in UK fisheries. Such data would not only establish temporal associations between the risk factors identified, but

also increase understanding of the timings of cohorts and stages of the parasite, thus aiding the development of effective, targeted management strategies.

This study aims to increase understanding of the population dynamics of *A. foliaceus* in a variety of UK stillwater trout fisheries by:

a) Elucidating detailed information on the parasites life-cycle.

b) Identifying correlations between stock turnover, water clarity and life-cycle of *A. foliaceus*.

c) Identifying potential target points for intervention in the parasites life-cycle.

3.2 Materials and Methods

A longitudinal study consisting of monthly visits to 5 UK stillwater trout fisheries was conducted between May 2002 and May 2003. Sites of varying size and management type were selected to establish correlations between stocking policies and the parasites population dynamics. Fish samples were obtained by seine netting. Obtaining fish samples through this method is invasive and disruptive to the fishery due to the manpower required, physical damage to the fishery associated with netting (erosion of banks and destruction of reed and weed beds) and the need to close areas of the fishery to anglers whilst the survey is conducted. As a result it was necessary to select waters where the managers would give their full cooperation throughout the year. This was a major constraint and limited the number of suitable sites available. Selected sites were identified from sites visited during the year 2001 cross-sectional study described in chapter 2, which provided a history and details of the site. Due to the commercially sensitive nature of the data collected during the study site names and exact locations cannot be given as a result of confidentiality commitments. No quantitative assessment of populations of species of fish other than trout could be made at each site, however, other pertinent details of the sites are provided below with a summary given in table 3.1.

Site 1: This was the smallest water surveyed, measuring 0.4 hectares in size and located in South Wales. The lake was three years old, averaged 1.5m in depth, and had little aquatic macrophyte cover. Water clarity was recorded in the previous year as

remaining relatively constant and not exceeding 1m. The lake had a standing stock of approximately 200 fish and was trickle stocked on a fortnightly basis with 750g rainbow trout. Of all of the study lakes this had the fastest rate of turnover of stock. According to the owners information, no fish species other than the stickleback (*Gasterosteus acueleatus*) were present in the fishery. This lake was downstream of, and directly connected to another trout lake that had experienced problem *A. foliaceus* infection in the two years prior to this study. Although the study lake had definitely been exposed to *A. foliaceus* from this lake in these years, the parasite had not been noted in it.

Site 2: This was a 3.6 hectare lake in Gloucestershire, holding a mixture of approximately 900 large rainbow and brown trout with an average weight of 4kg. This lake was trickle stocked on a weekly basis, and had an intermediate stock turnover compared to the other study sites. The lake had experienced problem *A. foliaceus* infections for over 10 years. In addition to trout the lake held large population of roach (*Rutilus rutilus*), perch (Perca fluviatilis), pike (*Esox lucius*) and other cyprinids. The lake had an average depth of 2.2m and over a third of the lakebed was covered with dense aquatic macrophytes. The lake was recorded as experiencing seasonal algal blooms that caused the water clarity to fluctuate between 3m and 0.9m.

Site 3: This lake was owned and run by the same manager as site 2, and was located less than half a mile away. This was a larger lake of 9 hectares with an average depth of 3.5m. *A. foliaceus* had been noted as present in the lake for over ten years but had

only caused occasional problems. The lake had a standing stock of approximately 3300 rainbow trout with an average weight of 2kg and held large numbers of roach, perch and pike. Stock turnover on this lake was the second highest of all of the sites. Although the lake was recorded as experiencing algal blooms it remained clear for the majority of the year with water clarity of up to 5m. Some extensive beds of aquatic macrophytes were present in the shallower regions of the lake.

Site 4: This was the largest study lake at 15 hectares, located in Staffordshire and held a standing stock of approximately 4000 rainbow trout with an average weight of 1kg. Although the lake reached depths of up to 6m it had an average depth of less than 2m. This had the second rate of slowest stock turnover of the study lakes and in year 2000 had suffered algal blooms that reduced the water clarity to less than 90cm and experienced a heavy infection of *A. foliaceus*. There was little aquatic macrophyte cover apart form in the shallowest regions. A large population of roach, perch and cyprinids were present.

Site 5: This was another small lake, measuring 0.6 hectares and located in Wiltshire. The lake is a privately owned water and not fished by the public. This lake was stocked once a year with a mixture of 200 brown and rainbow trout with an average weight of 500g. Also present in the lake was a resident population of circa 75 brown trout of between 1.5 and 3kg, and a small number of grass carp (*Ctenopharyngodon idella*) with an average weight of 10kg. A population of roach, perch and tench were also present. This lake had the slowest stock turnover of all of the sites as it was lightly fished and

those fish removed were not replaced until the following year. During the survey the only true stock turnover came in the form of sampling, where fish were caught, the parasite removed and the fish restocked free of *A. foliaceus*. Again this lake was downstream and connected to another lake experiencing problem infections, but this study lake had also persistently experienced problem *A. foliaceus* infections leading to trout mortality over the last ten years. The lake averaged 2m in depth and had little macrophyte cover. Water clarity had been noted as constant in previous years at approximately 1m.

Table 3.1. Details of study sites selected for May 2002 to May 2003 longitudinal study of the population dynamics of *A. foliaceus* in five UK stillwater trout fisheries.

Site Number	Size (Hectares)	Average Depth (m)	Maximum Water Clarity (m)	Rate of Stock Turnover (1= fastest)	Location
1	0.4	1.5	1	1	S. Wales
2	3.6	2.2	3	2	Gloucestershire
3	9	3.5	5	3	Gloucestershire
4	15	6	5	4	Staffordshire
5	0.6	2	1	5	Wiltshire

3.2.1 Temperature Data

Two Gemini data loggers accurate to 0.1°C were deployed into the deepest part of each of the study lakes and set to take a temperature reading at 3-hour intervals throughout the study. Loggers were attached to a rope secured to an anchor weight and a buoy. One sensor was set 30cm under the water surface, the other 30cm above the lakebed, at least 1.2m apart to provide "surface" and "bottom" temperatures, respectively. Before deployment all of the loggers used in the study were calibrated to ensure that they were recording within 0.1°C of each other. Data from each logger was downloaded using Gemini Logger Manager software on each site visit, after which they were reset and replaced in the same location in the lake. Average monthly surface and bottom temperatures were then calculated in Excel 2000 and the results graphed.

3.2.2 Water clarity

Each site was issued with a homemade Secchi disc and a record sheet. These were made from a 15 x 15 x 0.3cm white plastic square drilled through the centre. A 3.5m string knotted at 30cm intervals was passed through the disc and tied to a weight to allow the disk to sink. Each 30cm interval was recorded by the fishery owners as a Secchi disc unit (SDU) for simplicity.

To take a water clarity reading, the disc was lowered into the water until it just disappeared from sight. The depth was calculated by counting the number of knots that

had passed under the water surface. The distance from the final knot to be submerged to the surface was then measured and added to the total. A reading was taken on each site visit and fishery staff were shown how to take a measurement and asked to take a weekly reading from the same location in the lake. If the disc was still visible when it reached the lakebed they were asked to record 'bottom' and the depth.

3.2.3 Collection of *A. foliaceus* Egg Laying Data

To assess egg laying activity of *A. foliaceus* and depth of egg laying activity, foam-PVC boards were placed in each of the study lakes. Each board was grey in colour, 30x 30cm in size and drilled top and bottom. Boards were threaded onto a rope set at 30cm intervals. They were set to the lake depth with an anchor weight at one end and a buoy at the other. An additional board was attached to the buoy to float on the water surface. Up to 5 sets of boards were placed in each lake in deep water in different region around the lake where they did not interfere with angling. Boards were examined each month and any egg strings observed were counted and removed with a knife. After inspection, each board was scrubbed clean and replaced in the same location. The average depth of egg laying was calculated for each month and plotted as a percentage of the total lake depth using Excel 2000. Observations on the egg laying habits of *A. foliaceus* were also conducted on a drained fishery in Wiltshire that had a history of heavy infections by *A. foliaceus*.

3.2.4 Stock Data

Monthly stock turnover was defined as the number of fish removed and replaced each month and was assumed to be equal to the number of fish stocked in a month in all sites with the exception on site 5. As the latter was only stocked once a year there was no true stock turnover as fish were only removed but not replaced. In site 5 stock turnover was only recorded as the number of fish sampled as any argulids on these fish were removed and the fish restocked clean. In the other sites the number of fish stocked was calculated by the fishery manager based on the number of fish removed with a small adjustment for mortality, predation and poaching that varied between months and sites. Examination for correlations between the abundance of *A. foliaceus* and rate of stock turnover from sites 1-4 was conducted in SPSS, and the data graphed in Excel 2000.

3.2.5 Fish Capture

Fish capture is a difficult issue both in terms of the actual catching of fish, but also in the obtaining of a representative sample. There are many different methods of fish capture, all of which are biased to the capture of certain parts of the fish population, whether this be in terms of species, size or behaviour (Lagler, 1970). For the purpose of this project only two methods were deemed acceptable as they were non-destructive to the fish, and could be practically conducted given the manpower and resources available to the study. These were rod capture and seine netting.

Rod capture is likely to sample only healthy fish that are feeding, and bait choice is likely to determine the species that will be caught. It also requires a considerable time effort to catch large numbers of fish (Lagler 1970). Given the number of fish required at each sampling point and that one of the clinical signs associated with argulid infections was identified by the cross-sectional study to be reduced feeding/rod capture, this method was deemed unsuitable. Seine netting is not species specific, but it is argued that it may select the slowest or sickest fish (Bayley & Herendeen 2000). Although not a perfect method, it was deemed to be the most practical and unbiased method available for the purpose of the study.

A 30m long, 1.5m deep seine net with a $1cm^2$ mesh size was used on the two smallest waters, and a 75m long, 3.5m deep net with a $1.5cm^2$ mesh size on the three larger lakes. Netting was conducted in bays or inlets for ease of handling and to entrap the largest number of fish (Figure 3.1). The number of fish sampled at each site visit is detailed in Table 3.2.

Table 3.2. Numbers of rainbow trout netted from each site during the May 2002 to May 2003 longitudinal study.

	Site 1	Site 2	Site 3	Site 4	Site 5
May-02	7	11	9	7	11
Jun-02	22	20	19	5	7
Jul-02	17	13	20	2	13
Aug-02	15	8	3	2	10
Sep-02	6	20	20	5	14
Oct-02	20	15	3	3	15
Nov-02	30	16	10	10	12
Dec-02	25	5	0	16	8
Jan-03	30	19	9	14	7
Feb-03	30	7	30	21	10
Mar-03	25	7	8	10	8
Apr-03	11	11	14	10	13
May-03	13	12	8	11	7

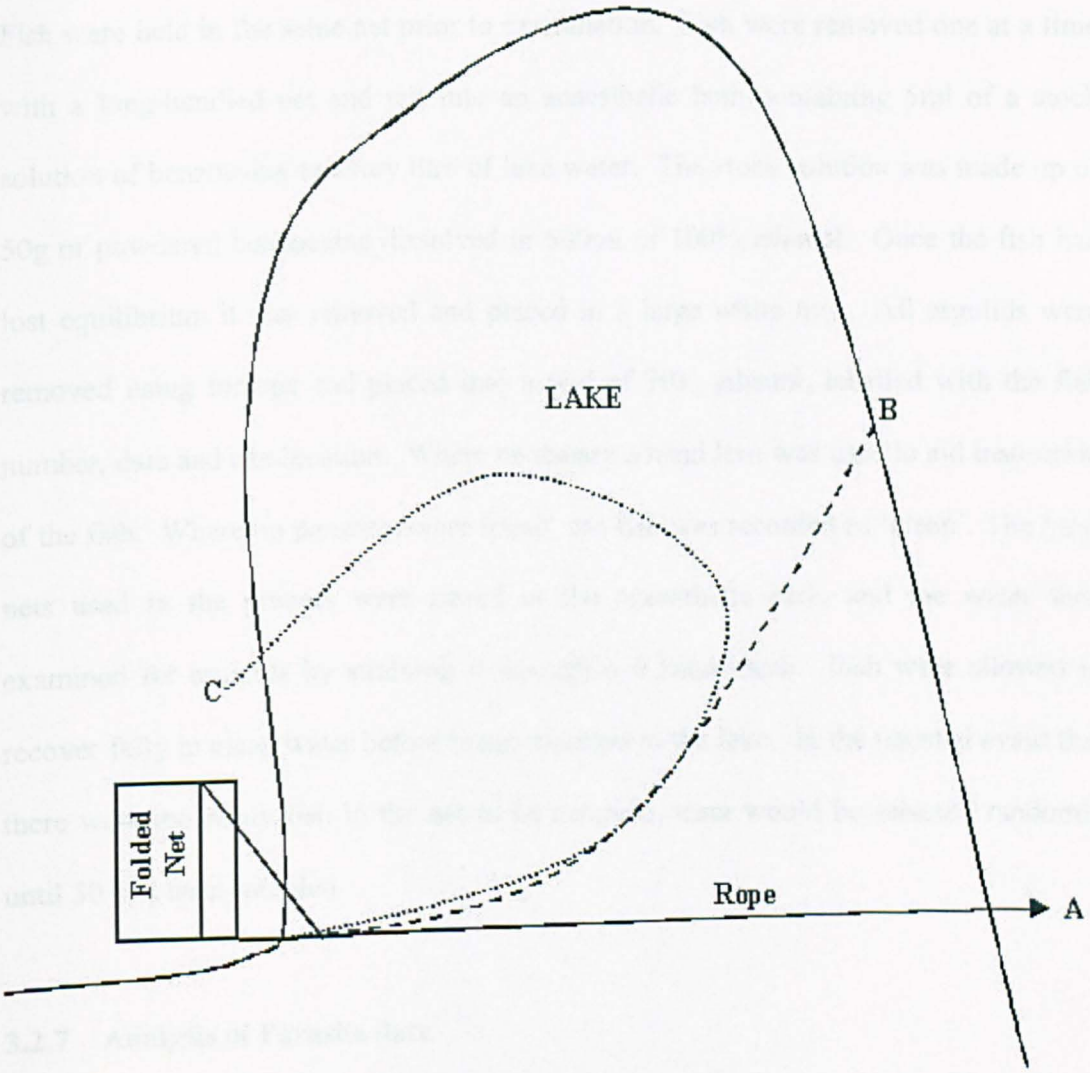


Figure 3.1. Diagrammatic representation of the seine netting process used to catch fish during May 2002 to May 2003 study. A) The point to where the net would be pulled. B) shows the net being walked around a bay. C) shows where the end of the net would be walked to, to form a horseshoe shape before being pulled in.

3.2.6 Parasite Sampling

Fish were held in the seine net prior to examination. Fish were removed one at a time with a long-handled net and put into an anaesthetic bath containing 5ml of a stock solution of benzocaine to every litre of lake water. The stock solution was made up of 50g of powdered benzocaine dissolved in 500ml of 100% ethanol. Once the fish had lost equilibrium it was removed and placed in a large white tray. All argulids were removed using forceps and placed into a vial of 70% ethanol, labelled with the fish number, date and site location. Where necessary a hand lens was used to aid inspection of the fish. Where no parasites were found, the fish was recorded as 'clean'. The hand nets used in the process were rinsed in the anaesthetic bath, and the water then examined for argulids by straining it through a 0.3mm mesh. Fish were allowed to recover fully in clean water before being returned to the lake. In the unusual event that there were too many fish in the net to be sampled, trout would be selected randomly until 30 had been sampled.

3.2.7 Analysis of Parasite data

The parasites in each vial from each site visit were counted. The computer package Quantitative Parasitology 2 (Rozsa, Reiczigel & Majoros 2000) was used to calculate the prevalence of infection and mean abundance. Relationships between abundance, site, stock turnover, temperature and water clarity were determined through scatter plots and analysis of covariance (ANCOVA) in SPSS.This data was then graphed using

Excel 2000. Definitions of prevalence and mean abundance were taken from Bush et al. (1997), and are as follows:

Prevalence = the proportion of individuals in the fish population infected with one or more parasites. In this study the fish equal trout and the parasite equals *A. foliaceus*.

Mean abundance = the total number of argulids counted on all of the trout examined, divided by the number of trout sampled.

3.2.8 Image Analysis

Image analysis was used to gain accurate information on the length, species and sex of specimens of *Argulus*. The image analysis equipment comprised a Pentium 4 personal computer connected to a Zeiss Axiocam MRC digital camera. This in turn was connected through a trinocular head on an Olympus SZ40 dissecting microscope. A macro to allow the necessary measurements to be taken was written in Zeiss KS300 software by Dr J. Bron of the Institute of Aquaculture, University of Stirling. The macro was written so that information could be gathered at either x 1 or 4 magnification and all data was automatically stored in a database.

To obtain measurements of parasites, vials, i.e. individual fish from each visit were randomly selected until either at least 300 parasites were analysed or, where less than

300 were present, all argulids collected in that site visit were analysed. Where 300 parasites had been analysed and specimens still remained in a vial, these were also measured so that no selection bias was introduced. This method of selection assumes that the proportion of size classes of argulids on each fish was similar. Comparisons of the size classes between two randomly selected fish in a sample suggested that this was the case (Mann-Whitney U, Z=-0.828, P=0.430). Alternative methods were attempted using a Stemple pipette to sub-sample from a population. The results showed the method to produce a sample biased towards the larger specimens in the sample when compared to the population data (Population mean = 3.07mm c.f Sample mean = 3.43mm Mann-Whitney U, Z=-2.430, P=0.015).

At the start of each session of measurements, the accuracy of the system was checked using a graticule. Specimens were removed individually from a vial and placed in a small Petri dish containing 70% ethanol under the dissecting microscope. The magnification was set to either x1 or x4 depending on the size of the specimen. Specimens were lit from above, and the light and focus adjusted until a clear image was visible on the computer monitor. Length and width were then entered by dragging a cursor across the specimen.

3.2.9 Length Frequency / Size Distribution Analysis and Population Growth

The mean size of each cohort was determined using the computer package Rmix (MacDonald 2002) to fit an appropriate statistical distribution (e.g. normal, log-normal)

to the data. This gives an estimation of the mean size and level of spread in each cohort and can also be used to discriminate cohorts if they merge. Overlapping distributions can be fitted to produce a model known as a mixture. This allows an estimate of the mean size and standard deviation of each cohort, and also an estimation of the proportion of the population that falls into each of the cohorts (Figure 3.2).

The technique enables cohorts to be followed through time, allowing estimates of growth rate, mortality and recruitment for each cohort. Growth was determined by taking the mean cohort size from one month, subtracting the mean size from the previous month and dividing by the number of days between visits to give growth per day. The difference in cohort size was also divided by degree-days to correct for the effect of temperature; the results of this analysis were plotted in Excel 2000. Growth per day was calculated for each cohort at each site, by dividing the amount of growth by the number of days between sampling trips, this data was plotted against temperature. Analysis of covariance (ANCOVA) was used to determine differences in the relationship between growth rates and temperature in each site. Regression analysis was used to describe the relationship in SPSS v.11. Mortality and recruitment to a cohort was assessed by comparing the numbers of parasites found in a cohort between samples. This was calculated based on the mean parasite abundance and the proportion of the total population that a cohort represents e.g. if the mean abundance is 100 and the cohort makes up 60% of the population then 60 parasites per fish are from this cohort, which can be compared with the value from the previous month.

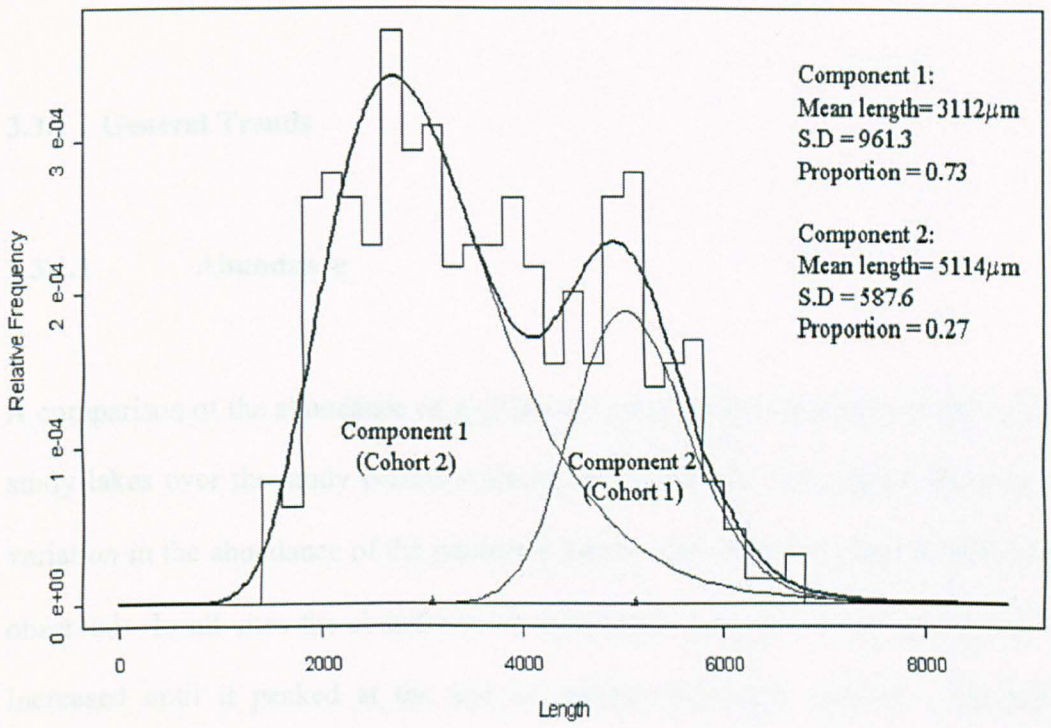


Fig. 3.2. Example of a mixture model fitted to length frequency data using two lognormal components.

Mixture models were fitted following the examples shown in Du (2002). Fitting these models involves a certain amount of subjectivity so proportions and mean size values obtained through the models were checked against data from the previous months samples and other sites to see if they were realistic. Hanging rootograms were used to visualise the degree by which the observed data deviated from the expected data (Friendly, 2000), and analysis of variance (ANOVA) functions contained within the Rmix package were also used to test how well the mixture model fitted the data.

3.3 Results

3.3.1 General Trends

3.3.1.1 Abundance

A comparison of the abundance of *A. foliaceus* on seine netted rainbow trout in all five study lakes over the study period is shown in Figure 3.3. The figure shows a large variation in the abundance of the parasite between sites, however general trends can be observed. In all sites the abundance of *A. foliaceus* was low in the spring, and then increased until it peaked at the end of summer and early autumn. The parasite abundance then dropped to low levels over the winter months before increasing once again in the following spring.

3.3.1.2 Water Temperature

Figure 3.4 shows a comparison of the mean monthly water temperature profiles of all the lakes over the study period. The data shows that all the lakes had very similar profiles over the year. In all lakes the temperature peaked in August at between 18-21°C and was lowest in February at 3-5°C. The temperature remained below 10°C (the minimum temperature at which *A. foliaceus* eggs are thought to hatch) from November 2002 to April 2003 in sites 1-4. In site 5 the temperature did not reach 10°C until May 2003. The greatest level of variability was seen between sites 2 and 5 where there was

a difference of 3.5°C in September 02 and April 03. Variability was very low throughout the winter, but was lowest in March, with only a 1.2°C difference between sites 2 and 5.

3.3.1.3 Stock Turnover

The percentage of stock turnover differed greatly between all 5 sites (Figure 3.5), but overall was highest in site 1 and lowest in site 5. Turnover fluctuated greatly in site 1 throughout the year (probably due to the small size of the lake and the capture methods used, i.e. anglers were not restricted to fly only methods), but over 100% of the standing stock of trout was removed and replaced each month (i.e. the entire stock of trout in the lake were replaced at least once in a month). Turnover was highest from June to August and lowest in the winter months, reaching a low in December when the lake froze over.

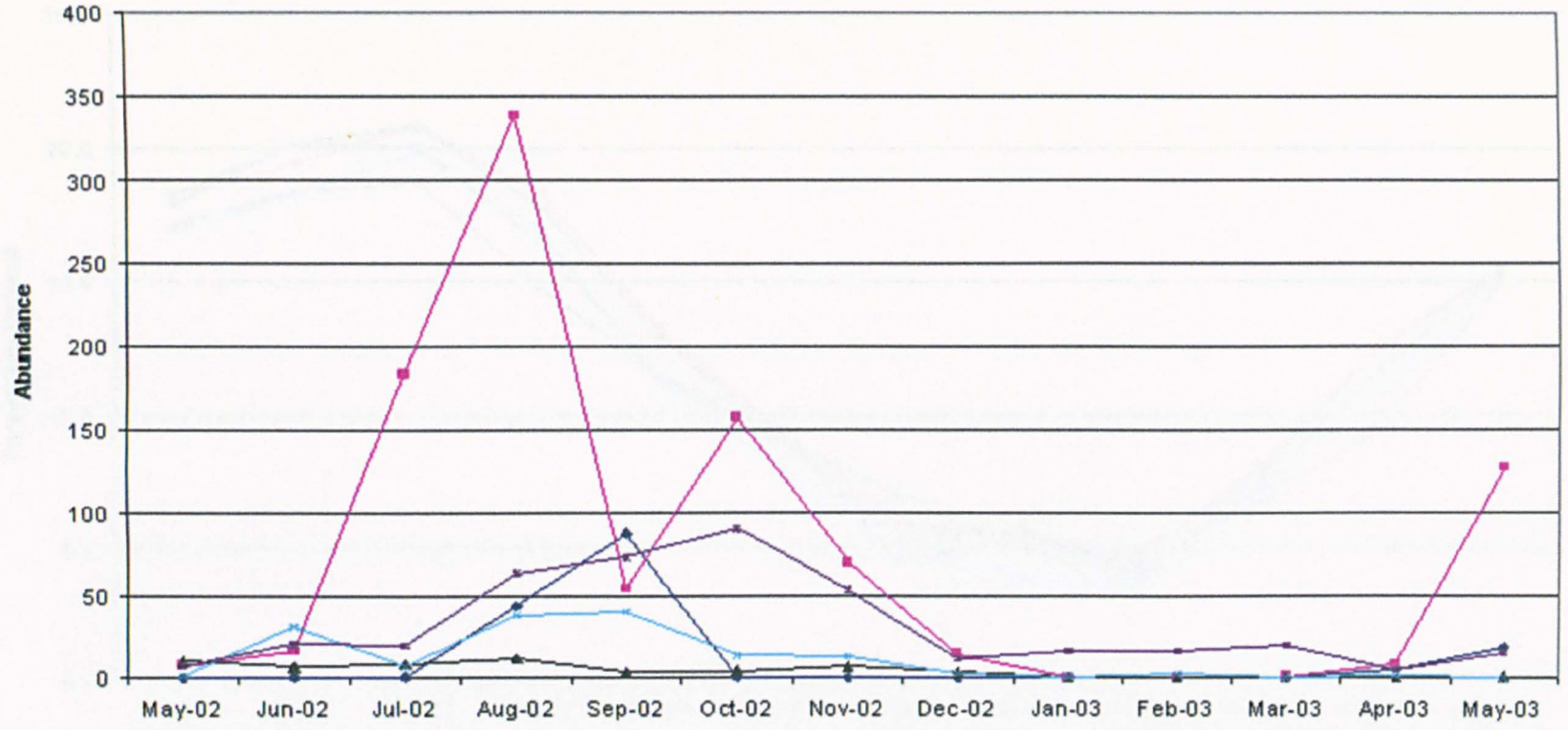
Stock turnover was fairly constant in site 2, except in July and August when it dropped substantially. Stock turnover was constant throughout the study year in sites 3, 4 and 5, although it was substantially higher in site 3 than in 4 and 5. In site 5, stock turnover was solely due to the investigator removing fish each month and returning them after parasites were removed. Although low numbers of trout were caught and removed from this lake, no fish were stocked to replace them until the following spring.

3.3.1.4 Water clarity

Water clarity in sites 1 and 2 showed a clear seasonal pattern with water clarity being lowest in summer and highest in winter (Figure 3.6). Sites 3 and 4 remained the clearest throughout the study year with water clarity fluctuating between 10 and 15 Secchi disc units. Site 5 had a water clarity reading that remained at 6.5 Secchi disc units throughout the study, except for the final three months when it dropped, reaching a low of 2.5 Secchi disc units in April 2003.

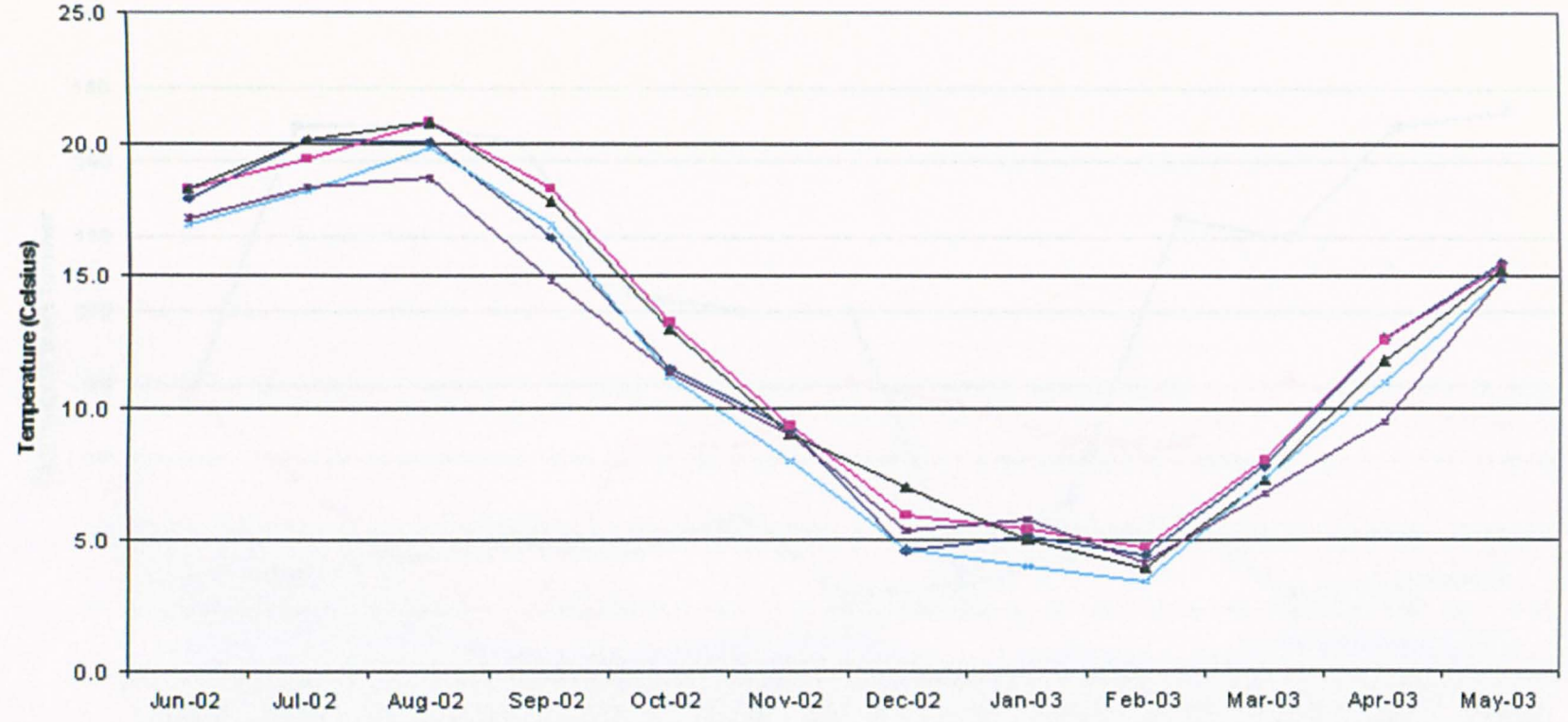
3.3.1.5 Egg Laying

In 2002 egg laying by *A. foliaceus* was highest in September in site 1, June in sites 2 and 3, July in site 4, and August in site 5 (Figure 3.7). Egg laying stopped in site 3 after August, but continued into September in sites 2 and 4. In sites 1 and 5 egg laying continued at low levels into October. Sites 1 and 5 are the two smallest lakes and had the lowest temperature profiles for the three months prior to the cessation of egg laying. No egg laying was observed in any of the sites between November and March. Egg laying was first noted in April in sites 4 and 5. In sites 1 and 2 egg laying did not occur until May, and in site 3 no egg laying was recorded by the end of the survey in May 2003.



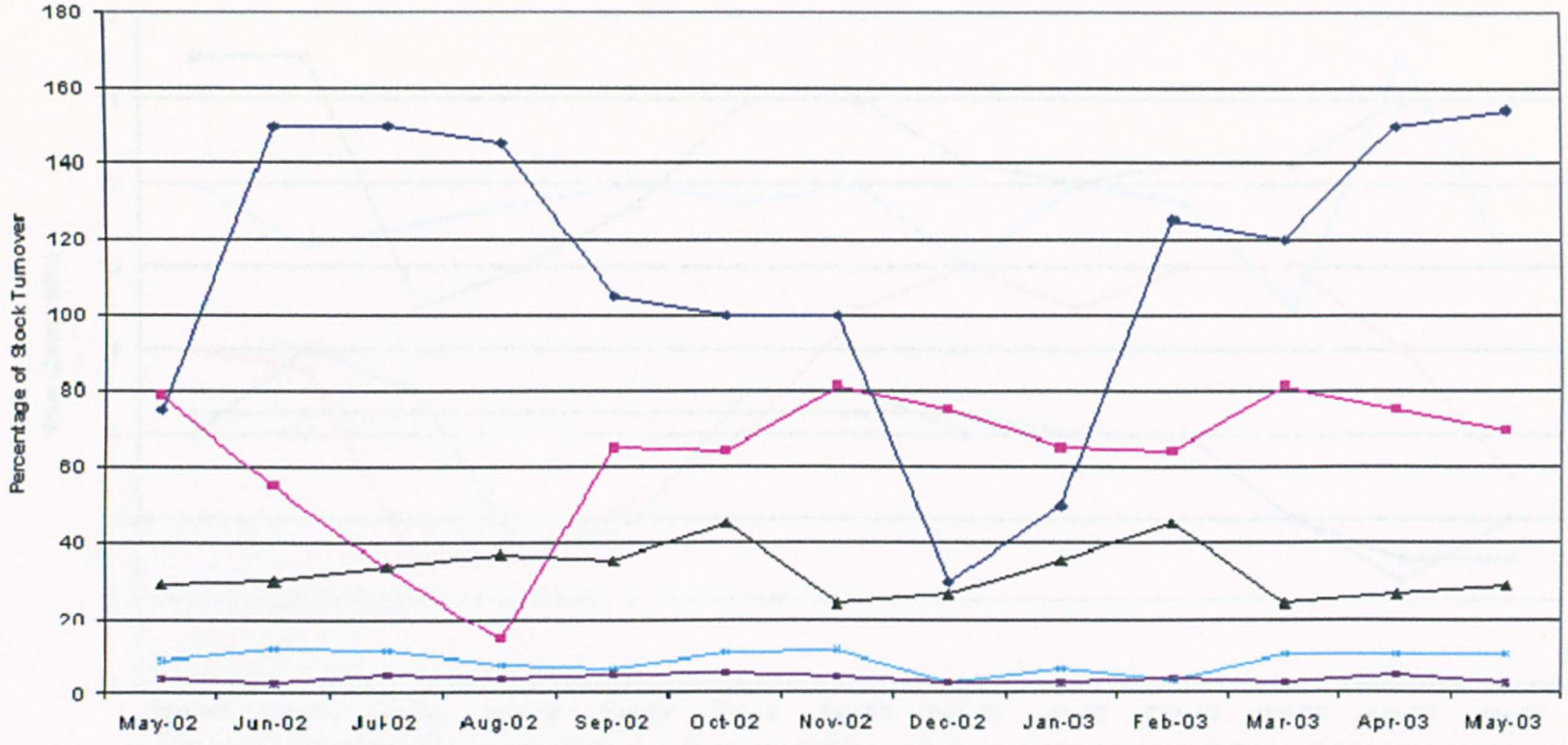
Month	May-02	Jun-02	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Site 1	0	1.23	1.24	43.4	87.83	0.95	0	0	0	0	0	5.36	19.08
Site 2	7.45	16.65	183.85	338.88	54.2	157.13	69.31	14.33	0.21	0.14	0.71	8.27	127.25
Site 3	9.89	7	8.3	12	3.75	4.67	7.2	3.4	0.22	0.1	0.13	0.36	0.63
Site 4	1.57	30.8	7	39	40.6	13.33	13	2.31	0.79	2.24	0.4	1.4	0.09
Site 5	7.91	21	20.15	63.5	72.86	90.47	53.08	12.11	15.89	16.6	19.38	5	15.29

Figure 3.3. The monthly abundance of *A. foliaceus* on rainbow trout in the five study sites May 2002 to May 2003.



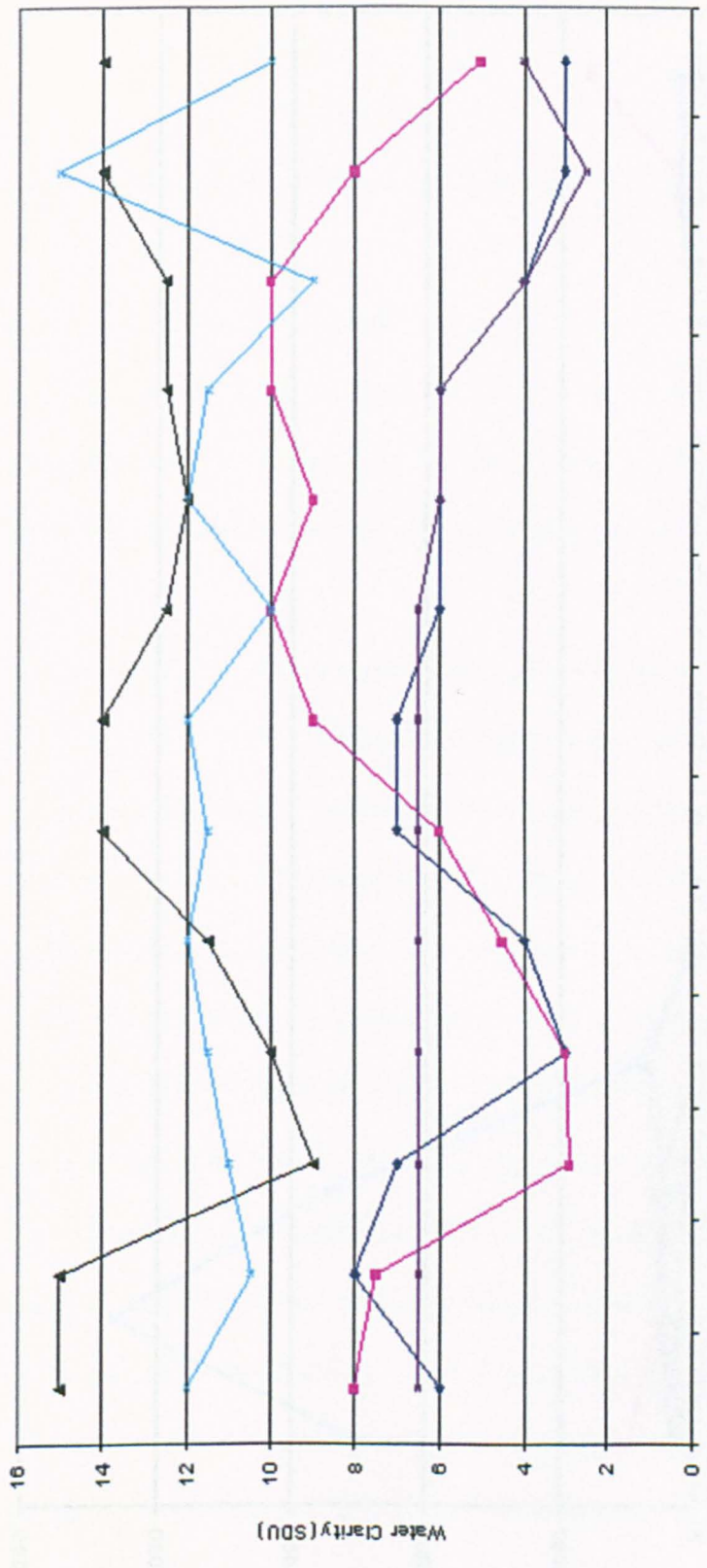
Month	Jun-02	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Site 1	18.0	20.2	20.1	16.5	11.5	9.2	4.6	5.1	4.4	7.8	12.6	15.6
Site 2	18.3	19.5	20.9	18.3	13.2	9.3	5.9	5.4	4.7	8.0	12.6	15.4
Site 3	18.4	20.2	20.8	17.8	13.0	9.0	7.0	5.0	3.9	7.3	11.8	15.3
Site 4	17.0	18.3	19.9	17.0	11.2	7.9	4.6	4.0	3.4	7.3	11.0	14.9
Site 5	17.2	18.4	18.7	14.8	11.3	9.0	5.3	5.7	4.1	6.8	9.5	15.0

Figure 3.4. Temperature profiles of the five study sites from May 2002 to May 2003.



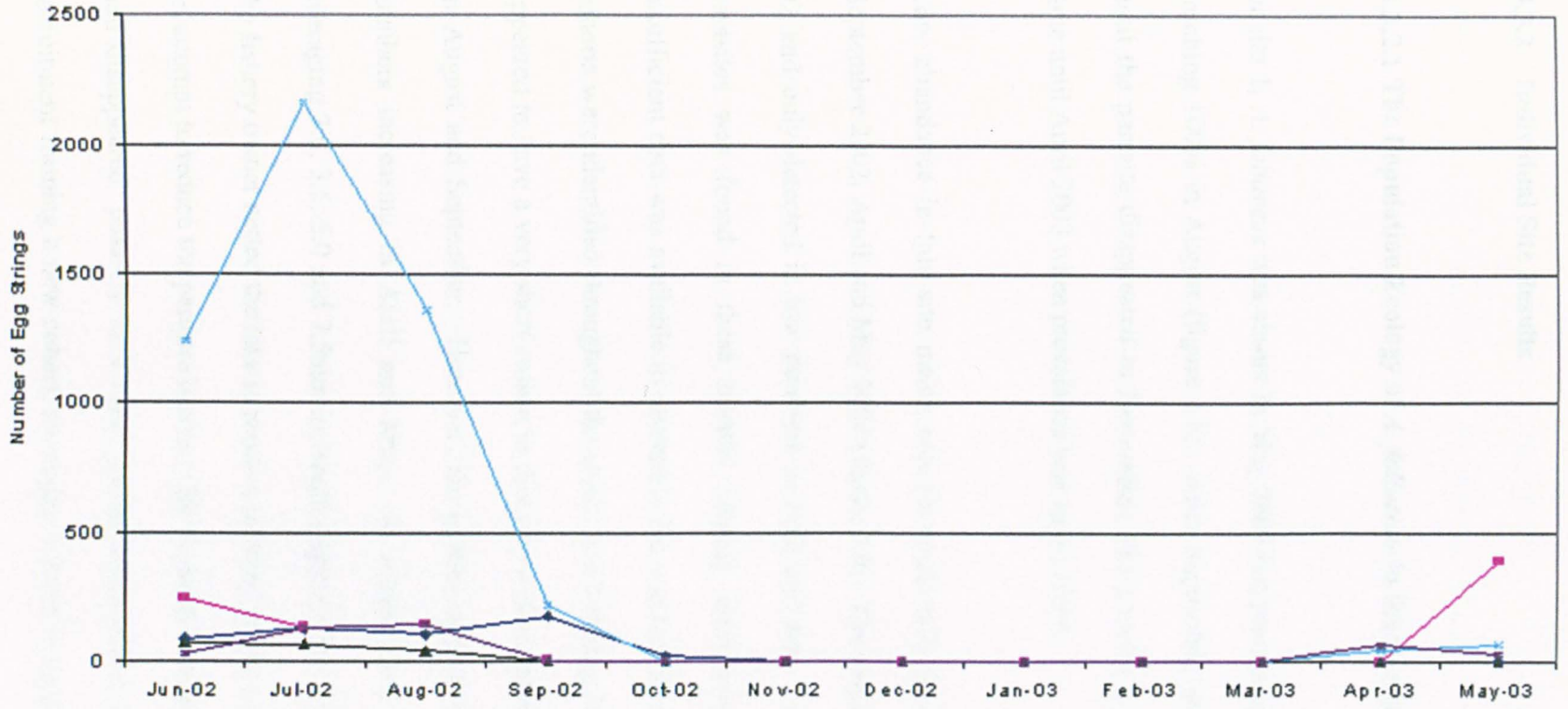
	May-02	Jun-02	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Site 1	75	150	150	145	105	100	100	29.5	50	125	120	150	154
Site 2	78.7	54.7	33.1	14.3	64.8	64.0	81.1	75.0	64.8	64.0	81.1	75.0	69.2
Site 3	29.3	29.9	33.4	36.7	35.3	45.1	24.0	26.3	35.3	45.1	24.0	26.3	28.5
Site 4	8.2	11.8	10.9	7.3	6.4	10.7	11.5	2.8	6.7	3.3	10.2	9.9	10.2
Site 5	4.0	2.5	4.7	3.6	5.1	5.5	4.4	2.9	2.5	3.6	2.9	4.7	2.5

Figure 3.5. The percentage of stock turnover each month in the 5 study sites from May 2002 to May 2003.



Month	May-02	Jun-02	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Site 1	6	8	7	3	4	7	7	6	6	6	4	3	3
Site 2	8	7.5	2.9	3	4.5	6	9	10	9	10	10	8	5
Site 3	15	15	9	10	11.5	14	14	12.5	12	12.5	12.5	14	14
Site 4	12	10.5	11	11.5	12	11.5	12	10	12	11.5	9	15	10
Site 5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6	6	4	2.5	4

Figure 3.6. Water clarity profiles of the 5 study sites from May 2002 to May 2003.



Month	Jun-02	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Site 1	93	128	106	177	22	0	0	0	0	0	0	5
Site 2	245	135	145	4	0	0	0	0	0	0	0	392
Site 3	74	71	44	0	0	0	0	0	0	0	0	0
Site 4	1242	2161	1358	220	0	0	0	0	0	0	48	72
Site 5	30	128	143	5	8	0	0	0	0	0	65	34

Figure 3.7. The egg laying activity of *A. foliaceus* in the 5 study sites from May 2002

3.3.2 Individual Site Results

3.3.2.1 The Population Ecology of *A. foliaceus* in Study Site 1

In site 1, *A. foliaceus* was absent in May 2002 but prevalence then increased steadily, reaching 100% in August (figure 3.8). After September, prevalence dropped rapidly until the parasite disappeared in November. The parasite was not detected from this time until April 2003 when prevalence was again 100%.

Low abundance in this site meant cohorts could only be discriminated in August, September 2002, April and May 2003 (figure 3.9). The parasite was not found in May 02 and only detected in low numbers in June and July. A wide size range of the parasites was found in these months ranging from juvenile to adult, however, insufficient data was available to determine the number of cohorts present. In total 7 cohorts were identified throughout the study, and 5 during 2002. In 2002 the parasite appeared to have a very short season in this site, with substantial numbers only present in August and September. However, the pattern appeared to change in 2003 with numbers increasing in April and May. In August four cohorts were identified, averaging 2.0, 3.5, 5.0 and 7.5mm in length (figure 3.10). After the August site visit, the fishery owner netted the lake to remove infected stock, restocking with clean fish in an attempt to reduce the parasite burden. By September the two largest August cohorts had disappeared, possibly as a result of the intervention, but there was substantial recruitment forming a new cohort, averaging 1.8mm in length. This caused the total

abundance to peak at 88. During this month the lake experienced an algal bloom reflected by a drop in water clarity. The bloom was found to be toxic and caused high mortality amongst the trout population. As a result, many of the fish sampled in this month were moribund and thus may have led to biased estimates of abundance. Abundance dropped markedly in October and only the fifth cohort remained, which had grown substantially to an average size of 4mm. No recruitment was seen through the winter months and the parasite had apparently died out by November. In April 03 two juvenile cohorts are present, the growth of which can be followed into May 03.

The abundance of *A. foliaceus* in this site followed a quite different pattern from all of other study sites. Much larger parasites were detected at this site and the number of eggs laid appeared to be disproportionately high compared to the other sites. High levels of egg laying were observed from the start of the study until September, even though the parasite's abundance was close to one from May to July, and very few adult parasites were present.

As no cohorts could be followed for more than two months at this site, it is difficult to make firm conclusions about parasite growth. The gradient of the line is similar between cohorts 3&4 and 6&7 suggesting that growth is linear (figure 3.10), with smaller and larger parasites growing at a similar rate. Cohort 5 has a slower growth rate corresponding to the drop in water temperature after September. Also of interest in this site is the observation that the specimens in cohorts 1&3 were greater than 7.5 mm in length, much larger than in any of the other sites.

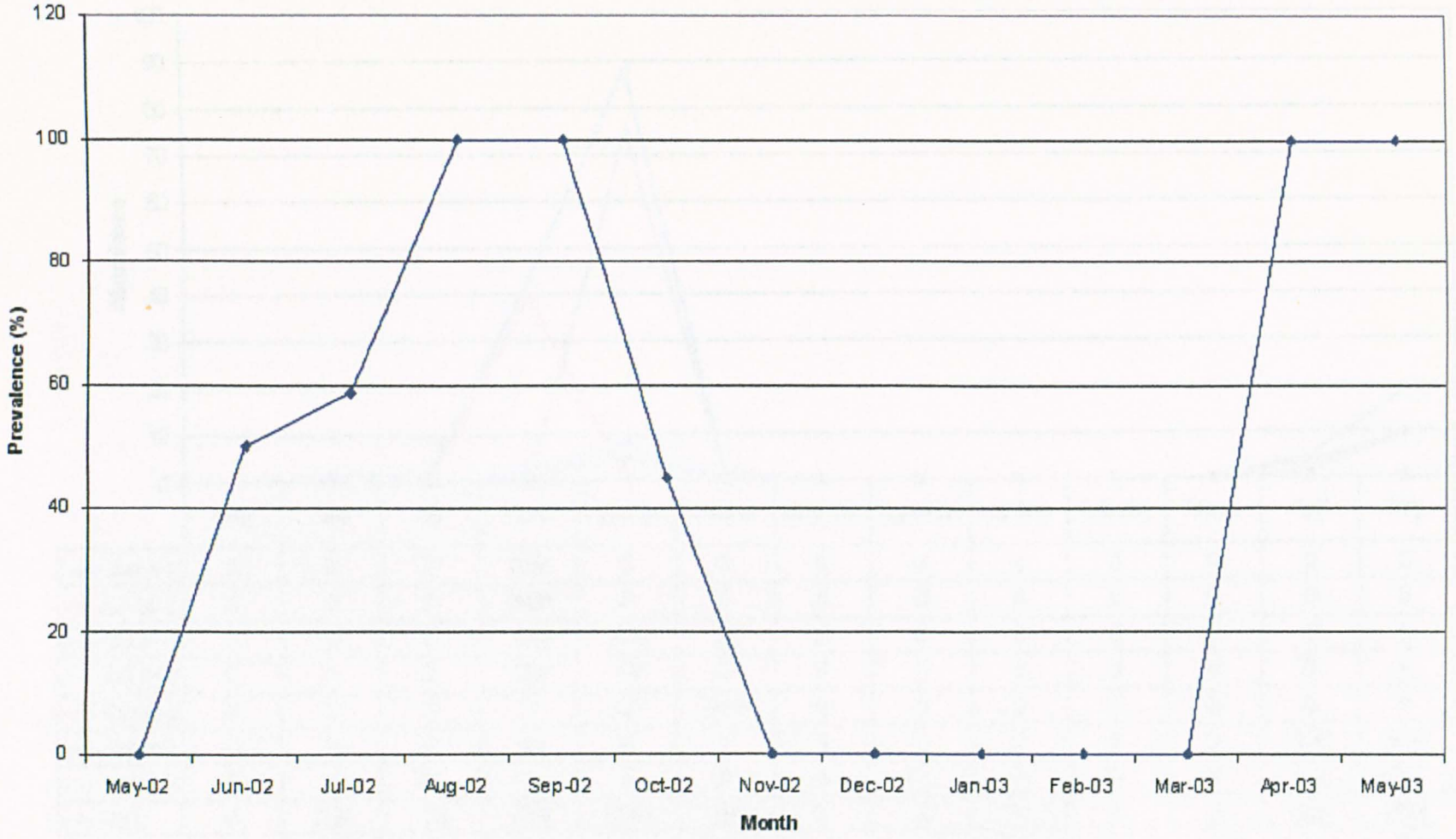
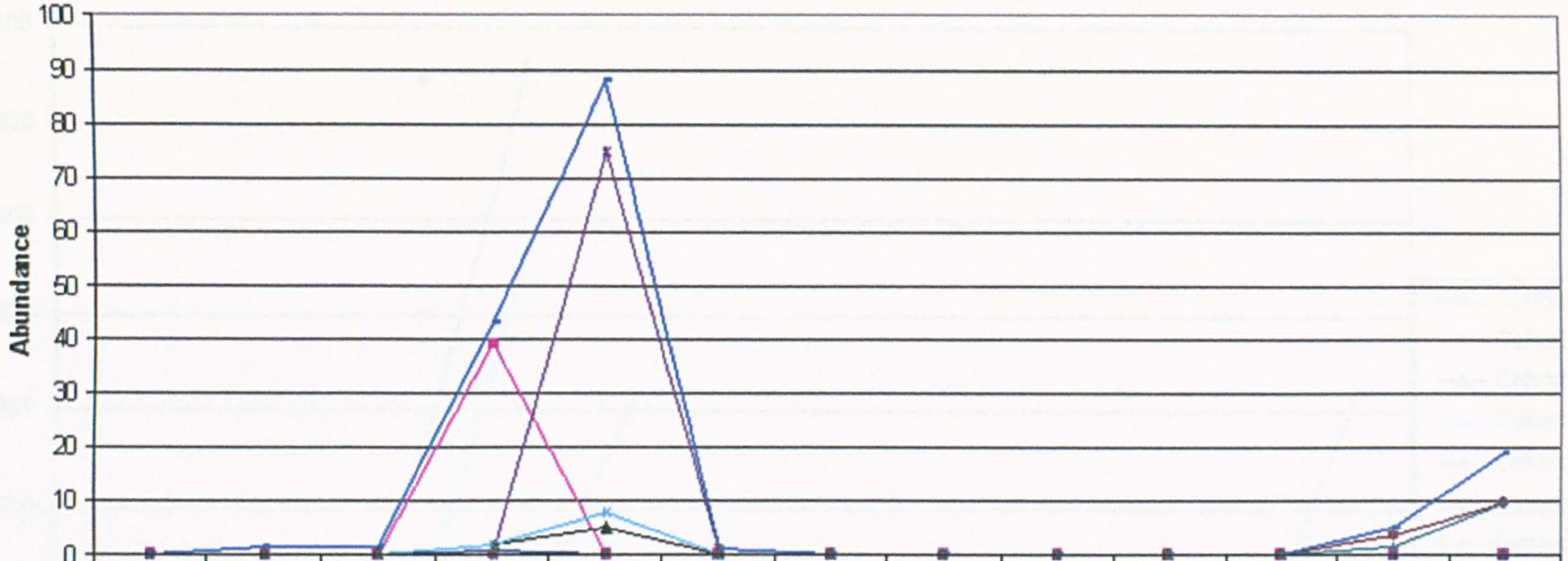


Figure 3.8. The prevalence of *A. foliaceus* in study site 1 from May 2002 to May 2003



	May	June	July	August	September	October	November	December	January	February	March	April	May
Cohort 1	0	0	0	0.78	0	0	0	0	0	0	0	0	0
Cohort 2	0	0	0	39.06	0	0	0	0	0	0	0	0	0
Cohort 3	0	0	0	1.78	5.3	0	0	0	0	0	0	0	0
Cohort 4	0	0	0	1.61	7.9	0	0	0	0	0	0	0	0
Cohort 5	0	0	0	0	74.7	0.95	0	0	0	0	0	0	0
Cohort 6	0	0	0	0	0	0	0	0	0	0	0	3.96	9.54
Cohort 7	0	0	0	0	0	0	0	0	0	0	0	1.4	9.54
Total	0	1.23	1.24	43.4	87.83	0.95	0	0	0	0	0	5.36	19.08

Figure 3.9. The total abundance and relative abundance of each cohort of *A. foliaceus* in site 1 from May 2002 to May 2003

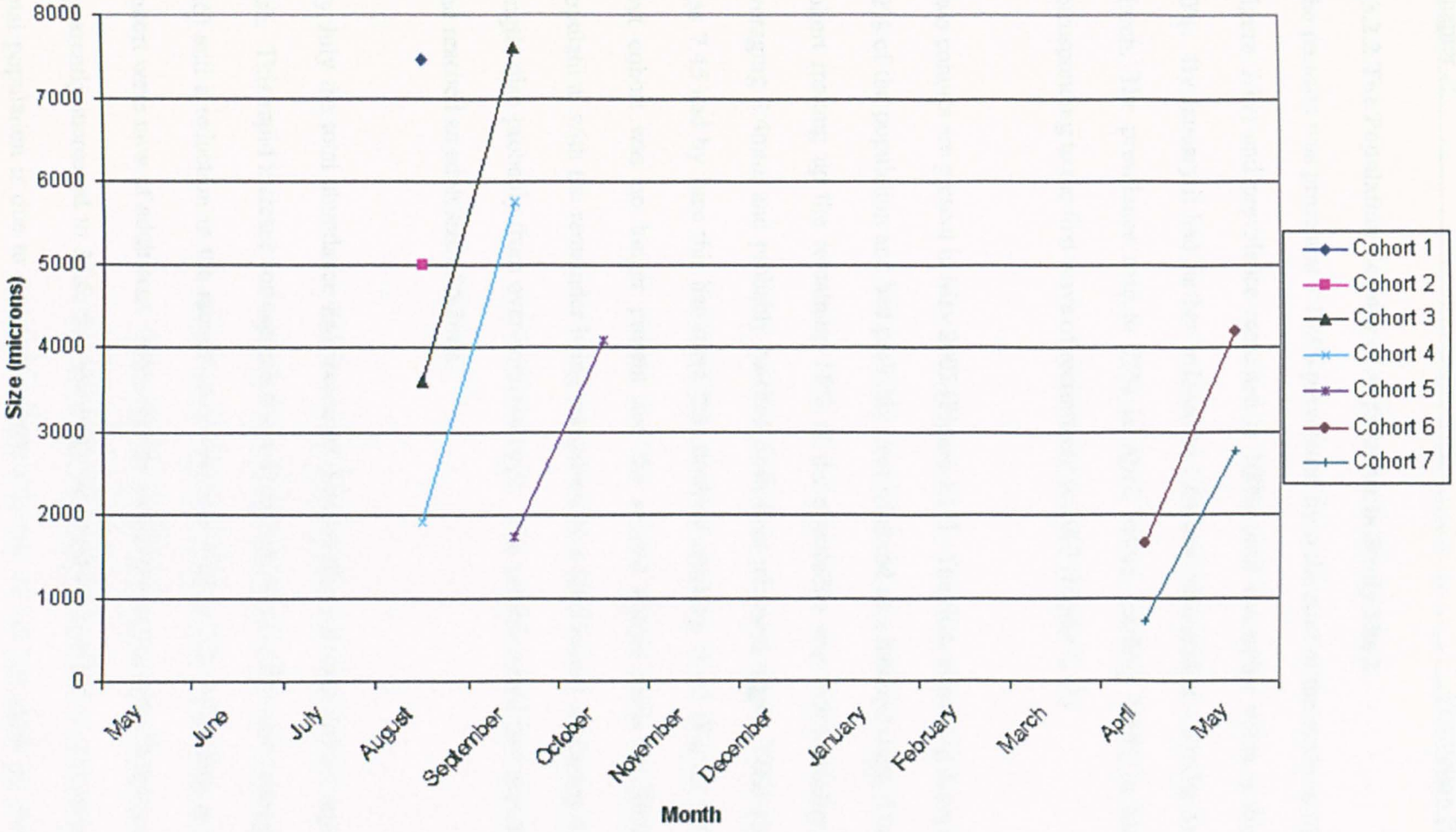


Figure 3.10. The mean size and growth of each cohort of *A. foliaceus* in study site 1 from May 2002 to May 2004

3.3.2.2 The Population Ecology of *A. foliaceus* in Study Site 2

The parasite was present at a 100% prevalence from the start of the study in May 2002 (figure 3.11) and prevalence remained at 100% until December when it dropped to 50%. By January it had further reduced to 10% and remained at a similar level until March. The prevalence rose to 75% in April before reaching 100% in May 2003 corresponding to the first wave of recruitment in 2003 (Figure 3.12).

Two cohorts are present in May 2002 (Figure 3.13). The first, averaging 4mm made up 82% of the population and had probably over wintered as a hatched stage. The second cohort making up the remaining 18% of the population was much smaller in size, averaging 2.4mm, and probably hatched from over-wintered eggs. Total abundance was 7.45 and by June this had more than doubled reaching 16.65 (Figure 3.12). The first cohort was no longer present and the second cohort made up 28% of the population, with the remainder being comprised of a third cohort averaging 2.7mm in length, also probably from over-wintered eggs. The second cohort had grown rapidly and reached an adult size of 5.3mm.

By July the total abundance had increased dramatically reaching 183.85 argulids per fish. This rapid increase corresponded to a sharp reduction in the water clarity (Figure 3.6) and a reduction in the rate of stock turnover (Figure 3.5). Parasites in the third cohort were now of adult size. Although the abundance of parasites in this cohort has apparently increased to 31.6, the reason for this marked increase in abundance in the total population is due to recruitment from a fourth cohort that made up 83% of the

total population. Total abundance continued to increase sharply into August reaching 338.8 with the arrival of a fifth cohort. The fourth cohort had reduced in number and had an abundance of 91.29, but was now mature at 5.1mm. The parasite population became male biased (figure 3.14), suggesting females had left their hosts to lay eggs, thus explaining the reduction in numbers. This peak in abundance in August corresponded to a low in stock turnover (Figure 3.5).

After the site visit in August the fishery owner put an intervention into place in an attempt to reduce the abundance of the parasite. Results from the September visit suggested the intervention had been successful in terms of reducing the numbers of hatched *A. foliaceus* to 54.2 per fish. The fourth cohort was no longer present and the fact that little egg laying (Figure 3.7) occurred between the visits suggests that these parasites had been killed by the intervention and were not just absent from the fish whilst laying eggs. Abundance within the fifth cohort was also substantially reduced to 3.56. The intervention also led to an increase in the rate of stock turnover, with levels returning to those at the start of the study. The intervention appears to have had little or no effect on eggs already laid, as demonstrated by the emergence of a sixth and final cohort of the year making up 93% of the population. Recruitment to this cohort continued into October raising the total abundance to 157.13. The number of parasites in the fifth cohort remained relatively constant at 4.22. Interestingly, stock turnover remained constant despite this increase in abundance. Water clarity, however, increased to 6 Secchi disc units and temperature dropped below 15°C.

A substantial reduction in abundance of the fifth cohort to 0.69 was observed in November, corresponding to the cohort becoming adult and the water temperature dropping below 10°C. To a lesser extent, the abundance of parasites in the sixth cohort was also reduced, to 68.62. At this time the growth rate of this cohort appeared to slow, corresponding to a drop in temperature. By December the prevalence of the parasite had reduced to around 50% and the population was made up solely of individuals from cohort 6. The total abundance had decreased to 14.22 and continued to fall into January, along with prevalence. From January to March the population had a low total abundance of less than 1 and a prevalence of between 10 & 15%. In April the water temperature rose above 10°C and a new cohort (cohort 7) with an average length of 1.5mm from over-wintered eggs and increased the prevalence to 75%. There also appeared to be an increase in the number of parasites in the 6th cohort. Recruitment to cohort 7 continued into May 2004 bringing the prevalence back to 100% and increasing the abundance greatly to 166. The fifth cohort was no longer present, however, egg laying was observed between the April and May site visits, which must be attributed to this cohort as it alone had reached adult size. The rapid increase in total abundance corresponded to a drop in water clarity and an increase in water temperature to 15.4°C. Stock turnover however, remained high at 69% between April and May.

The sex ratio of the *A. foliaceus* population on the fish was equal or female biased during periods when no egg laying was observed i.e. May 2002 and October 2002 to March 2003 (Figures. 3.14 & 3.15). During periods of egg laying the population shifted to equal or male biased, reflecting the leaving of adult females from their hosts

to lay eggs. This was observed from June to September 2002 when the first three cohorts of the year reached maturity, and in April 2003 corresponding with over-wintered parasites leaving their hosts to lay eggs.

In the majority of cohorts in this site, growth can only be followed over two months. In cohorts 2,3,4 &7 (Figure 3.13 & 3.16) the gradient of the lines were similar both in terms of real time and degree days. Growth in cohort 5 appears to be exponential, however the intervention after the August visit may have masked the actual relationship. After this time only low numbers of parasites from this cohort were present, which may have affected the reliability of the estimates. Cohort 6 shows a seasonal growth curve with growth slowing over the cooler winter months and increasing with temperature in spring. When growth is followed in degree-days, increases in length were not linear but slowed between November and March when the water temperature dropped below 9°C.

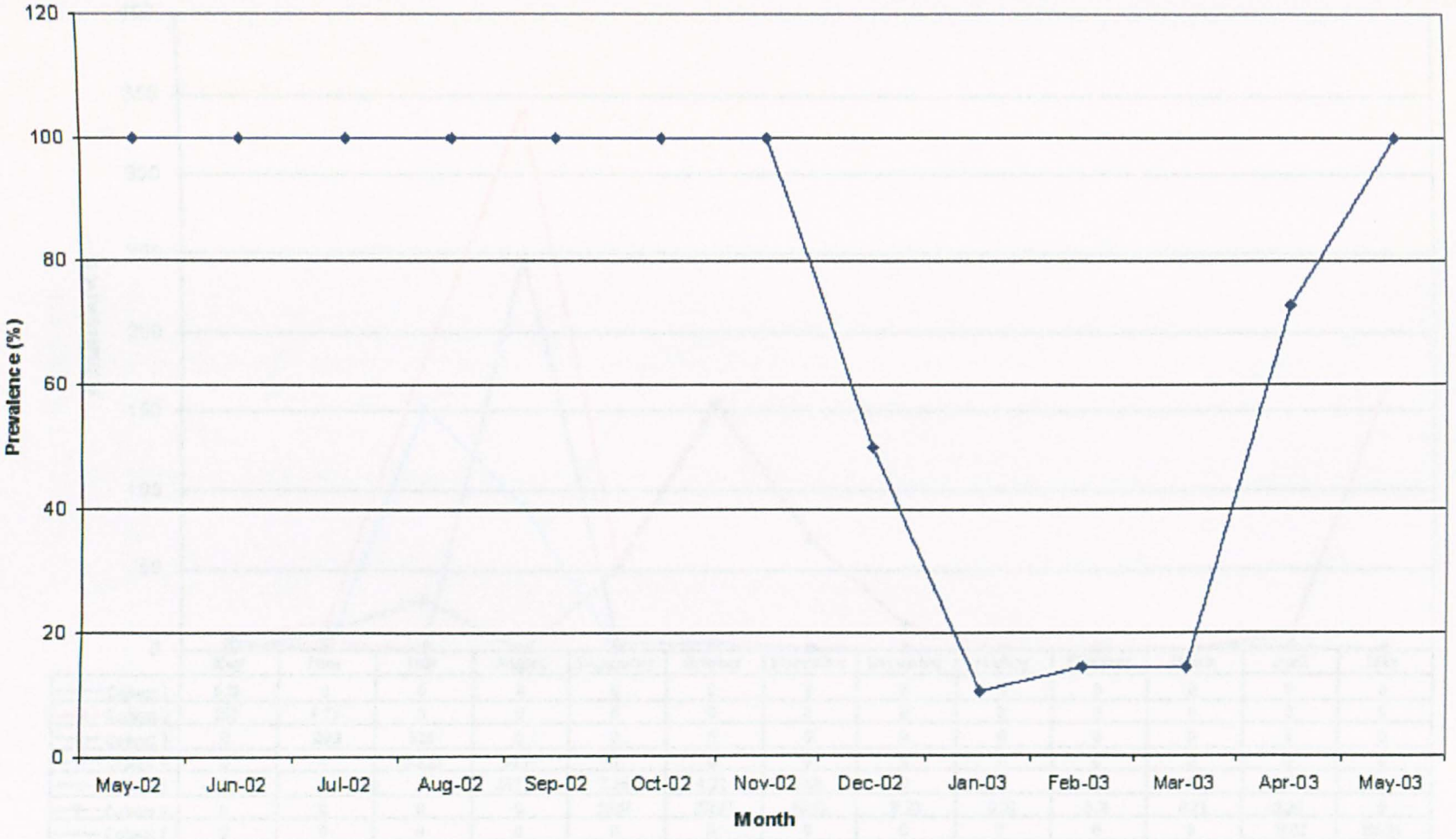
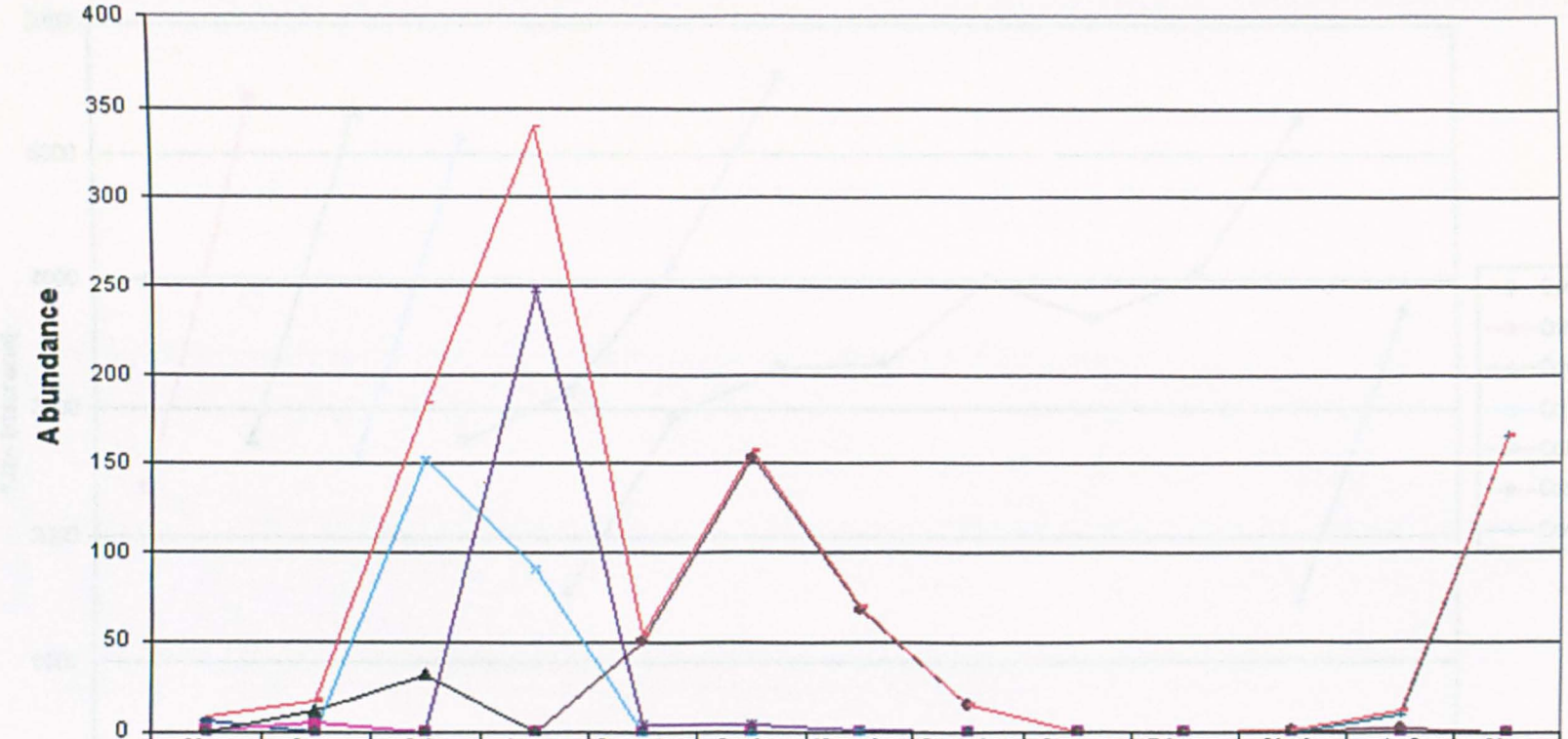


Figure 3.11. Prevalence of *A. foliaceus* in study site 2 from May 2002 to May 2004



	May	June	July	August	September	October	November	December	January	February	March	April	May
Cohort 1	6.12	0	0	0	0	0	0	0	0	0	0	0	0
Cohort 2	133	4.73	0	0	0	0	0	0	0	0	0	0	0
Cohort 3	0	11.92	3.16	0	0	0	0	0	0	0	0	0	0
Cohort 4	0	0	152.25	91.29	0	0	0	0	0	0	0	0	0
Cohort 5	0	0	0	247.59	3.56	4.22	0.69	0	0	0	0	0	0
Cohort 6	0	0	0	0	50.64	152.91	68.62	14.33	0.21	0.14	0.71	2.26	0
Cohort 7	0	0	0	0	0	0	0	0	0	0	0	10.01	166.25
Total	7.45	16.65	183.85	338.88	54.2	157.13	69.31	14.33	0.21	0.14	0.71	12.27	166.25

Figure3.12. The total abundance and relative abundance of each cohort of *A. foliaceus* in site 2 from May 2002 to May 2003

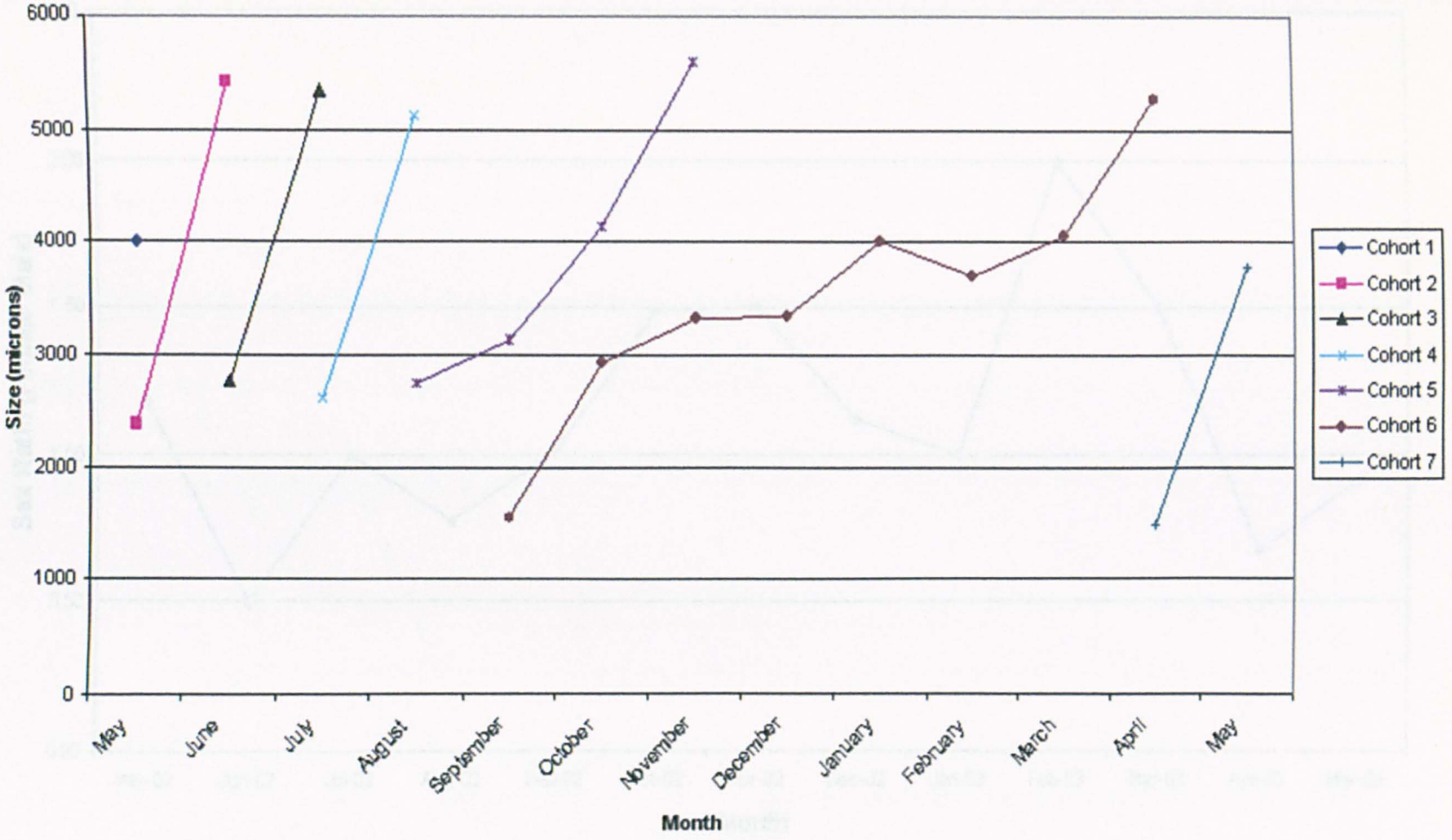


Figure 3.13. The size and growth of each cohort of *A. foliaceus* in study site 2 from May 2002 to May 2003.

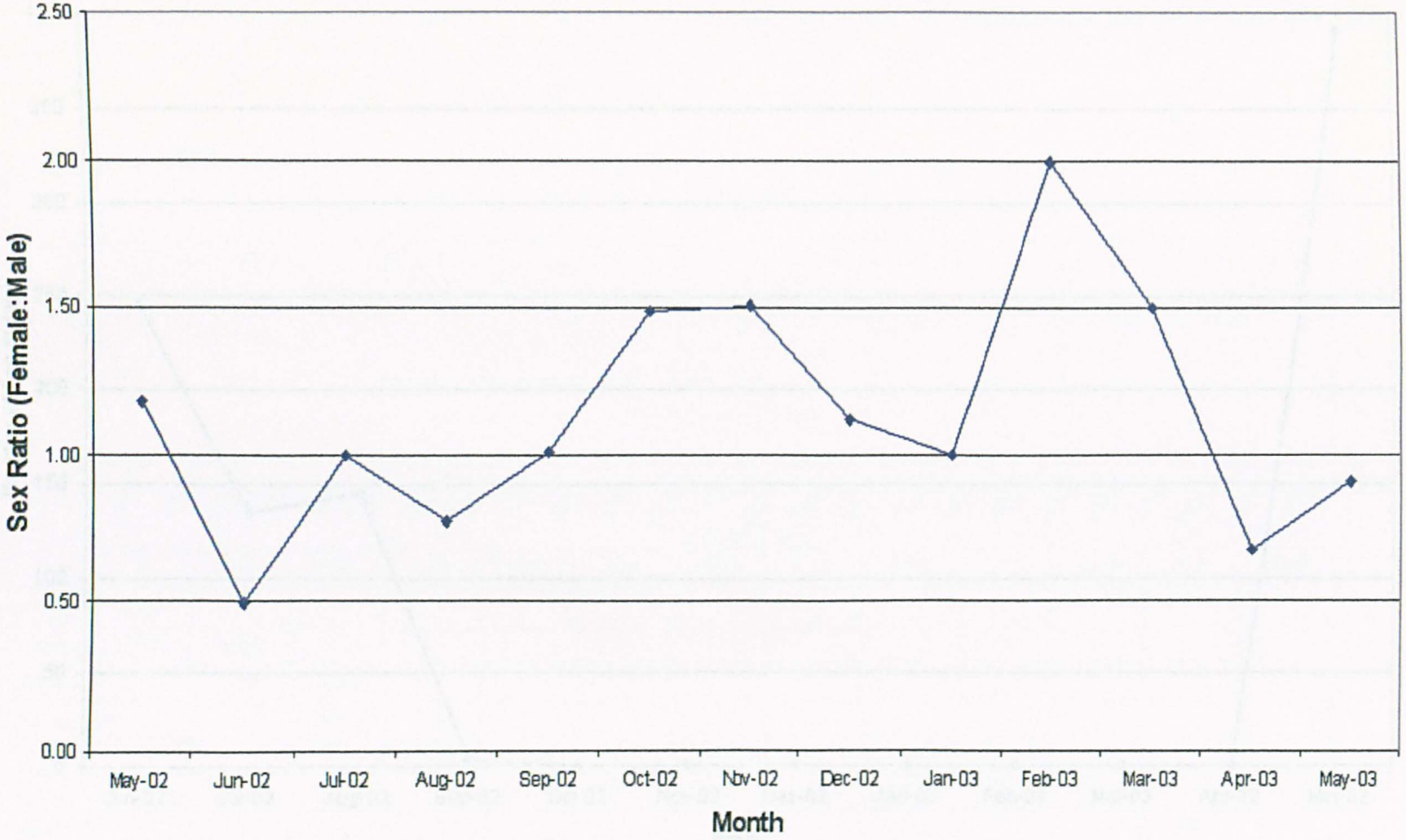


Figure 3.14. Sex ratio of *A. foliaceus* in site 2 from May 2002 to May 2003. 1= Equal, <1=Male Biased, >1=Female Biased.

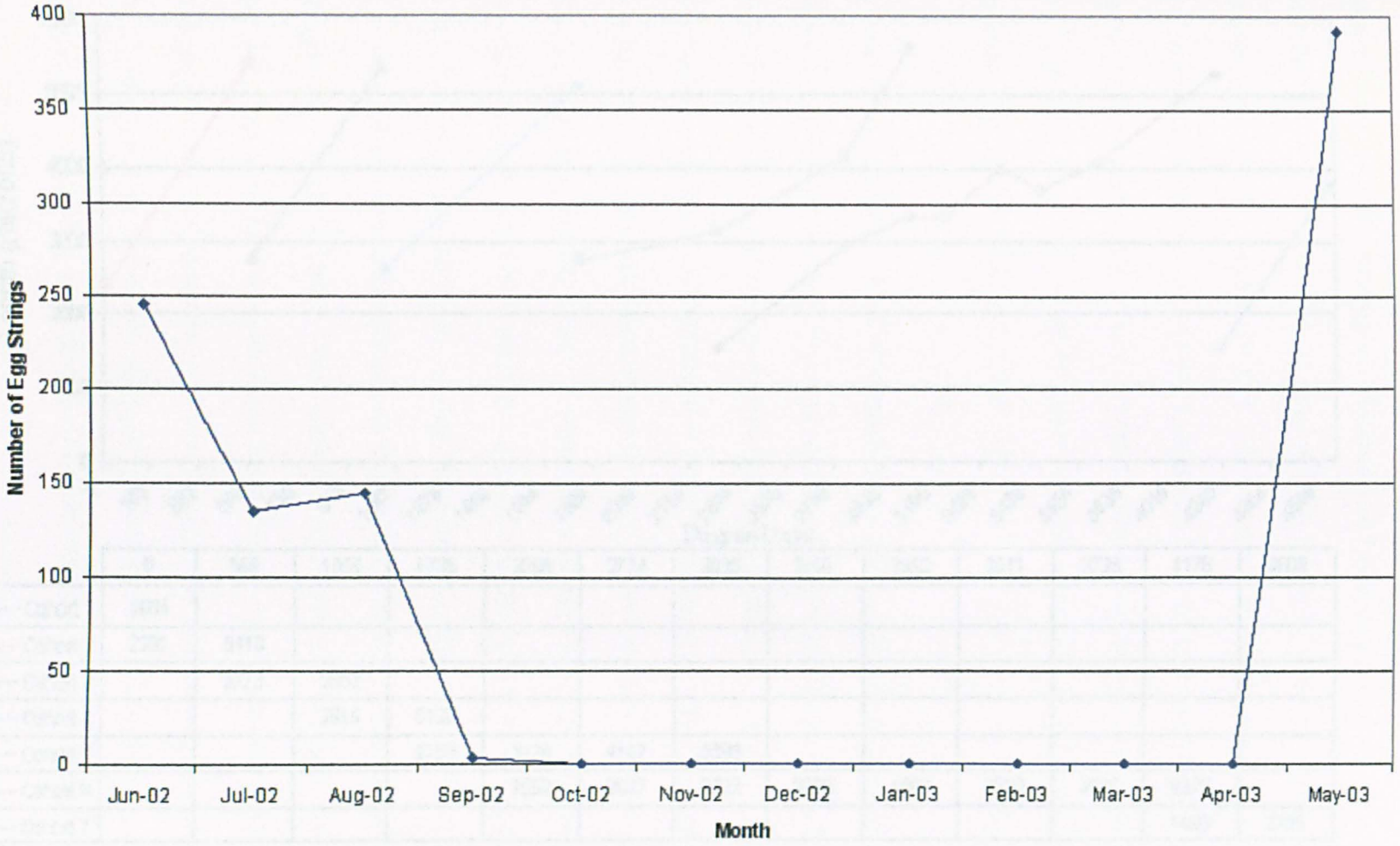
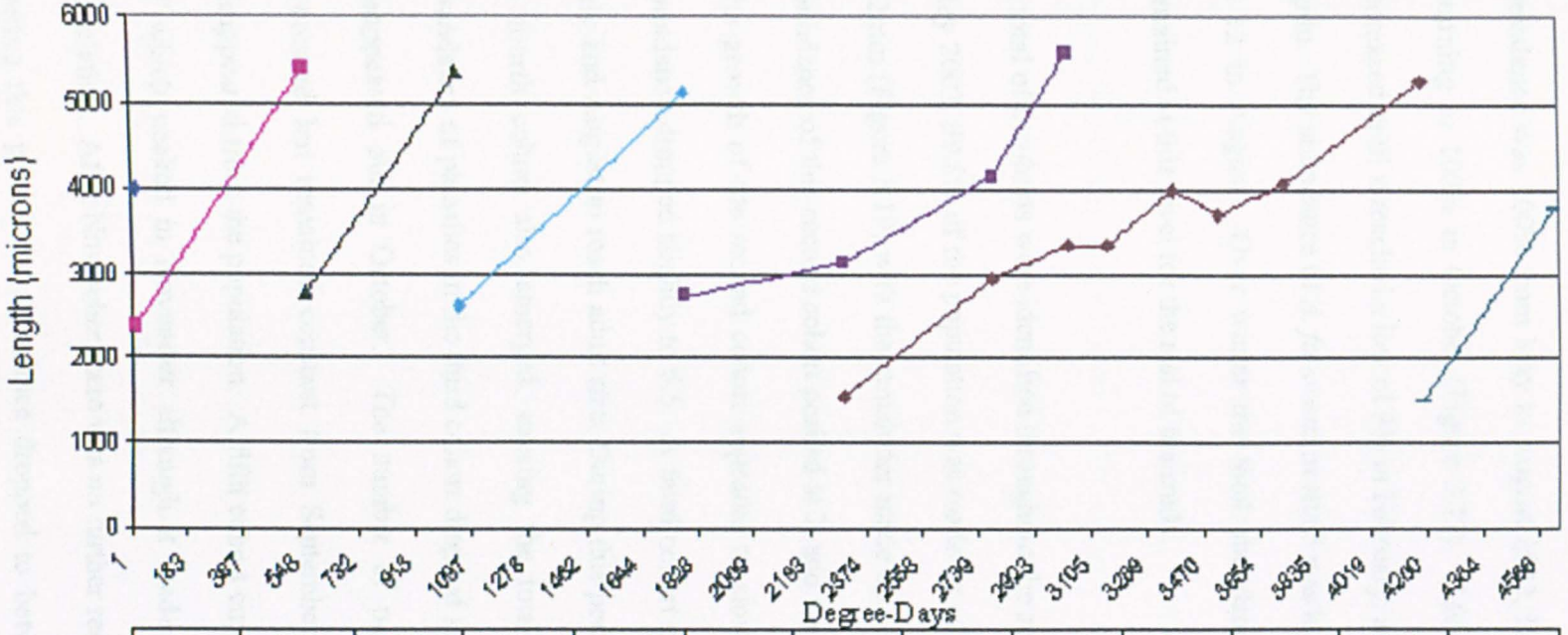


Figure 3.15. Number of *A. foliaceus* egg strings laid in study site 2 from May 2002 to May 2003.



	0	568	1055	1785	2298	2774	3035	3159	3352	3511	3736	4176	4608
Cohort 1	4004												
Cohort 2	2381	5418											
Cohort 3		2773	5352										
Cohort 4			2615	5123									
Cohort 5				2753	3126	4147	5593						
Cohort 6					1552	2927	3322	3329	4000	3700	4060	5275	
Cohort 7												1493	3783

Figure 3.16. The size (microns) and growth of each cohort of *A. foliaceus* in degree-days in study site 2 from May 2002 to May 2003

3.3.2.3 The Population Ecology of *A. foliaceus* in Study Site 3

Prevalence was 100% from May to August 2002, falling to 80% in September before returning to 100% in October (Figure 3.17). After October the prevalence steadily decreased until it reached a low of 8% in February, after which it slowly began to climb again. The abundance of *A. foliaceus* in site 3 was low from May to November peaking at 12 in August. Over winter the total abundance dropped to less than one and remained at this level for the rest of the study.

A total of 6 cohorts were identified throughout the study, with 5 occurring in a year. In May 2002, 99.8% of the population was made up of a cohort with an average length of 3.2mm (Figure 3.19) with the remainder made up of an emerging cohort. By June the abundance of this second cohort peaked at 7, and the first cohort was no longer present. The growth of the second cohort appeared to slow between June and July, and the abundance dropped slightly to 6.5. A third cohort emerged and grew rapidly between July and August to reach adult size. During this period the second cohort disappeared. A fourth cohort also emerged, causing the total abundance to peak at 12. The abundance of parasites in the third cohort dropped substantially by September before it disappeared out in October. The number of parasites in the fourth cohort also decreased but remained constant from September to November, after which they disappeared from the population. A fifth cohort emerged in September, the abundance of which peaked in November although it made up fewer than 50% of the total population. After November there was no further recruitment to the system until April. During this period the abundance dropped to between 0.03 and 0.22, as only low

numbers of cohort 5 over-wintered. Growth in this cohort appeared to slow over the colder months.

Figureure 3.20 shows that egg laying was only detected between May and August 2002, suggesting the third cohort was the last of the year to lay eggs. During June and July when egg laying activity was highest, the sex ratio was male biased (Figureure 3.21), but for the rest of the year, with the exception of March, the ratio was either female biased or almost equal. The strongly male biased population in March represents only two specimens found in this month, both of which were male.

Low numbers of parasites in each cohort made an accurate assessment of growth difficult. However the gradient of the lines suggests that all 6 cohorts develop at a similar rate in terms of degree-days. Growth of cohort 5 appeared linear until the parasite reached adulthood and it took approximately 1160 degree-days to reach adult size after hatching.

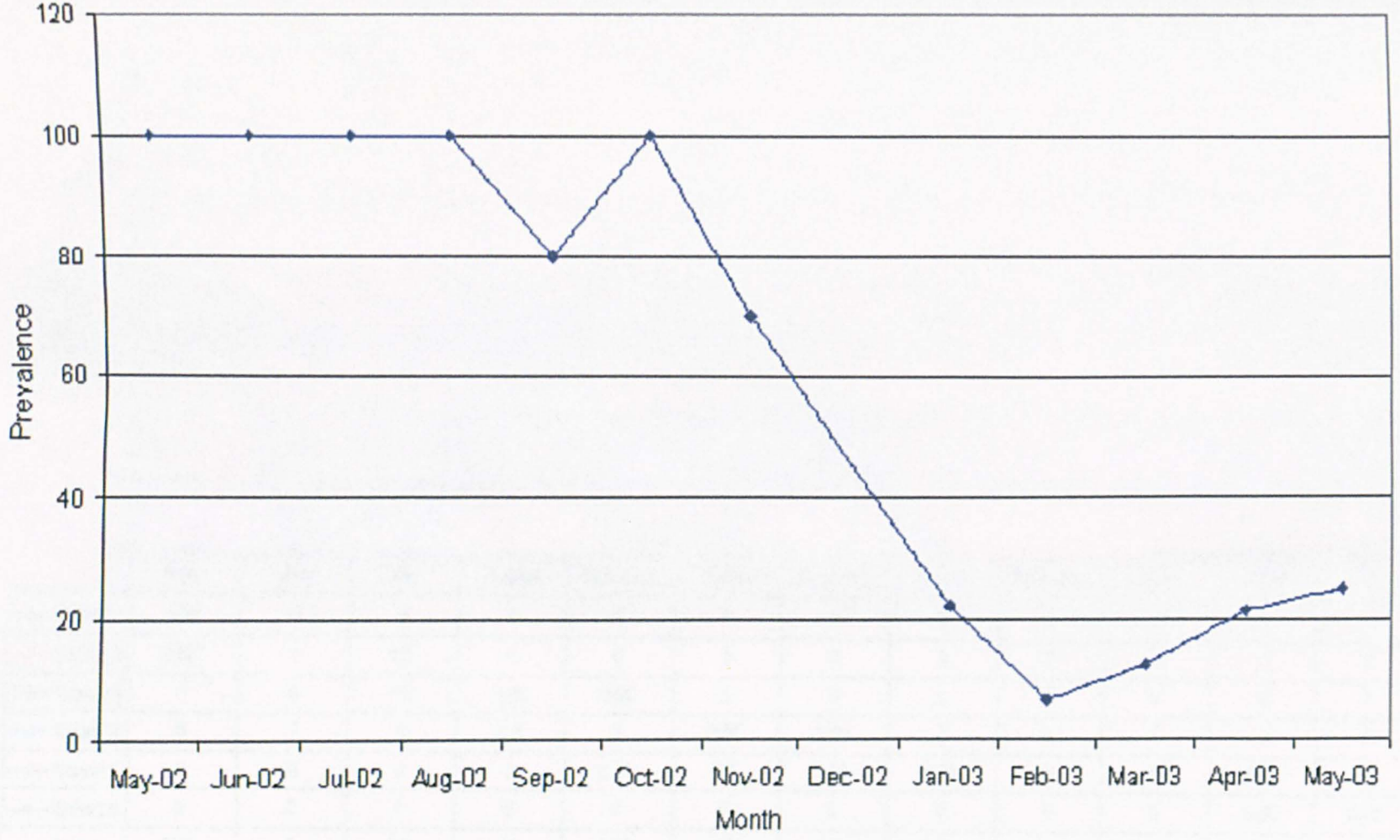
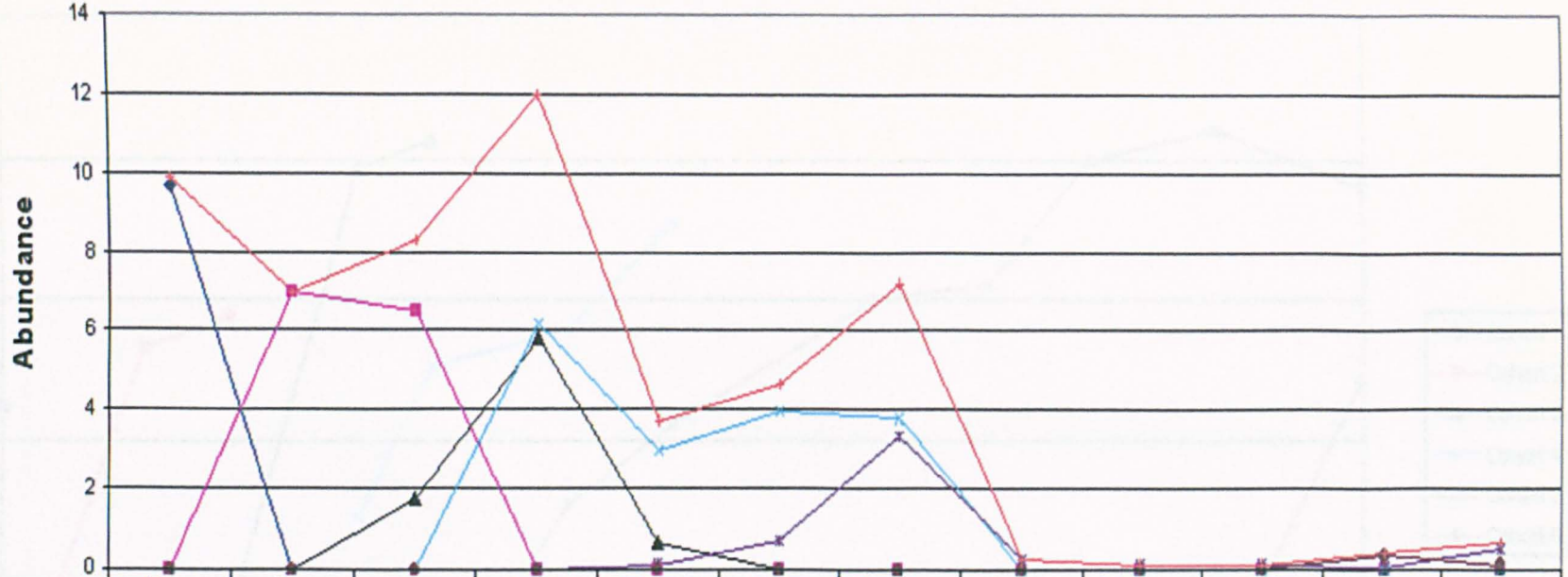


Figure 3.17. Prevalence of *A. foliaceus* in study site 3 from May 2002 to May 2003.



	May	June	July	August	September	October	November	January	February	March	April	May
Cohort 1	9.66	0	0	0	0	0	0	0	0	0	0	0
Cohort 2	0.023	7	6.5	0	0	0	0	0	0	0	0	0
Cohort 3	0	0	1.8	5.8	0.64	0	0	0	0	0	0	0
Cohort 4	0	0	0	6.2	3	3.97	3.82	0	0	0	0	0
Cohort 5	0	0	0	0	0.11	0.7	3.38	0.22	0.1	0.13	0.03	0.5
Cohort 6	0	0	0	0	0	0	0	0	0	0	0.33	0.13
Total	9.89	7	8.3	12	3.75	4.67	7.2	0.22	0.1	0.13	0.36	0.63

Figure 3.18. The total abundance and relative abundance of each cohort of *A. foliaceus* in site 3 from May 2002 to May 2003

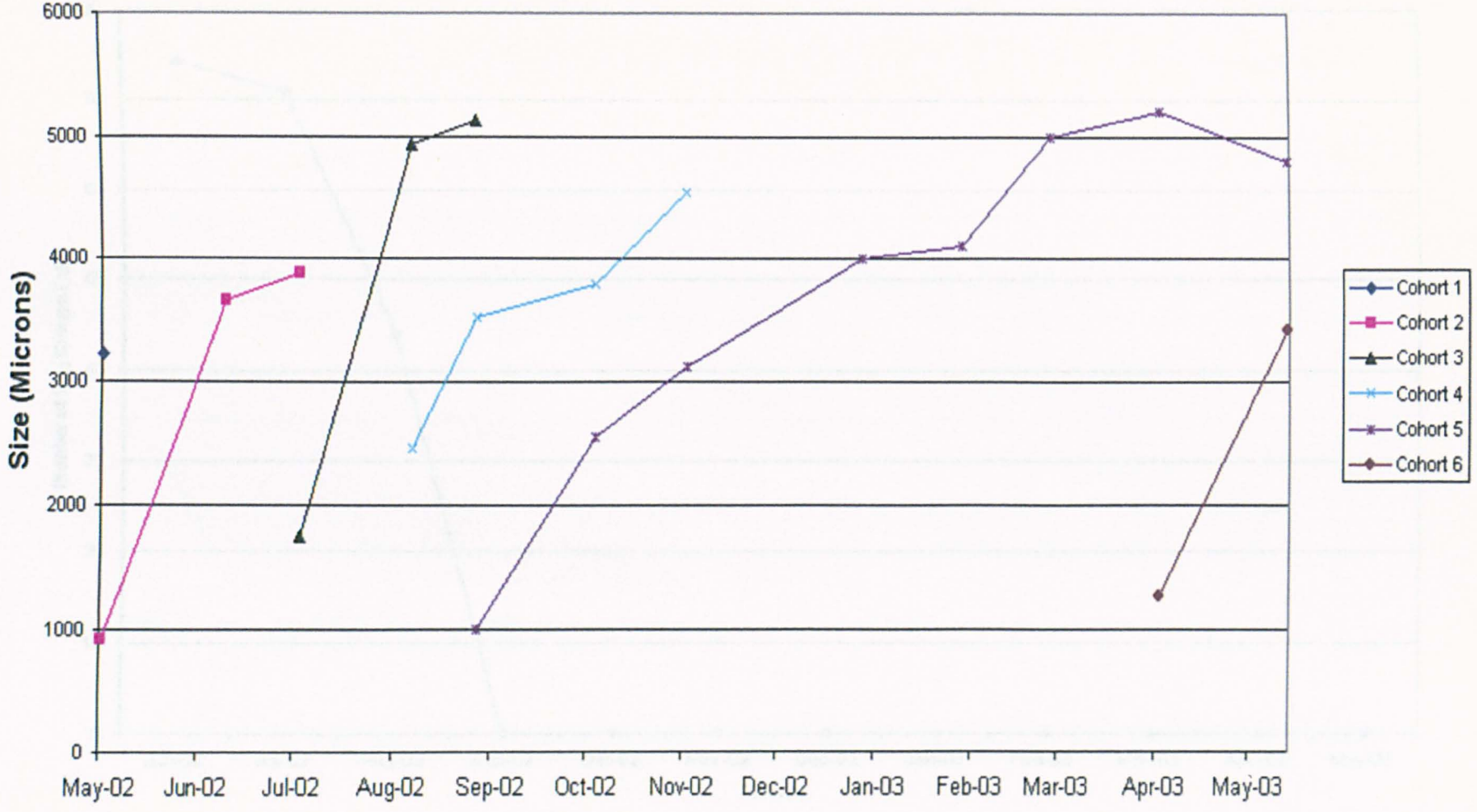


Figure 3.19. The size and growth of each cohort of *A. foliaceus* in study site 3 from May 2002 to May 2004

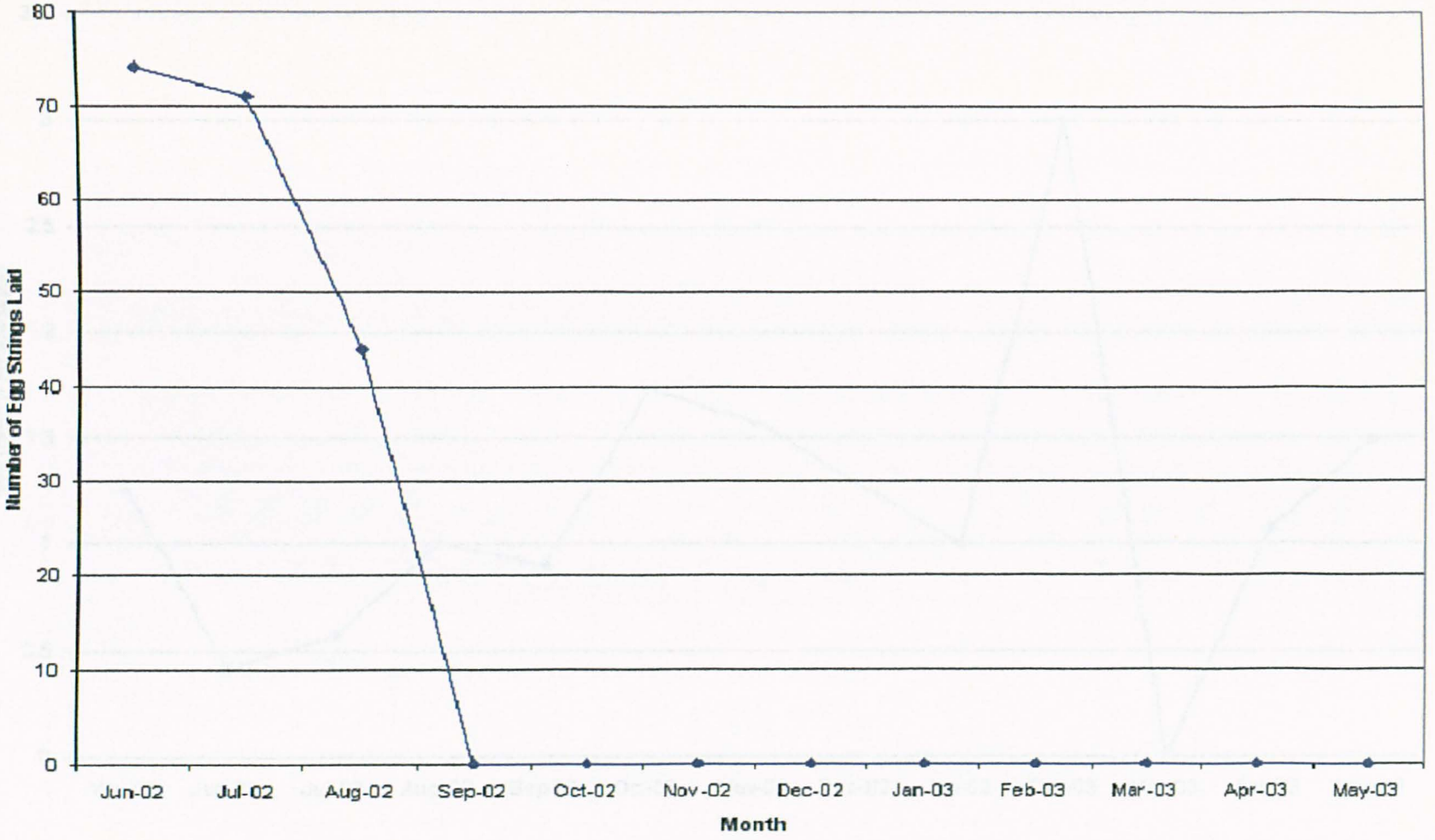


Figure 3.20. Number of *A. foliaceus* egg strings laid in study site 3 from May 2002 to May 2003.

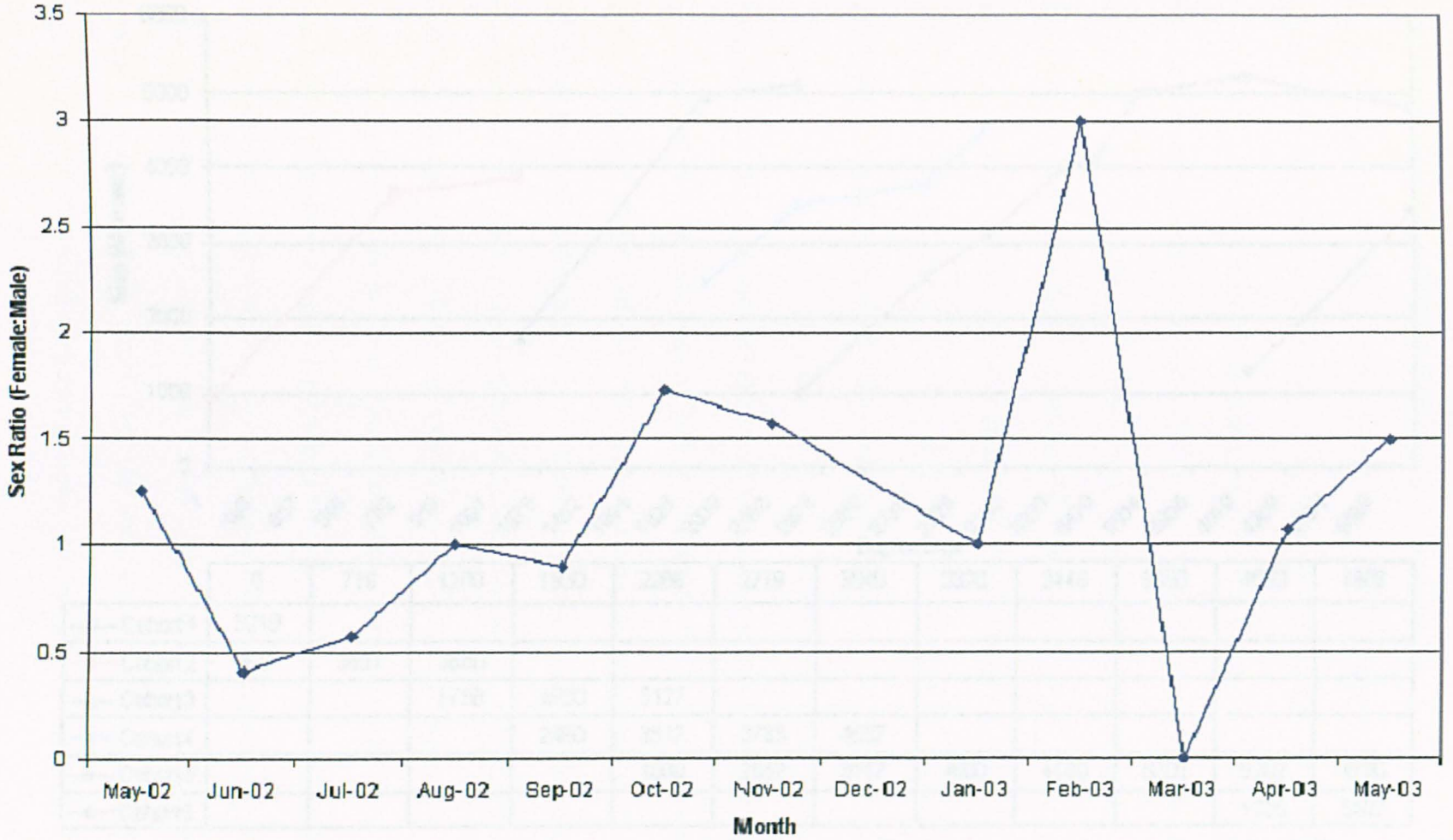
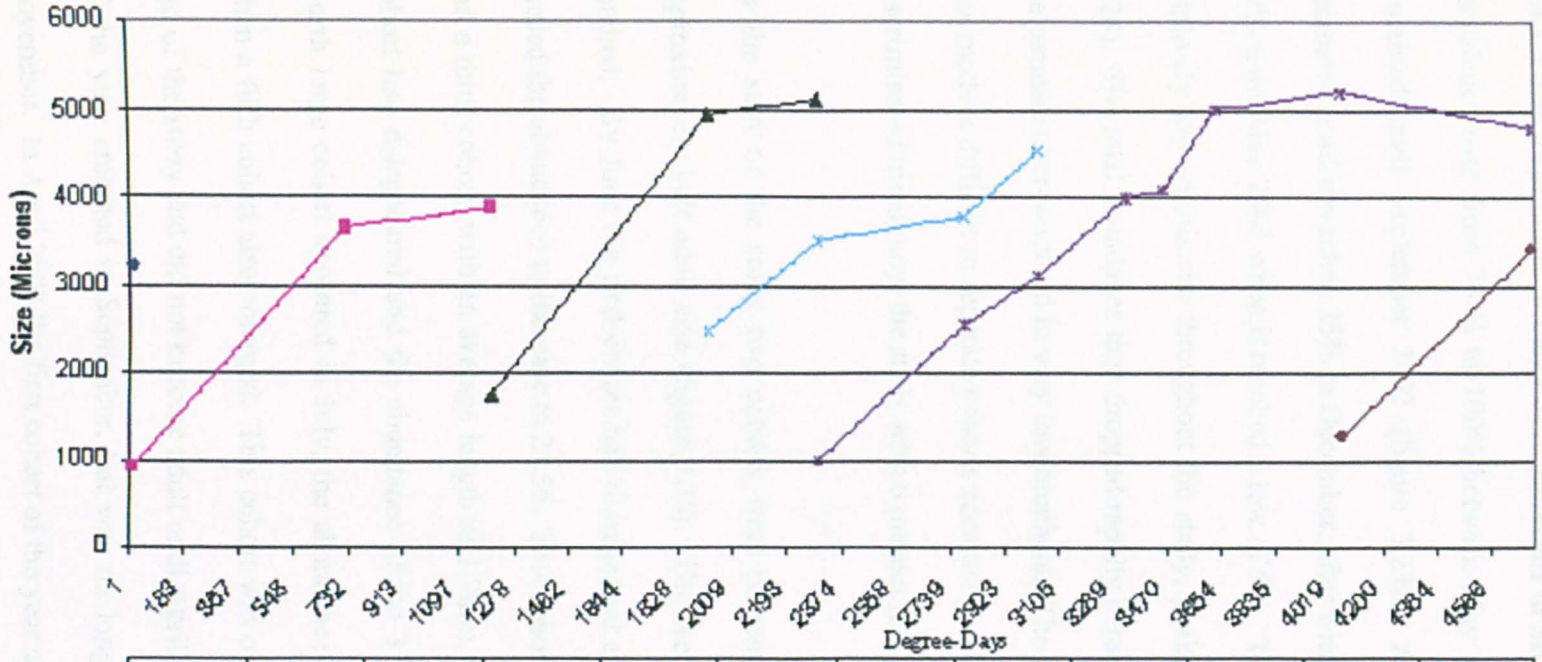


Figure 3.21. Sex ratio of *A. foliaceus* in site 3 from May 2002 to May 2004. 1=Equal, <1=Male Biased, >1=Female Biased.



	0	716	1200	1930	2286	2779	3040	3320	3446	3650	4063	4689
Cohort 1	3219											
Cohort 2	920	3657	3880									
Cohort 3			1738	4930	5127							
Cohort 4				2460	3517	3783	4537					
Cohort 5					1000	2552	3117	4000	4100	5000	5202	4796
Cohort 6											1260	3409

Figure 3.22. The size (microns) and growth of each cohort of *A. foliaceus* in degree-days in study site 3 from May 2002 to May 2003.

3.3.2.4 The Population Ecology of *A. foliaceus* in Study Site 4

Prevalence rose from 70 % to 100% between May and June 2002, at which level it remained until September 2002 (figure 3.23). After September the prevalence decreased until it reached 25% in December, after which it fluctuated between 38% and 49% until May 2003 when it reached a low of 9%. The abundance of the parasite was relatively low in this site throughout the study, peaking at 40.6 in September 02 (fig 3.24). The total abundance then dropped rapidly between September and December, as the parasite over-wintered in very low numbers. The low abundance and small sample size made it difficult to separate cohorts accurately. However, a total of 8 cohorts were discriminated throughout the study with 6 present in a year.

At the start of the study two cohorts were present, the first in low numbers and approximately half adult size (figure 3.25). The second cohort was small and newly hatched. By June the first cohort had disappeared and the recruitment to the second caused the abundance to increase to 21.56. This cohort now averaged 3.8mm in length, and a third cohort, with an average length of 1.7mm, was present. By July the second cohort had disappeared and the abundance of the 3rd cohort had dropped to 1.4. A fourth large cohort appeared in July, the abundance of which increased into August when a fifth cohort also emerged. This cohort was observed at each site visit until the end of the study and did not become adult until April 2003. The sixth and final cohort of the year emerged in September, but was no longer present in the population by November. In April 2003 the first cohort of the year appeared, followed by a second in May. Despite this recruitment the parasites abundance remains low in spring 2003.

The gradient of the lines shown by cohorts 2,3,4 & 7 suggests growth in terms of degree-days occurred at a similar rate (figure 3.26). Cohort 5 followed a similar rate of growth until it reached 4mm in November. After this time, growth appeared to slow corresponding to the water temperature decrease to below 9°C, and did not increase again until the temperature rose above this level after March 2003. By studying the growth of cohort 5 in real time one can see that it followed a seasonal growth pattern slowing over the winter months (figure 3.25). Growth in cohorts 1, 6 and 8 could not be assessed, as only one data point was obtained for each.

By following the growth of cohort 2 shown in figure 3.25 it is clear that it reached adult size prior to the June visit. This corresponds to the egg laying observed between the May and June 2002 site visits (Figure 3.27). Egg laying was recorded until September after which it stopped. This coincided with cohorts 1 to 4 becoming adult and laying eggs. Egg laying was first detected between March and April 2003 (Figure 3.27), coinciding with the fifth cohort reaching adulthood.

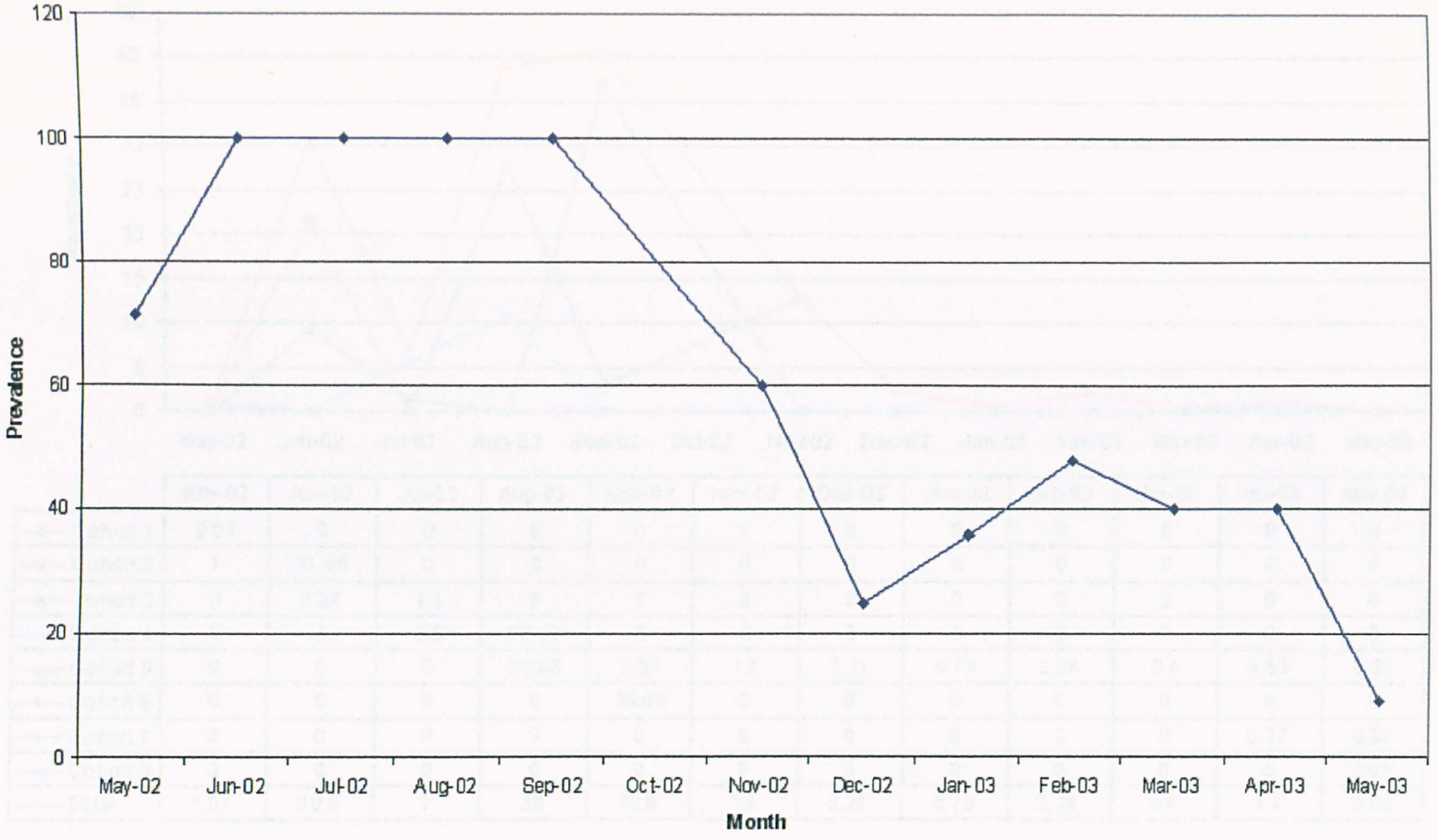
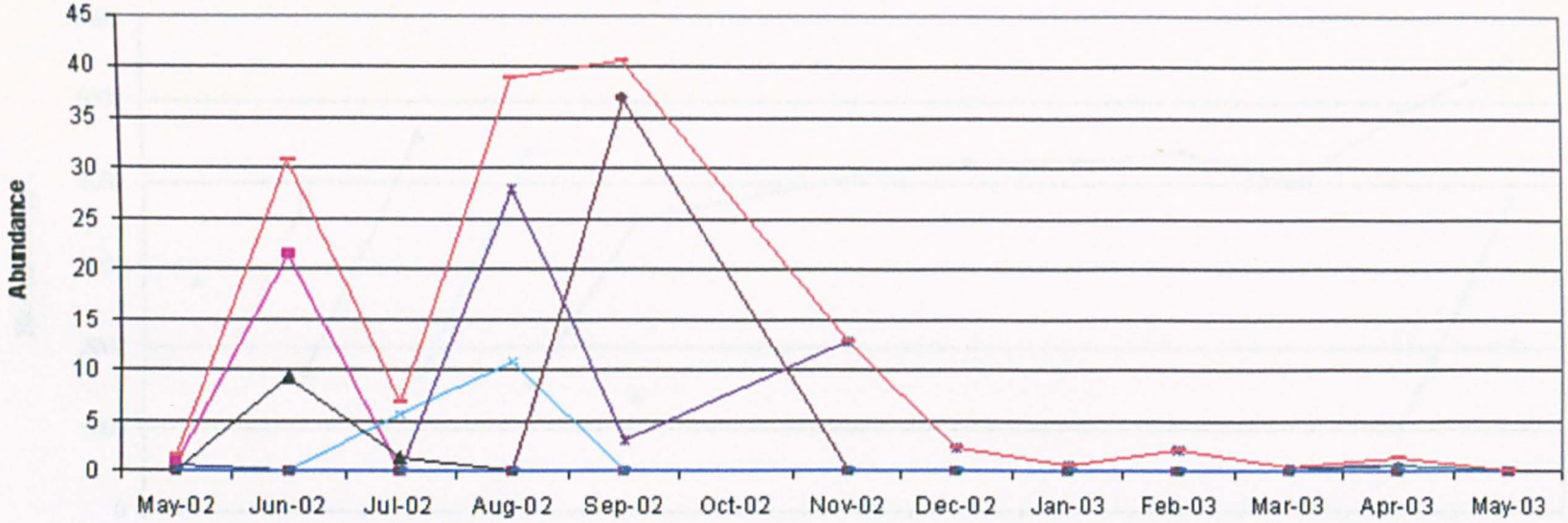
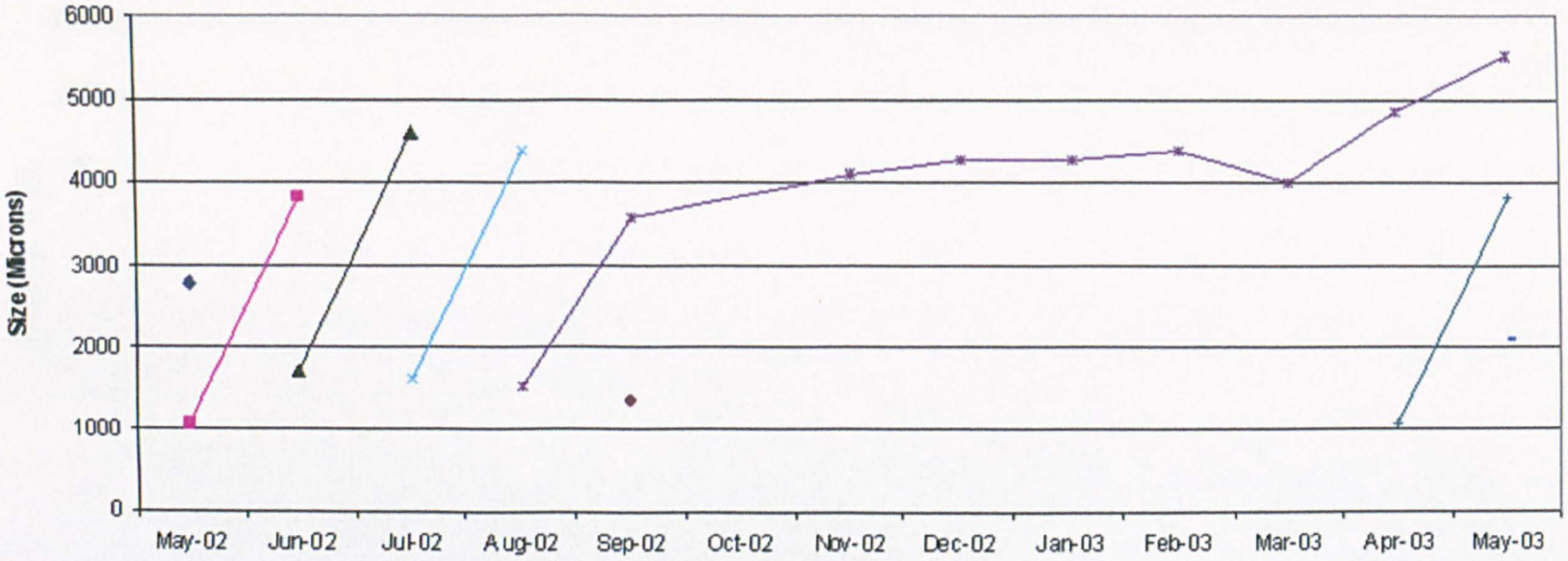


Figure 3.23. Prevalence of *A. foliaceus* in study site 4 from May 2002 to May 2003.



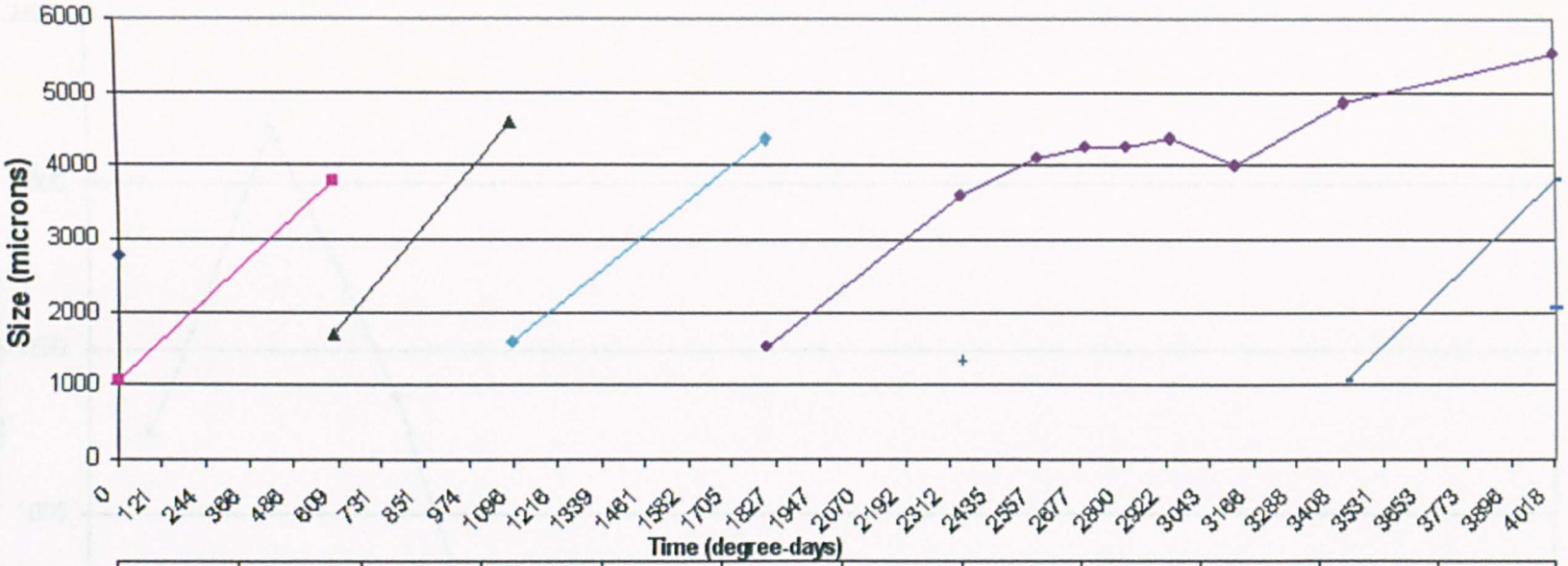
	May-02	Jun-02	Jul-02	Aug-02	Sep-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Cohort 1	0.57	0	0	0	0	0	0	0	0	0	0	0
Cohort 2	1	21.56	0	0	0	0	0	0	0	0	0	0
Cohort 3	0	9.34	1.4	0	0	0	0	0	0	0	0	0
Cohort 4	0	0	5.6	10.92	0	0	0	0	0	0	0	0
Cohort 5	0	0	0	28.08	3.05	13	2.31	0.79	2.24	0.4	0.63	0.01
Cohort 6	0	0	0	0	36.95	0	0	0	0	0	0	0
Cohort 7	0	0	0	0	0	0	0	0	0	0	0.77	0.07
Cohort 8	0	0	0	0	0	0	0	0	0	0	0	0.01
Total	1.57	30.8	7	39	40.6	13	2.31	0.79	2.24	0.4	1.4	0.09

Figure 3.24. The total abundance and relative abundance of each cohort of *A. foliaceus* in site 4 from May 2002 to May 2003.



	May-02	Jun-02	Jul-02	Aug-02	Sep-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Cohort 1	2766											
Cohort 2	1073	3810										
Cohort 3		1696	4600									
Cohort 4			1603	4382								
Cohort 5				1520	3582	4111	4279	4270	4376	4000	4855	5537
Cohort 6					1342							
Cohort 7											1063	3830
Cohort 8												2078

Figure 3.25. The size (microns) and growth of each cohort of *A. foliaceus* in study site 4 from May 2002 to May 2003.



	0	595	1088	1803	2346	2560	2699	2810	2935	3118	3425	4021
Cohort 1	2766											
Cohort 2	1073	3810										
Cohort 3		1696	4600									
Cohort 4			1603	4382								
Cohort 5				1520	3582	4111	4279	4270	4376	4000	4855	5537
Cohort 6					1342							
Cohort 7											1063	3830
Cohort 8												2078

Figure 3.26. The size (microns) and growth of each cohort of *A. foliaceus* in degree-days in study site 4 from May 2002 to May 2004

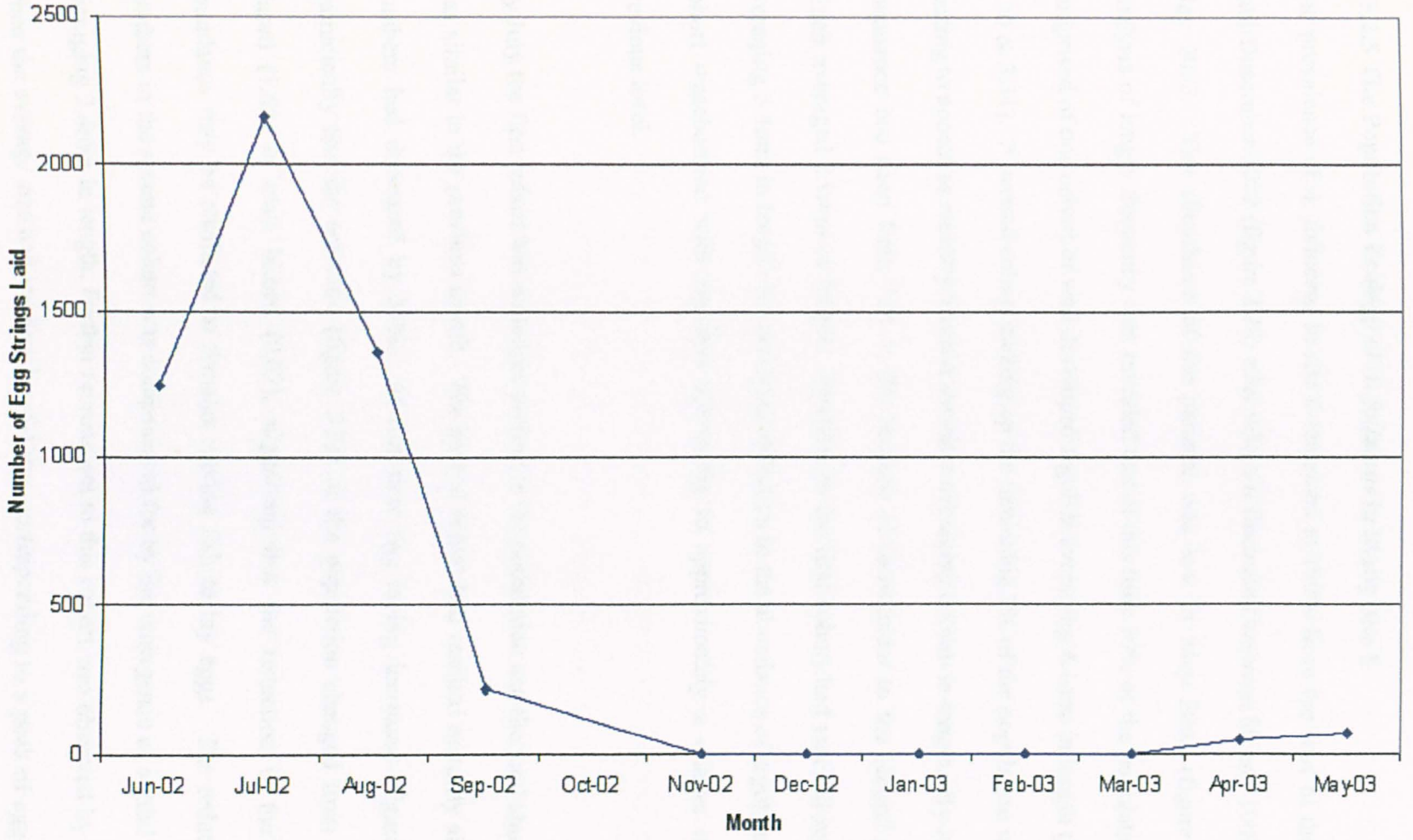


Figure 3.27. Number of *A. foliaceus* egg strings laid in study site 4 from May 2002 to May 2003.

3.3.2.5 The Population Ecology of *A. foliaceus* in Study Site 5

The prevalence of *A. foliaceus* in site 5 remained at 100% from the start of the study until December 2002 (figure 3.28) after which it fluctuated between 80 and 100% until May 2003. The abundance of the parasite was low in May 2002 (figure 3.29). Analysis of length frequency data revealed that at this time 99% of the population was comprised of one cohort of well developed argulids averaging 4.1mm in length (figures 3.30 & 3.31). A second cohort making up the remaining 1% of the population was just starting to appear as recently hatched parasites averaging 0.8mm in length. By June the abundance had risen from 7.91 to 21, because of recruitment to the second cohort, which averaged 2.9mm in length. Parasites in the first cohort had reached maturity, averaging 5.4mm in length. A substantial reduction in the abundance of argulids in this cohort wasobserved with numbers decreasing to approximately a quarter of their previous level.

By July the first cohort was no longer present in the population and the total abundance was similar to the previous month. The second cohort had reached maturity although numbers had decreased by 31%. At this time egg laying increased (figure 3.32) dramatically and the sex-ratio (figure 3.33) of the population changed from female biased (1.65) to male biased (0.62), suggesting that the reduction in the cohort abundance may be attributed to females leaving fish to lay eggs. The reduction in numbers in the second cohort was compensated for by the emergence of a third cohort, averaging 2.4mm in length. Further recruitment to this cohort was observed by August when the average size had increased to 5.1mm, corresponding to a peak of egg laying.

The second cohort is no longer present in the population, but a fourth cohort had emerged, with an average length of 3.1mm, and making up 74% of the population and causing more than a 3-fold increase in the total abundance of the parasite to 63.5. A male biased sex ratio is still present at this time.

Abundance continued to increase into September with the emergence of a fifth cohort that made up 51% of the total population. Cohorts 3 and 4 were both still present although their numbers had reduced. At this time, although still male biased, the sex ratio was closer to equality. Egg laying stopped between October and February 2003. Abundance peaked in October at 90.47 (two months after temperature peaked and at least one month later than the other sites) with the appearance of a sixth and final cohort of the year making up 15% of the population. Only very low numbers of the third cohort remained, however the abundance of the fourth and fifth cohorts, neither of which had reached maturity, remained constant. From October to February the sex ratio remained equal or female biased corresponding to a cessation in egg laying.

By November the total abundance dropped sharply to 53.08, corresponding to the reduction in prevalence observed in figure 3.28. This reduction can be attributed to the disappearance of the fourth cohort from the population, as the abundance of the fifth and sixth cohorts remained constant. Abundance reached a low in December, due to a reduction in the numbers of the fifth cohort, corresponding to this cohort reaching adult size. The sixth cohort averaged a length of 3.5mm by December, and remained constant until March, as did the total abundance of the *A. foliaceus* population.

Growth in cohorts 5 and 6 clearly show a seasonal pattern, slowing over winter until April when growth rate increased with temperature (figure 3.7). In April the first cohort of 2003 began to appear (seventh detected by the study), however, the total abundance dropped. This was due to the abundance of parasites in the sixth cohort dropping from 14.77 to 1. During March and April the population became male biased, corresponding to the start of egg laying by cohort 6 in April. By May 2003 the total abundance of *A. foliaceus* had increased from 5 to 15.29. There had been no further recruitment to the seventh cohort, and although an eighth cohort comprising 20% of the population had appeared, the majority of the increase can be explained by female parasites from the sixth cohort returning to the population after egg laying. The sixth cohort appeared to have reduced in length, perhaps due to the larger parasites in the cohort dying thus reducing the average length. At this time the sex ratio was slightly female biased and the fifth cohort had disappeared from the population.

Figures 3.30 & 3.31 show that in all cohorts, except 3 and 5, the populations did not appear to reach a size bigger than 5.3mm. In cohort 3 the population began to reduce in abundance after the parasite reached an average size of 5.1mm in August, and only small numbers reached an average size of 6.8mm in October. Both the fifth and sixth cohorts followed a clear seasonal growth curve, with growth slowing during the winter months. In terms of degree-days growth appeared to be linear until the temperature fell below 6°C between December and February. During this time, growth in both of these cohorts nearly stopped and no egg laying occurred, even though the parasites in cohort 5 have reached adult size. After February 2003 the temperature increased, as did the

growth rate. Only low numbers remained in cohort 5; these reached an average length of 6.4mm in April, after which the cohort disappeared. The abundance of parasites in cohort 6 dropped to one in April 2003, coinciding with the start of egg laying and an average water temperature of 9.5C.

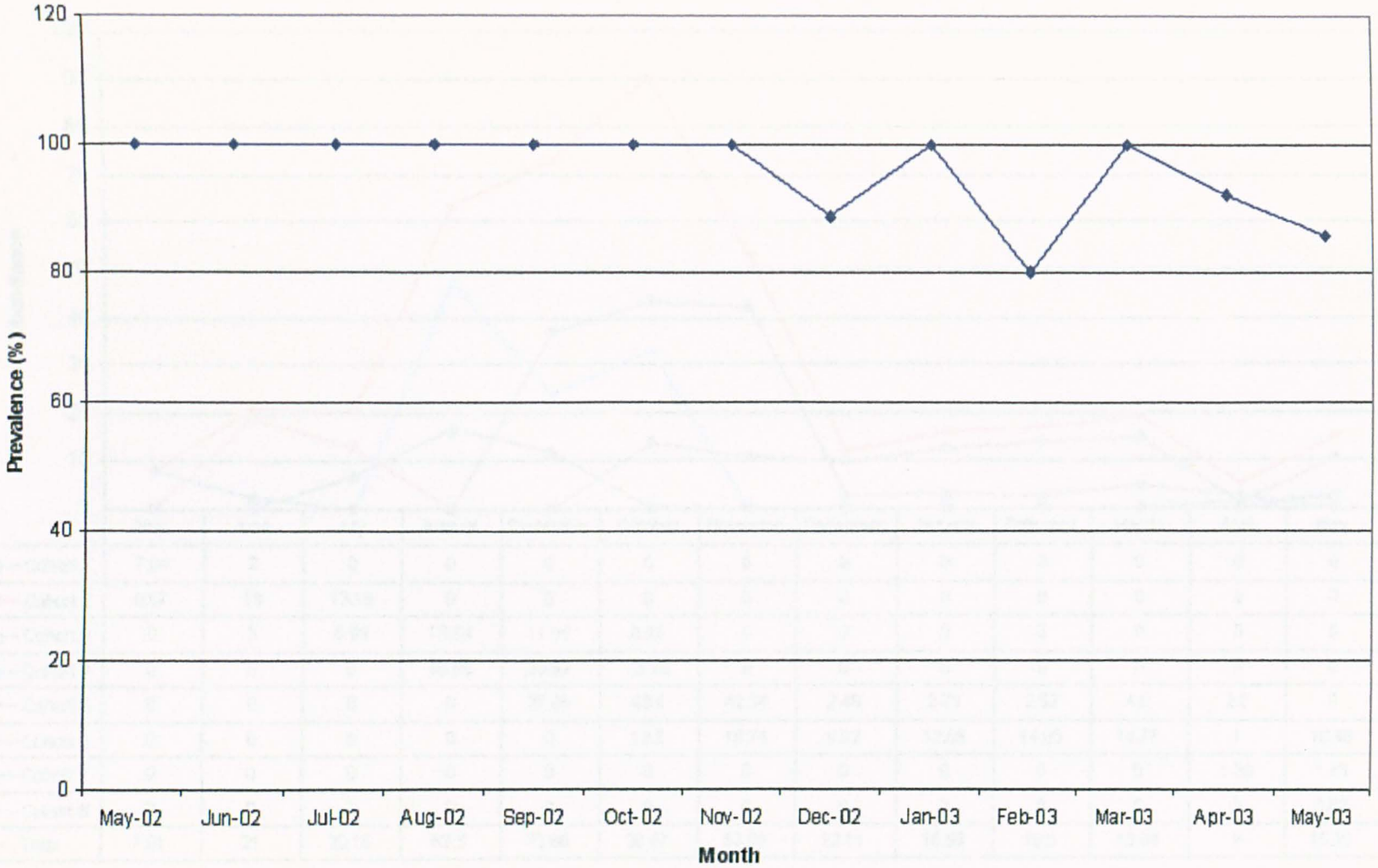
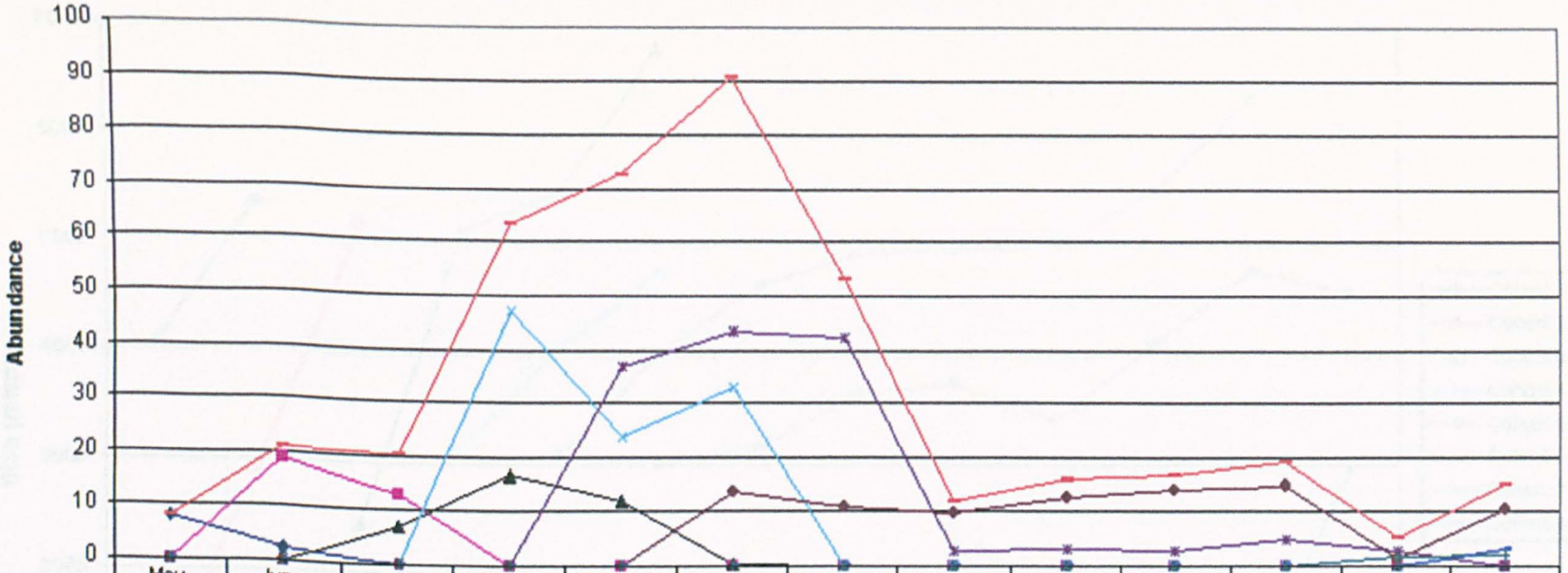


Figure 3.28. Prevalence of *A. foliaceus* in study site 5 from May 2002 to May 2003.



	May	June	July	August	September	October	November	December	January	February	March	April	May
Cohort 1	7.84	2	0	0	0	0	0	0	0	0	0	0	0
Cohort 2	0.07	19	13.16	0	0	0	0	0	0	0	0	0	0
Cohort 3	0	0	6.99	16.54	11.98	0.45	0	0	0	0	0	0	0
Cohort 4	0	0	0	46.96	23.82	33.15	0	0	0	0	0	0	0
Cohort 5	0	0	0	0	37.06	43.4	42.34	2.49	2.73	2.52	4.6	2.6	0
Cohort 6	0	0	0	0	0	13.6	10.74	9.62	12.86	14.08	14.77	1	10.48
Cohort 7	0	0	0	0	0	0	0	0	0	0	0	1.39	1.79
Cohort 8	0	0	0	0	0	0	0	0	0	0	0	0	3.02
Total	7.91	21	20.15	63.5	72.86	90.47	53.08	12.11	15.89	16.6	19.38	5	15.29

Figure 3.29. The total abundance and relative abundance of each cohort of *A. foliaceus* in site 5 from May 2002 to May 2003

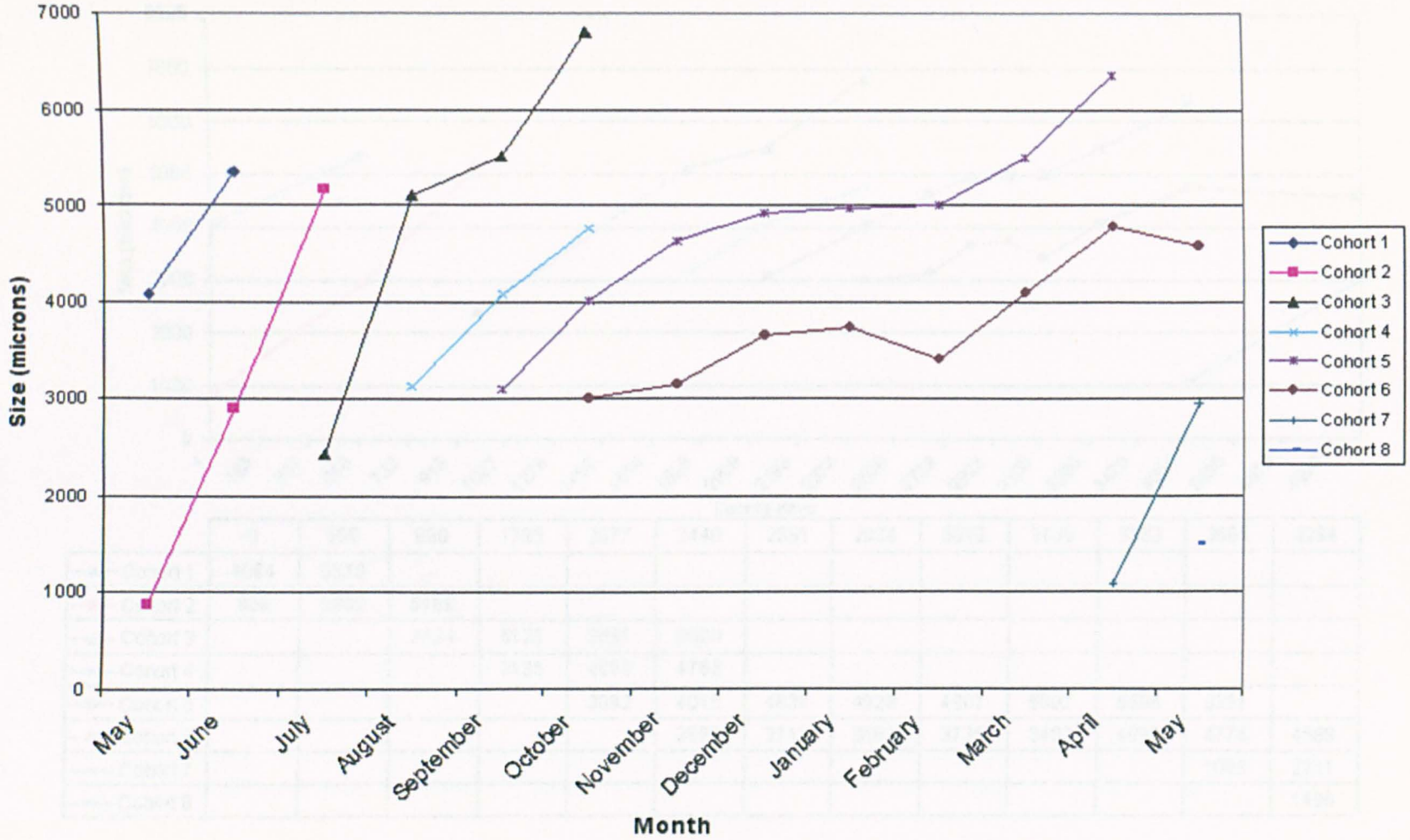
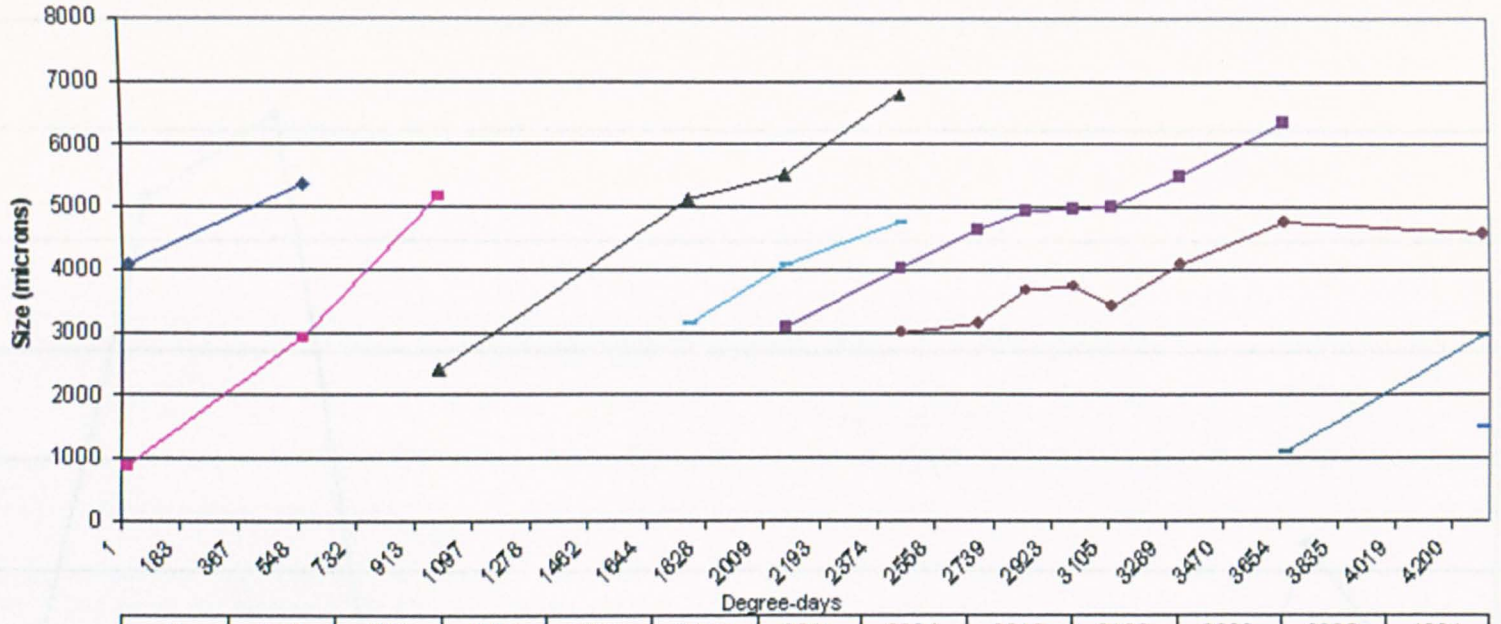


Figure 3.30. The size and growth of each cohort of *A. foliaceus* in study site 5 from May 2002 to May 2003.



	0	568	990	1795	2077	2440	2691	2834	3012	3130	3333	3665	4294
Cohort 1	4084	5370											
Cohort 2	869	2908	5189										
Cohort 3			2424	5125	5531	6800							
Cohort 4				3135	4085	4762							
Cohort 5					3092	4015	4638	4929	4967	5002	5506	6351	
Cohort 6						2999	3142	3662	3735	3403	4097	4774	4589
Cohort 7												1088	2941
Cohort 8													1496

Figure 3.31. The size (microns) and growth of each cohort of *A. foliaceus* in degree-days in study site 5 from May 2002 to May 2003.

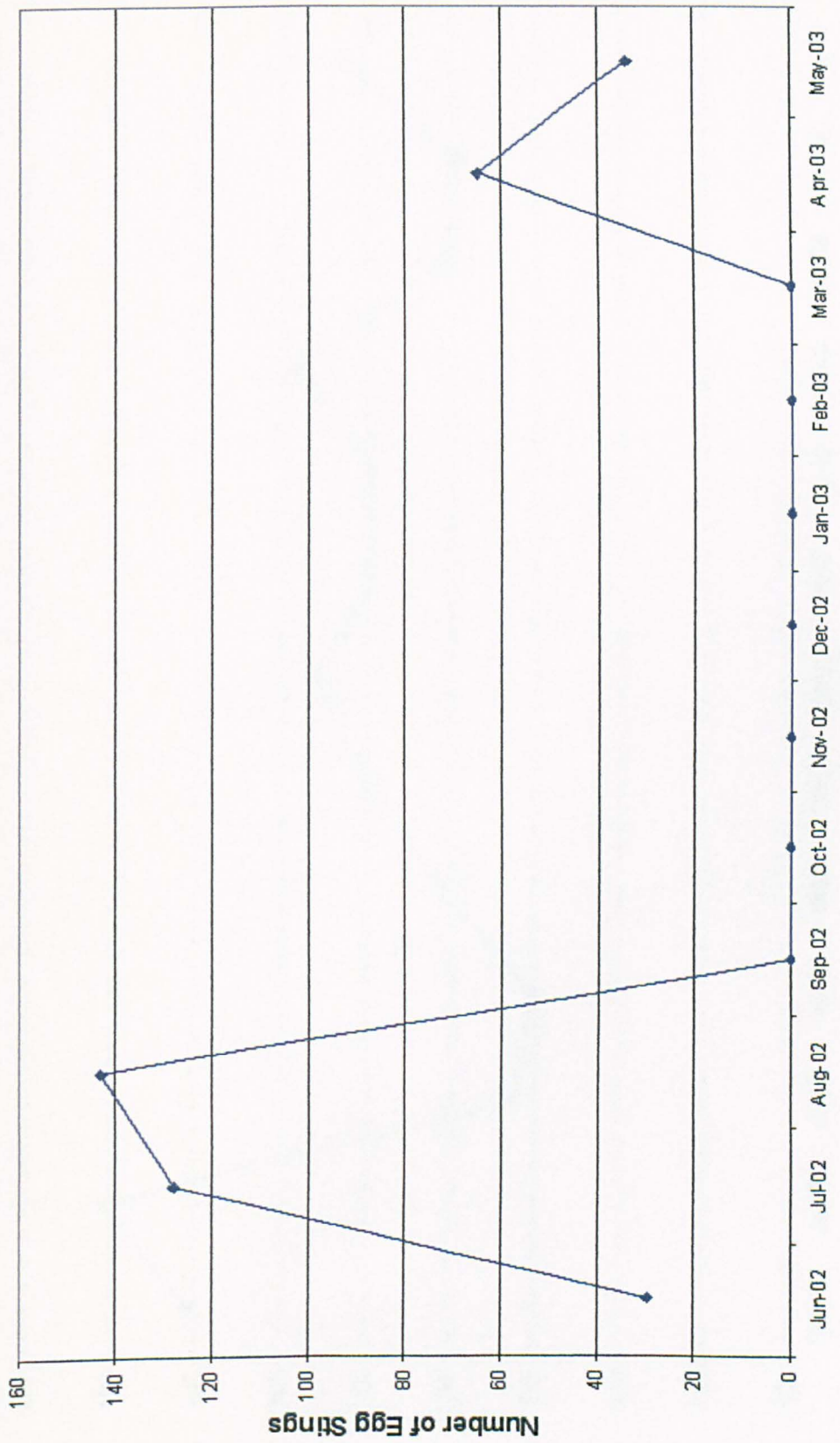


Figure 3.32. Number of *A. foliaceus* egg strings laid in study site 5 from May 2002 to May 2003.

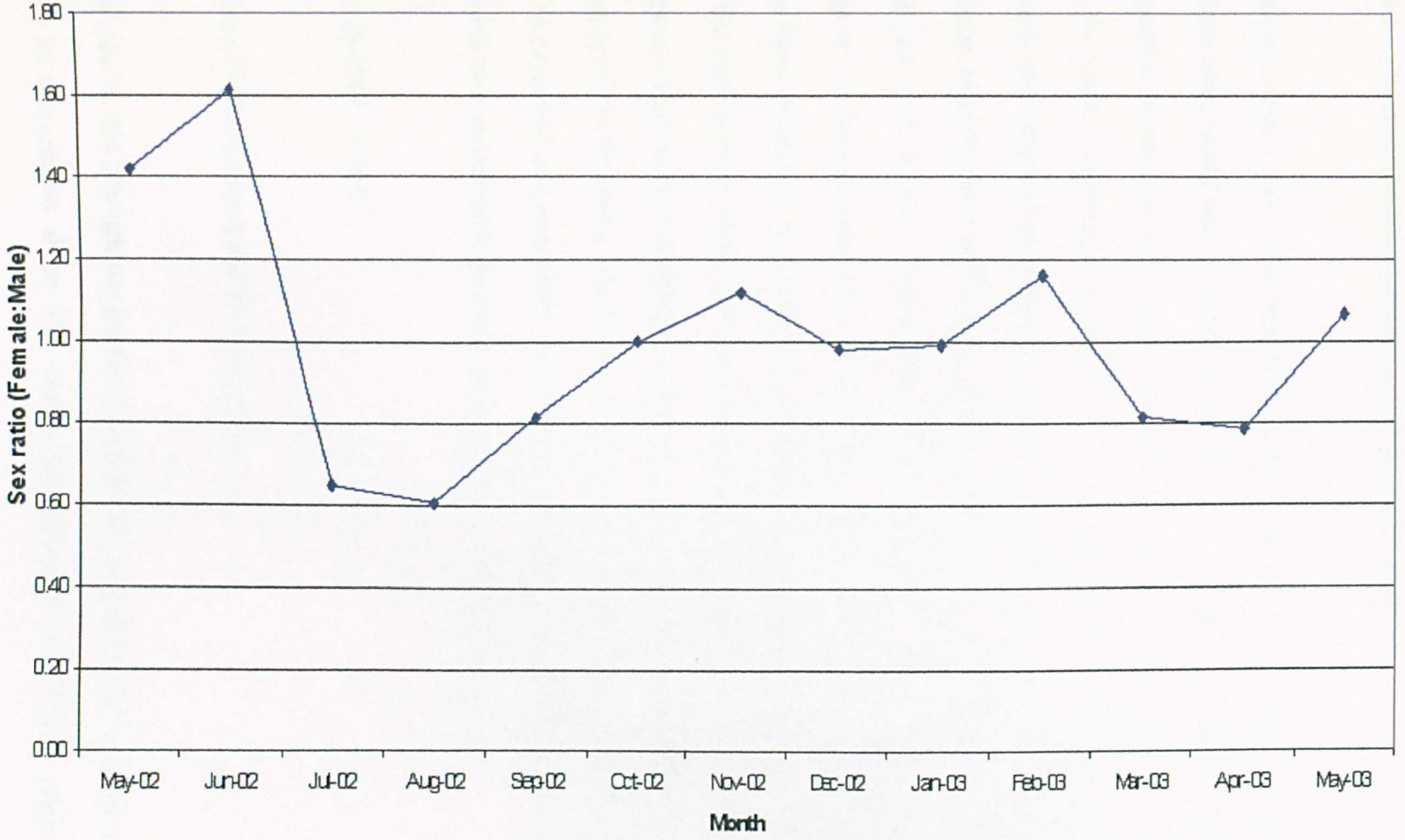


Figure 3.33. Sex ratio of *A. foliaceus* in site 5 from May 2002 to May 2004. 1=Equal, <1=Male Biased, >1=Female Biased.

3.3.3 The Relationship between Growth and Temperature

Data from sites 1 and 3 were removed from this analysis as scatter plots showed small sample sizes caused large variations in the data sets. Normality tests showed the data on growth per day was not normally distributed (Kolmorgorov-Smirnov = 0.164, d.f. = 45, P= 0.004). Scatter plots showed the data to be heteroskedastic i.e. variability in the growth rates increased as temperature increased. Square root transformation produced a linear, homoskedastic relationship and normalised the data (Kolmorgorov-Smirnov = 0.09, d.f. = 45, P= 0.2). Levene's test showed the data between the three remaining sites to be homogeneous (F=2.35, P=0.108). ANCOVA showed there to be no significant differences in the slope (F=0.222, P=0.802) or intercept (F=0.089, P=0.915) of the lines between sites. Data was pooled and subjected to linear regression. This showed a significant relationship between temperature and the root transformed growth rates (t=10.00, P=0.000). The R^2 value showed 69.9% of the variability in the dataset to be explained by temperature. The analysis showed the relationship between daily growth rates and temperature was described by the following equation:

$$G = (0.434T + 0.361)^2$$

Where: G=Daily growth and T= Temperature.

This shows that although the graphs of growth vs. degree-days for each site suggest that, at temperatures above 9°C, growth rate increases in a linear fashion with

temperature (as the gradient of the lines for each cohort is similar), the relationship is in fact exponential. The time taken from hatching to adult can be estimated by the equation:

$$DT = 3900 / G$$

Where: DT = Development time and G =Daily growth (as calculated above).

3.3.4 Correlations Between Risk Factors

Site data was pooled and scatter plots of parasite abundance against temperature, water clarity and turnover revealed a non-linear relationship. Abundance data and stock turnover were natural log (ln) transformed, which produced a linear relationship. Scatter plots of ln abundance of the parasite, against temperature, water clarity and ln stock turnover revealed some level of correlation between the parasites abundance and the factors of interest. ANCOVA showed a significant association between the ln abundance of the parasite and water clarity, temperature and site (table 3.3). Also included in the model was the natural log of the parasites abundance in the previous month, as this was likely to strongly affect the abundance in the subsequent month. A significant relationship was also observed between these two variables. In total, the four variables explained 74.1% of the variation observed in the data set (R^2=0.741). No statistically significant relationship was observed between rate of stock turnover and the ln abundance of the parasite. Levene's test showed the data to be homogeneous

between sites (F=0.977, df1=4, df2=55, P=0.428) and no statistically significant interaction terms were found between any of the variables, showing that the assumption of homogeneity in slope was met. The scatter plots showed a negative correlation between the parasites abundance and water clarity, and a positive relationship between abundance in the previous month and temperature.

Table 3.3. ANCOVA showing variables associated with the natural log abundance of *A. foliaceus* in the 5 study sites from May 2002 to May 2003.

Dependent Variable: Ln Abundance

Source	Type III Sum of Squares	d.f.	Mean Square	F	P-Value
Corrected Model	116.75	7	16.68	21.21	0.00
Intercept	2.22	1	2.22	2.82	0.10
Water clarity	4.06	1	4.06	5.17	0.03
Temperature	18.18	1	18.18	23.12	0.00
Ln Abundance previous month	19.05	1	19.05	24.23	0.00
Site	8.40	4	2.10	2.67	0.04
Error	40.89	52	0.79		
Total	438.44	60			
Corrected Total	157.64	59			

R Squared = .741 (Adjusted R Squared = .706)

ANCOVA was run using ln stock turnover as the dependent variable against water clarity and temperature measurements from the same month, and ln parasite abundance from the month prior to the stock turnover reading of interest. Differences between sites were also assessed. This was to determine whether these variables had any influence on stock turnover. Statistically significant associations were observed

between water clarity and site (Table 3.4). These two variables explained 93.8% of the variability in the data set (R^2=0.938). Data between sites was homogeneous (Levene's test: F=0.977, df1=4, df2=34, P=0.259) and no significant interaction was found between the variables. Scatter plots showed a positive correlation between the rate of stock turnover and water clarity.

Table 3.4. ANCOVA showing variables associated with the natural log stock turnover rate of trout in the 5 study sites from May 2002 to May 2003.

Dependent Variable: Ln Stock Turnover

Source	Type III Sum of Squares	d.f.	Mean Square	F	P-Value
Corrected Model	54.58	5	10.92	99.54	0.00
Intercept	13.70	1	13.70	124.95	0.00
Site	54.51	4	13.63	124.25	0.00
Water clarity	0.50	1	0.50	4.53	0.04
Error	3.62	33	0.11		
Total	401.18	39			
Corrected Total	58.20	38			

R Squared = .938 (Adjusted R Squared = .928)

3.3.5. Observations on the egg laying habits of *A. foliaceus*

Observations on the egg laying habits of *A. foliaceus* were conducted on a drained fishery in Wiltshire that had a history of heavy infections by *A. foliaceus*. The lake averaged 90cm in depth. Eggs were found on any firm, silt free surfaces i.e. rocks,

concrete, sunken plastic and wood. A preference for smoother surfaces over rough was also observed i.e. wood over concrete. Eggs were not found on silt or fine gravel. As a general rule, eggs were laid in deeper water, with no eggs found within the top 22cm of the lake depth. Eggs also appeared to be laid in shaded regions out of direct sunlight. The majority of eggs were found to be concentrated around features that were likely to be natural fish holding habitats, such as sunken boats and logs, as opposed to areas of open rocky bottom. Low numbers of eggs were found on the bases of marginal reeds, but were not found on any other aquatic macrophytes. Eggs were also found on swan mussels and, interestingly, on the carapace of signal crayfish (*Pacifastacus lenuisculus*) in the lake. Of 20 crayfish examined in 2001, 7 were found to have argulid eggs on their carapace (figure 3.37). Due to their overland migrations this makes crayfish a possible transmission vector for argulids.

Data from the egg laying boards at each of the study sites suggests that depth of egg laying varies through the season (figure 3.38). In site 1 egg laying occurred in mid-water in June 02. All egg laying in the following month was noted at the water surface on the floating egg boards. Depth of egg laying increased in each subsequent month until October when it stopped for the year. Egg laying in site 1 began again in May 03 at 20% of the lake's total depth. In site 2 egg laying in June 02 occurred at 12% of the total water depth. The depth of egg laying increased in the subsequent two months before occurring higher up the water column in September 02, when it stopped for the year. Egg laying in site 2 started again in May 03 at 10% of the lake's total depth. Egg laying in site 3 was only noted between June and August in 2002, when it occurred at

between 72 and 82% of the lakes total depth. Egg laying was noted again in site 3 in May 03 on boards on the lake surface. Profiles of egg laying depths for sites 4 and 5 were similar. Egg laying occurred at 34% (site 4) and 39% (site 5) of the lakes total depth in June 02. Egg laying occurred in deeper water in each subsequent month until September 02, when it stopped in site 4 at 85% of the total depth. In site 5, egg laying continued into October 02 at the bottom of the lake (100% depth). Egg laying was noted in both sites 4 & 5 in April and May 03, at between 20 & 35% of the lakes total depth. In general, egg laying appears to start in the upper water layers at the beginning of the egg laying season and then moves deeper as the season progresses and the water temperature increases. Egg laying occurred in deeper water in the two study sites with lowest water clarity, sites 2 and 4.

Figure 3.37 Signal crayfish caught in a Wiltshire trout fishery. Arrows show egg strings of *A. foliaceus* laid on the carapace.

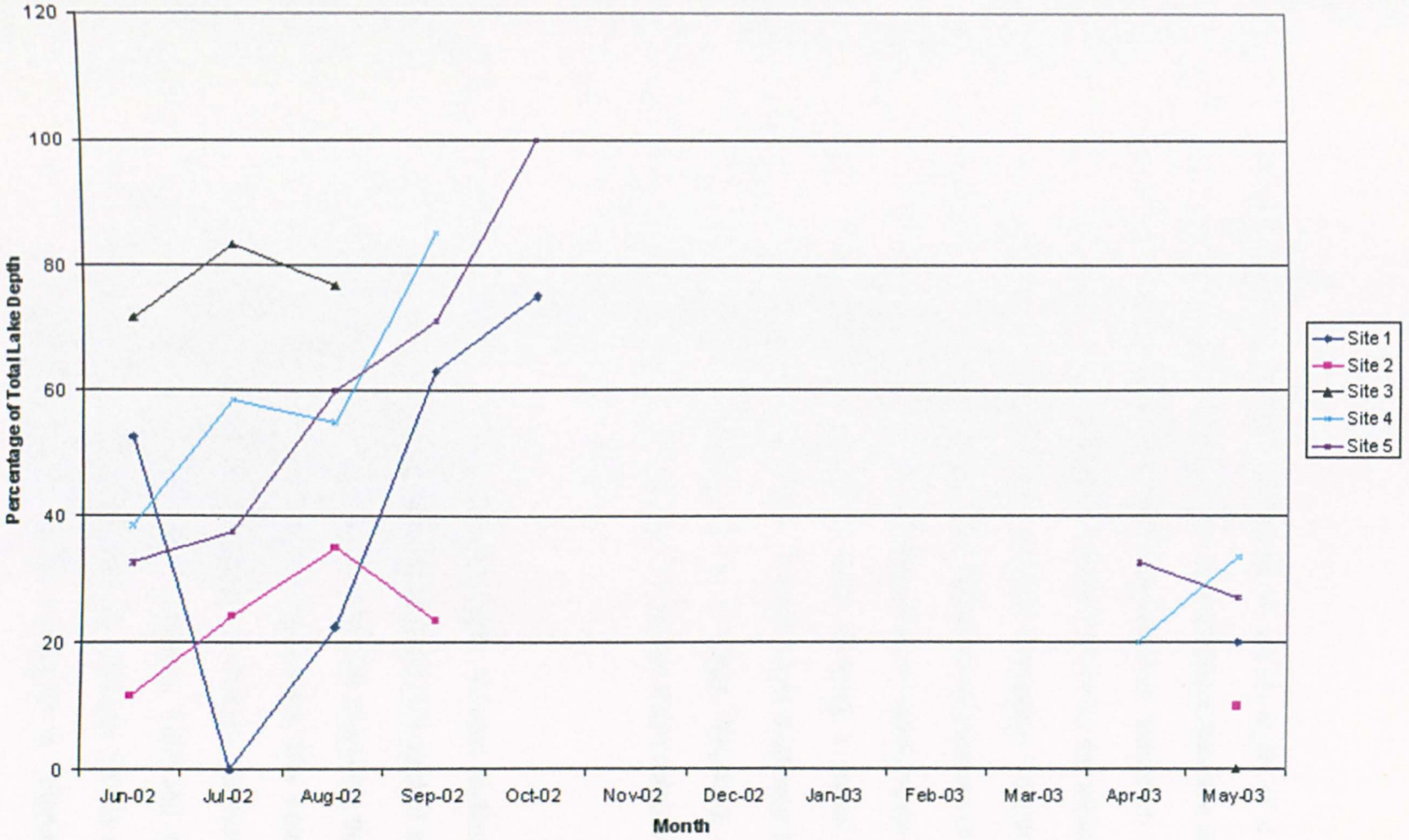


Figure 3.38. The average depth of egg laying of *A. foliaceus* in the five study sites from May 2002 to May 2003

3.4 Discussion

This study has successfully elucidated information on the life-cycle of *A. foliaceus* populations in five UK stillwater trout fisheries. A relationship between temperature and water clarity on the abundance of *A. foliaceus* has been identified. Although Rushton-Mellor & Boxshall (1996) differentiated each moult in the life-cycle of *A. foliaceus*, distinguishing each moult is difficult and time consuming. Length frequency data of the populations of *A. foliaceus* under study, suggested the presence of cohorts as opposed to continuous recruitment. The use of image analysis increased the sensitivity and accuracy of measurements that could be taken, allowing a greater degree of flexibility in the choice of bin size used when plotting length frequency histograms. This proved important to allow accurate analysis of length frequency data to be conducted. The subsequent use of mixture analysis on this data proved a successful technique for discriminating overlapping cohorts.

Although length frequency data has been used in many parasite studies when life staging is difficult, this is first time that mixture analysis has been applied to a parasite system. This method proved successful and has allowed data on growth, the origins of cohorts, and the dynamics of individual cohorts to be quantified. This would not have been possible using more subjective length frequency analysis, as studies on the population dynamics of *A. coregoni* (Hakalahti & Valtonen, 2003) and *A. japonicus* (Shafir & Oldewage, 1992) demonstrate. Hakalahti & Valtonen (2003) used length frequency data to study the population structure of *A. coregoni* in a Finnish fish farm

over a two-year period. No form of further analysis was, however, used to formally discriminate cohorts. Although their data showed when recruitment was occurring and the proportion of parasites in each size group at any time of year, it did not provide information that could be used to determine the number of cohorts present at any time and, therefore, could not make any quantitative judgement of growth or mortality.

Shafir & Oldewage (1992) went further in their analysis of length frequency data, using probability paper and following the methods described by Harding (1949) and Cassie (1954). The method elucidated cohorts and followed their growth over a 16-month period, however, according to Macdonald & Pitcher (1979), interpretation of data using this method is subjective, difficult to reproduce and varies between analysts. The reliability of Shafir & Oldewage's (1992) estimates are cast into doubt when compared to findings of laboratory studies by Kimura (1970) on the development and growth of *A. japonicus*. Shafir & Oldewage (1992) estimated that cohorts grew between 2.6 and 4.4mm over a sixth month period, during which time water temperature was greater than 20°C. Based on Kimura's (1970) estimates, the time taken to grow to 4.3mm from hatching to adult at 20°C was 35 days, around 150 days less then Shafir & Oldewage (1992) observed. This suggests that either the growth of the parasite is much slower in the wild, or the estimates obtained through the methods used are unreliable.

The data obtained from this study has identified differences in the dynamics of the parasite between waters and elucidated some of the mechanisms behind this variation. Information gained by the study allows key points in the life-cycle to be identified,

around which management strategies could be targeted. If more regular samples could be taken, then, by applying the analytical techniques used in this study, accurate life table data could be elucidated. This would allow mortality within cohorts to be assessed in regular size classes, in relation to environmental factors. This may allow key factor analysis to be conducted in the same way that Begon, *et al.* (1996) used it to identify factors and stages in the life-cycle of the Colorado potato beetle responsible for the greatest levels of population change.

3.4.1 Population Ecology in Individual Sites

Site 1: Prior to the study *A. foliaceus* had not been noted in this lake. It was however exposed to the parasite as it received water directly from an upstream lake that was known to suffer problem infections. This upstream lake may have influenced the population dynamics of the parasite observed in the study lake. Abundance of *A. foliaceus* was very low during May, June and July 2002. This implies that only extremely low levels of eggs or hatched *A. foliaceus* over-wintered, however, high numbers of egg strings were found in June and July suggesting that the parasite was present in the lake in higher numbers than detected by sampling. These parasites may have been washed into the study water from the upstream lake and laid eggs without parasitising fish in the study lake. In August there was a sudden increase in the abundance of *A. foliaceus*, composed of a cohort already of adult size. During this month the lake suffered an algal bloom, reflected by a decrease in water clarity. This may explain the high infection success but does not explain the origin of the parasite.

There are several possible explanations for the appearance of this cohort. Parasites could have hatched since the previous visit, grown and reached adulthood by the August visit. However, this would mean that the growth rate was much faster than in the other sites. The parasite could have been imported on fish from the stocking farm, but fish in the site stock pond were free of *A. foliaceus*. The most likely explanation is that the parasite came from a reservoir source. As the study lake was directly connected to the upstream lake that was known to suffer a heavy *A. foliaceus* infection. This seems the most likely source of infection, as adult parasites leaving their host to lay eggs may have been washed into the study lake. An alternative source was the large number of sticklebacks present in the study lake, which were infected with low numbers of juvenile *A. foliaceus*. No other fish species were present in the lake. Prior to the August visit a mass mortality of sticklebacks had been noted. These were a possible source of infection, becoming infected before the trout, and with the parasite leaving these hosts before or after their death. Mikheev, *et al.* (2004) found evidence that *A. coregoni* change their host preference from roach to brown trout halfway through their development. They hypothesise that this is due to a change in host finding mechanisms, with juvenile stages being reliant on vision and the older stages relying more on olfactory cues to detect a host. It maybe that similar mechanisms occur in the case of *A. foliaceus*, but to date no studies have been conducted to support this.

After the August site visit, the fishery owners removed all of the fish from the lake by netting. The lake was then stocked with fish free of *A. foliaceus* in an attempt to

eradicate the parasite. This did not remove eggs already laid in the lake and in September, the abundance of *A. foliaceus* peaked as a large cohort of metanauplii hatched from eggs laid between the July and August samples. Recruitment was possibly aided by immigration of metanauplii from the upstream lake. The intervention was thus mistimed. To be effective it should have been repeated in the subsequent month to remove new recruits or conducted earlier in the year to remove the parasite before it could lay eggs, i.e. April/May. After September, there was no recruitment for the rest of the year and the parasite population dropped to very low levels. This was probably due to the very fast stock turnover resulting in the removal of fish and parasites, to the extent that 105% of the standing stock of trout in the lake was removed and replaced per month. There was a subsequent drop in the stock turnover rates in the lake during December and January due to cold weather and ice reducing angler numbers but, by this point, the *A. foliaceus* population had almost died out. Recruitment of *A. foliaceus* began in April from over-wintered eggs most probably laid by the September cohort.

Site 2: This site suffered the highest abundance of *A. foliaceus* of all those sampled, even though it had a relatively fast stock turnover. A rapid increase in the abundance of *A. foliaceus* was observed between June and July 2002. This corresponded with an algal bloom that caused the water clarity to reduce from 7.5 SDU to 2.9 SDU. If reduced water clarity were to increase infection success by some means, then this, in combination with large fish able to carry high numbers of *A. foliaceus* and the observed reduction in stock turnover as anglers could not catch fish, might explain the high

abundance observed. Fish in this site were, on average, four times larger than those in other sites, and thus the potential carrying capacity of the host is likely to be increased. Tucker, Sommerville & Wootten (2002) observed this to be the case in Atlantic salmon infected with the sea louse, *Lepeophtheirus salmonis*. They found that the total abundance of the parasite increased with fish size although the relative density of the parasite per unit host surface area decreased as fish got larger.

The *A. foliaceus* population peaked in August but dropped rapidly by September. After the August visit, the site owner carried out an intervention, which appeared to be successful as numbers of *A. foliaceus* decreased and no egg laying occurred. However, the intervention did not kill eggs already present in the lake as further recruitment occurred in September causing numbers to peak again in October. This cohort was removed over winter due to the fast stock turnover and, as a result, no egg laying was observed in April. High levels of recruitment were observed in May 03 and corresponded with another algal bloom and reduced water clarity. This cohort would be expected to emerge from eggs laid by the August cohort in September. However, this cohort was apparently killed off before it could lay eggs, and thus the high spring recruitment is difficult to explain. It is possible that a proportion of eggs from earlier cohorts had a delayed period of hatching. Pasternak *et al.* (2000) suggests this may occur in *A. foliaceus* populations in Finland, although they suggest hatching in over-wintered eggs to be relatively synchronised. Hakalahti, Hakkinen & Valtonen (2004) also suggest this to be the case with *A. coregoni* populations in Finland. However studies by other authors have found relatively conserved hatching periods of one to

three weeks in the cases of *A. foliaceus* and *A. japonicus* (Tokioka 1936, Kollasch 1959, Stammer 1959, Shafir & van As 1986, Rahman 1995). Another possible explanation is that the intervention did not impact upon the *A. foliaceus* on reservoir hosts in the lake, as it was only likely to target trout. Although little egg laying was observed following the intervention, it is possible that other species of fish inhabited different parts of the lake and that the egg boards used would not have detected egg laying, e.g. pike inhabit reed and weed beds, any *A. foliaceus* leaving these fish to lay eggs are unlikely to use boards in open water.

Sites 3: In this lake the water remained comparatively clear throughout the survey, and the site experienced the lowest abundance of *A. foliaceus* of all the study sites. Due to its size, fish capture in this site was difficult and sample sizes often small. As a result care must be taken when interpreting the data. Abundance of *A. foliaceus* appeared to remain constant and low from May 02 to August 02, and then dropped to about 50% of this level from September to December, and then to almost zero in January, where it remained for the rest of the study. This drop in abundance in September corresponds to the cessation of recruitment and fast stock turnover removing infected fish, which prevented hatched parasites from over-wintering.

Due to low sample sizes the length-frequency data is difficult to interpret, however using mixtures some information was gained. Two cohorts were discriminated in May 02, and only low levels of recruitment occurred in June, suggesting few hatched *A. foliaceus* or eggs successfully over-wintered. Slightly higher levels of recruitment were

observed in July and August which were probably the progeny of the first two cohorts to hatch in 2002 (April & May). No recruitment occurred in September suggesting the progeny of the June cohort had not successfully established. Only low numbers of *A. foliaceus* emerged in April 03 and none in May 03 due to the small July and August cohorts laying few over-wintering eggs. There was no egg laying in April or May 03, reflecting the low number of over-wintering hatched parasites and low April recruitment.

Site 4: This was the largest study lake and again difficulties in fish capture meant sample sizes were often small. As with site 3 the lake remained very clear throughout the year. Stock turnover was constant but was much slower, taking approximatley 200 days. This lake was also much shallower than site 3. The site had suffered a very heavy infection of *A. foliaceus* in the year prior to the study. Although it had been managed in exactly the same way as in the study year, it had experienced an algal bloom from the start of July until the end of September 2001.

Only low numbers of *A. foliaceus* were observed in May 02, and spanned a wide range of size classes. Two cohorts were discriminated, one of recently hatched juveniles and the other of parasites mid-way through development. These two cohorts are likely to have emerged from over-wintered eggs. Substantial recruitment had occurred by June when two clear cohorts were observed and the parasite abundance had increased, suggesting that substantial numbers of *A. foliaceus* over-wintered and laid eggs in April. The abundance of *A. foliaceus* was relatively constant from June to September

due to recruitment of a succession of cohorts. Recruitment occurred in each of these months and the analysis indicates that five cohorts hatched during the year with the final cohort over-wintering in a hatched state. Recruitment stopped after September and did not occur again until April. The sixth cohort of the season, emerging in September, was only observed in this month. It may be that this final cohort of the year died out due to their small size and the rapid decrease in temperature between September and October. Kimura (1970) found survival to be low in the final cohort of *A. japonicus* of the season. It is possible that the small stages of the parasite have insufficient energy reserves to maintain them through the winter. As *Argulus* spp. hatch at an advanced metanaupliiar stage they would not retain extensive energy reserves on hatching. Atkinson (1998) noted that marine copepods that undergo diapause during the winter months tend to have extensive lipid stores. It is not currently known whether parasitic *A. foliaceus* or other *Argulus* species continue feeding on the fish in low water temperatures or whether they become reliant on energy stores. The total numbers of hatched *A. foliaceus* over-wintering were small and this was reflected by the low levels of egg laying observed in April 03.

Site 5: This was an interesting study population as it demonstrated how a long established population of *A. foliaceus* behaves on trout when there is little or no management of the fishery. Water clarity remained constant during the entire survey, with the exception of April 03 and thus water clarity probably had little impact on changes in parasite abundance.

Recruitment from over-wintered eggs laid in 2001, and eggs laid by over-wintered hatched stages occurred between April and June, causing parasite numbers to increase steadily. Although recruitment occurred, abundance was constant into July, probably due to adults from the June cohort dying or leaving the fish to lay eggs, thus counteracting the effect of recruitment from the third cohort. There was a significant increase in abundance in August corresponding with the emergence of a fourth cohort. The abundance continued to rise slowly until it reached its peak in October after which recruitment stopped. A total of six cohorts were observed in 2002. There was a substantial drop in the abundance of *A. foliaceus* in November, corresponding to the water temperature falling below 10°C and suggesting that, as no egg laying was observed and there was no real stock turnover, that the low temperature was responsible for the apparent high mortality. This finding corresponds with Kimura's (1970) observation that *A. japonicus* that hatched in the autumn were very likely to die over the winter. After the initial drop in numbers in November, the abundance of the parasite remained constant, suggesting that those parasites surviving the initial mortality persisted through the winter.

In the following April there was another significant drop in the abundance of *A. foliaceus* on fish, associated with the start of egg laying. The first metanauplii of the season were also found to hatch in April, but numbers were very low compared to the other study lakes, with the exception of site 4. This may be because the temperature had not risen above 10°C, which is considered as the critical temperature for hatching to occur (Hindle 1948, Gault *et al.* 2002).

3.4.2 General Features of the Population Ecology of *A. foliaceus.*

3.4.2.1 Abundance and Growth of *A. foliaceus.*

Temperature positively affects both the development rate of eggs and the growth of hatched stages of *A. foliaceus*. Site 5 was used as a model system to investigate this relationship, due to its comparative lack of management and intervention, and because it provided large samples of both fish and specimens of *A. foliaceus* throughout the study year. Figure 3.39 summarises this data and illustrates growth rates and periods of recruitment to the *A. foliaceus* population throughout the study year. Using the data from figure 3.39 it becomes clear from which parent cohort a new cohort emerges, by determining when the former cohort reaches adult size and lays eggs. Red arrows on the figure show this relationship. *A. foliaceus* is able to lay eggs at approximately 4.7mm (Rushton-Mellor & Boxshall 1996), and the point at which each cohort lays its eggs is shown by the black arrows on figure 3.39. The metanauplii of the parasite are approximately 0.8mm in length upon hatching (Rushton-Mellor & Boxshall 1996) and the emergence of each cohort is shown on figure 3.39 by a black dashed line. As a general rule two cohorts emerge between a cohort laying eggs and its progeny emerging (figure 3.39).

The abundance of the parasite throughout the year varied between waters and appeared to depend on the ecology and management of the lake. This may explain the variability of the reports provided by fishery owners regarding periods when *A. foliaceus* was present in the lake when questioned in the cross-sectional study (chapter 2). However,

the study has shown that, in all cases, the parasites abundance followed a clear seasonal pattern with low numbers in spring, an increase throughout the summer, before a fall in early winter. In sites 4 & 5 where no interventions were implemented, a total of 6 cohorts were identified in a year. Two of these emerged from over-wintered eggs and a third from over-wintered hatched stages of the parasite. The subsequent three cohorts are the progeny of the first three. In turn, two of these produced eggs that over-winter, while the other over-winters in its hatched form. This would suggest three complete generations occur in a year, one more than suggested by Gault *et al.* (2002). This strategy of several distinct cohorts over-wintering and hatching at different intervals is likely to ensure the parasite survival in the subsequent year, should host availability be low. Details of the general pattern of how these cohorts were produced and develop can be observed in fig 3.39 and are described in Table 3.5. Few other ectoparasites leave their host to lay eggs in the same way as argulids, and those that do (e.g. the fish leech, *Piscicola geometra*) have received little study making it unclear whether similar mechanisms of dispersal in time occur i.e. staggering of cohorts. Other mechanisms for dispersal in time are observed in many parasite systems, e.g. *Ergasilus* spp. develop in the plankton with only adult females being parasitic (Kabata, 1970).

The results of the present study show that *A. foliaceus* lays eggs from April to September, depending on the development rate of the fifth cohort, which is the final cohort of the year to lay eggs. Recruitment of argulids to the population stops once the average temperature falls below 11.3°C after October, and does not start again until the temperature averages 9.5°C between March and April. However, it appears from the

timings of the cohorts that some egg laying by this fifth cohort must occur after September, which was not detected on the egg boards. This gives rise to the second cohort of the year, with eggs from the fourth cohort of the preceding year producing the first cohort of the year. Parasites in the sixth cohort become adult before the water temperature falls below 10°C, and over-winter as adults, laying their eggs between April and May once the temperature has increased above 10°C. In sites 1,2 & 3 where this sixth cohort did not successfully over-winter, egg laying was not recorded until a month later, and as a result no third cohort occurred in June (see site 3 results). Upon hatching, the cohorts appear to be female biased but once egg laying begins, this shifts to a male biased ratio, reflecting the movement of females off their hosts to lay eggs. In sites 1 and 5, egg laying continued furthest into the year. These are the two smallest lakes and had the lowest temperature profiles for the three months prior to the end of egg laying; this possibly delayed development of the August cohort thus delaying egg laying. Egg laying begins again in April if hatched stages of the parasite over-winter, or in May if the cohort originates from eggs which over-winter and hatch in April. This also suggests that egg laying activity does not occur at temperatures of less than 9.5°C, similar to the estimates from field studies by Stammer (1959) and Gault *et al.* (2002). This is 4-6°C lower than the temperatures suggested by Bauer (1969), Rizvi (1969) and Hoffman (1977). Egg laying appears to occur when the parasite reaches 5mm in length, similar to Rushton-Mellor & Boxshall's (1994) estimate of 4.67mm based on laboratory studies.

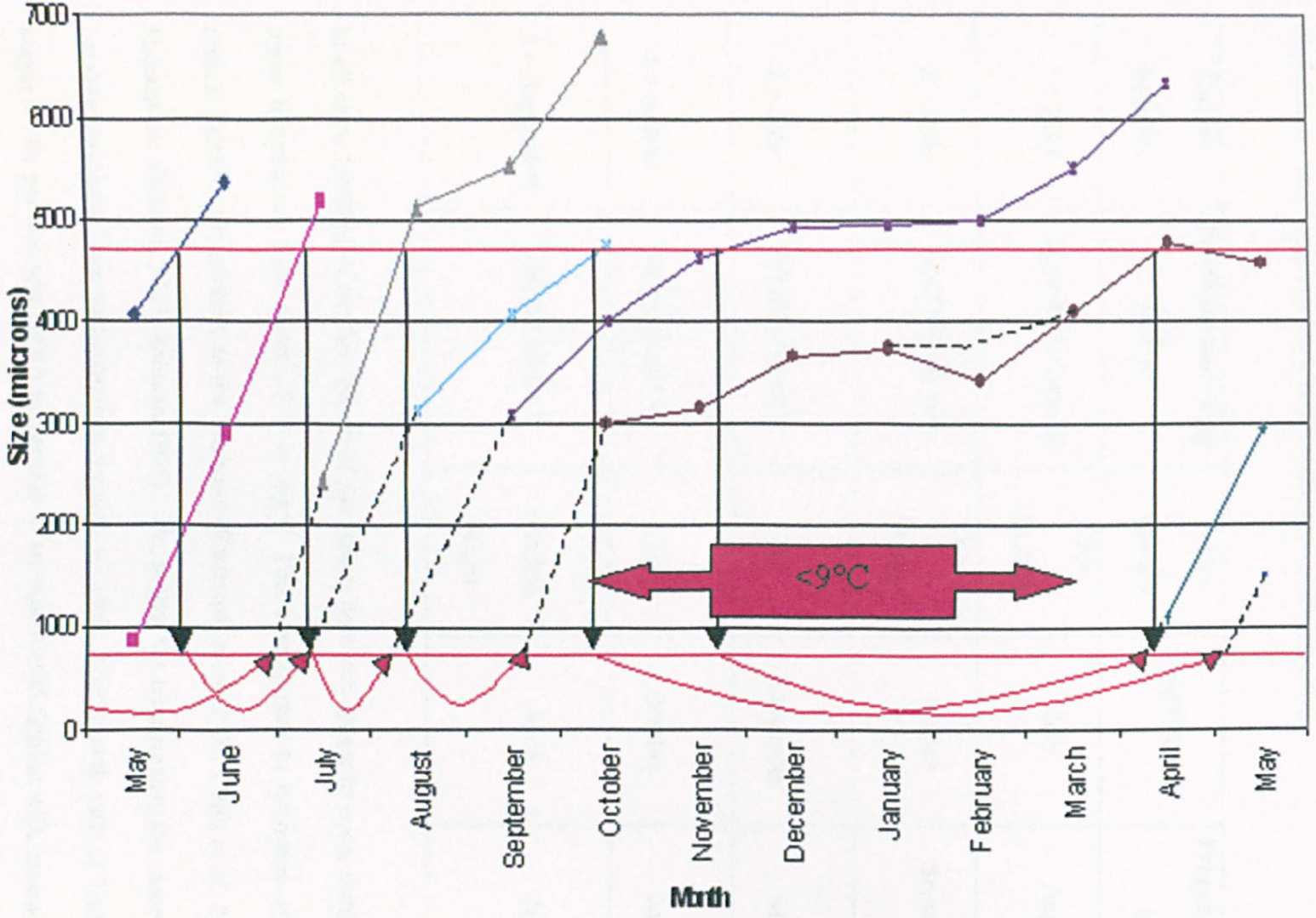


Figure 3.39. Growth of *A. foliaceus* at study site 5 between May 2002 and May 2003.
Dashed lines show extrapolated growth rates. Black arrows represent the point at which the parasite lays eggs.
Red arrows show point at which eggs hatch.

Table 3.5. Generalised timings of development and egg laying of cohorts of *A. foliaceus* hatching in a year in UK stillwater trout fisheries.

Cohort hatches	Produced from Eggs laid in	Over-winters	Lays eggs in	Progeny hatch in
1 = May	October by cohort 4	Yes As Eggs	July	August
2 = June	April by cohort 5	Yes As Eggs	August	September
3 = July	May by cohort 1	No	September	May
4 = August	June by cohort 2	No	October	June
5 = September	July by cohort 3	Yes As Hatched Stages	April	July

In all sites, hatching of the first cohort of the season does not appear to occur until the water temperature rises above 10°C in April. This corresponds to estimates of the critical hatching temperature in the literature (Pasternak *et al.* 2000, Gault *et al.* 2002, Hakalati & Valtonen 2003, Rahman 1995). The higher the temperature, the faster the parasite develops from metanaupliius through to adult. The growth rate of hatched stages of the parasite was shown to increase in an exponential fashion with increase in temperature. This was also observed in laboratory studies on *A. foliaceus* by Schluter

(1979), and on *A. japonicus* by Kimura (1970). With the exception of the September cohort (fifth cohort to hatch), the length frequency data shows that the time from hatching to egg laying is between 51 to 110, days depending on the water temperature. These estimates are based on extrapolation of data in figure 3.39 and shown in table 3.6. It can be seen from table 3.6 that observed development times for each of the cohorts are close to those predicted by the growth model developed in section 3.3.3. When estimates from the model are compared to Schluter's (1979) estimates from laboratory studies they compare favourably, showing the reliability of the model and the use of length frequency analysis in determining this information (table 3.7). When compared to Kimura's (1970) development times in *A. japonicus* the development of *A. foliaceus* appears to be much slower especially at low temperatures (table 3.7).

The data in figure 3.39 & Table 3.8 shows that the period between eggs being laid and hatching is between 30 and 42 days for first three cohorts of egg laid in the season when water temperatures are high. This period increases over 160 days in subsequent cohorts of eggs that over-winter, corresponding to decreasing temperatures. This concurs with the findings of Kimura (1970) in *A. japonicus*. The moderatlely conserved widths of cohorts observed in the length frequency data, suggest that hatching of eggs occurs in waves lasting between 2-3 weeks. This suggests that cohorts emerge from batches of eggs laid at different times and not from a prolonged hatching of a reserve of eggs as suggested for *A. coregoni* in Finland by Hakalahti. Hakkinen & Valtonen (2004). Pasternak *et al.* (2000) observed that variability in hatching periods differed between habitats and time of year in *A. foliaceus* populations in Finland. They

found hatching periods were conserved early in the season in both farm and lake habitats, and remained so in the farm throughout the year. In the lake environment, however, hatching of the second generation of the year lasted over 200 days. The authors acknowledged that other studies (Kollatsch 1959, Stammer, 1959 and Shafir & Van As, 1986) did not find this same level of variability and hypothesise that the degree of variability is driven by host availability. They suggest that, in environments with low host density, extended hatching periods occur to increase the chance of successful infection. Although in the current study, hatching periods did seem to increase later in the year, they did not extend to the degree observed by Pasternak *et al.* (2000) and any increase was probably due to variability in the time when the eggs were laid.

Insufficient data was available from this study to accurately predict the relationship between egg development and hatching. However, approximate hatching times can be gained by estimating the number of days taken from a cohort reaching adult (black arrows, figure 3.39) to its progeny hatching (red arrows, figure 3.39). These estimates of hatching times are presented in table 3.8, in relation to the average temperature during these periods. These data would suggest that small changes at high temperatures, lead to large changes in the rate of development. The accuracy of the estimates at the lower temperatures is less certain. These estimates also appear to be shorter than those of Pasternak *et al.* (2000) who found hatching to start at 25 days after laying at temperatures between 19-22°C and 20 days in the case of over wintered eggs once the temperature was increased to 24°C. The estimates are also shorter than those observed through laboratory studies by Rahman (1995) who noted hatching to take 17

days at 23°C. Estimates of hatching time from the current study are likely to be inaccurate as they were estimated by extrapolating from the cohort data to predict when egg laying and hatching would occur. Further investigation is required to establish the effect of temperature on hatching of *A. foliaceus* eggs, and the duration over which eggs hatch in the field.

Table 3.6. Development times of cohorts of *A. foliaceus* in study site 5. Observed times and times predicted by growth model.

		Days from hatching until adult	
Cohort	Average Temperature (°C)	Observed	Expected
2	17.8	51	59
3	18.6	64	55
4	14.9	94	84
5	13.3	110	104
6	8.3	211	248

Table 3.7. Development times from hatching to adult for *A. foliaceus* and *A. japonicus* as predicted by the current study and from other authors.

	Days from hatching until adult		
Temperature (°C)	Study Estimates	Schluter (1979) Estimates for *A.foliaceus*	Kimura (1970) Estimates for *A.japonicus*
16	73	79	47
20	48	52	35
24	34	32	26

Table 3.8. Estimated hatching times for egg batches laid in site 5.

Egg Batch	Average Temperature (°C)	Estimated Hatching Time (Days)
1	18.2	42
2	18.5	30
3	18.7	41
4	6.7	164
5	7.7	178

3.4.2.2 Correlations with Risk Factors

The abundance of the parasite in the study sites was correlated with the average temperature and water clarity. Temperature showed a positive correlation with abundance, a relationship that is well documented for many host parasite systems. Anderson (1976) suggests that this is due to the narrowing time interval between generations allowing numbers to build up and cohorts to overlap. This can clearly be seen to be the case from this data set as figure 3.39 shows hatching of cohorts to become closer to one another as temperature increases and that up to 3 cohorts persist at any one time during the warmer months, this leads to increased abundance. In addition, water temperatures high enough to induce stress in fish may suppress their immune system or change their behaviour making them more susceptible to infection. Alabaster & Lloyd (1980) note that the 'preferred' temperature for rainbow trout is between 9 and 17°C. Summer temperatures of up to 20.9°C were recorded during this study, probably causing stress to the fish. Temperature may also influence the infection success and survival of a parasite. Tucker, Sommerville & Wootten (2000) found that higher water temperatures increased infection success and survival of the sea louse, *Lepeophtheirus salmonis*. Laboratory studies detailed in chapter 4 show that, in the case of *A. foliaceus*, temperature did not affect initial infection success, but higher temperatures did increase the likelihood of the parasite surviving to adult.

A negative correlation was found between water clarity and parasite abundance. This suggests a temporal association, as water clarity readings were taken prior to the

abundance readings. There are several hypotheses that might explain this negative correlation. Low water clarity is also correlated to reductions in stock turnover, which results in the fish remaining in the lake longer and thus they have a greater opportunity to acquire higher parasite numbers. Changes in water clarity may also affect fish behaviour making them more or less susceptible to infection. Mikheev *et al.* (1998, 2000) found the behaviour of *A. foliaceus* to change between light and dark conditions. Under darkened conditions the parasite was shown to adopt an active search pattern, which was correlated to the behaviour of the host fish, which became sedentary in the darkened environment. Reduced water clarity may also cause trout to adopt a sedentary behaviour that would make them more susceptible to infection. Finally, water clarity may affect the level of predation on the parasite by fish. Herter (1927), Chen (1933), Bower-shore (1940), Kiselev & Ivleva (1953, cited in Bauer, 1970) and Thomas (1961) have all noted a variety of fish species predating upon *Argulus* spp.. Rainbow trout are highly visual feeders and Barrett, Grossman & Rossenfield (1992) found that, as water clarity decreased so did the reactive distance of the fish to visual prey items. Stuart-Smith, *et al.* (2004) found increased water clarity reduced the feed intake, and dietary composition of brown trout. Rowe (1984) also found that reduced water clarity altered the feeding behaviour of rainbow trout, causing them to feed more heavily upon benthic prey. Such changes in response and behaviour may reduce predation on the parasite by fish. Studies on the level of predation on *A. foliaceus* under varying water clarity conditions are warranted.

The cross sectional study suggested that a fast rate of stock turnover made waters less susceptible to a problem *A. foliaceus* infection. However, reduced stock turnover could have been an effect of the infection, as problem infections were associated with reduced feeding in fish hosts and thus reducing catchability. This study found no significant relationship in either direction, however a significant correlation was found showing that low water clarity led to reduced stock turnover. This would suggest that fish may be able to tolerate a heavy infection and still be caught if water clarity and other conditions are favourable. Data from site 2 supports this hypothesis as high stock turnovers were observed in October when the parasite burden was high but the lake remained clear. This would suggest that the perceived problem identified in the cross-sectional study is not solely attributable to *A. foliaceus* but to a combination of factors. This is advantageous from a management perspective as it may mean that other factors such as water clarity can be manipulated to increase the catch rate. Water clarity may affect turnover rates by reducing the distance a fish can see (Barrett, *et al.* 1992) and therefore the likelihood of it taking an angler's fly. The majority of variation in stock turnover was attributed to site differences. This is likely to be related to the number of anglers attending the fishery, as the greater the number of anglers, the higher stock turnover is likely to be. The results suggest that a better method of estimating the impact of the parasite and other factors on a fishery, would be to look for relationships between the average number of fish caught, per angler, per month, and each variable of interest.

Although no significant relationship was found in this study, it is likely that fast stock turnovers will remove a greater number infected fish from a site and therefore more parasites. This may explain the over-winter die out of the sixth cohort in the sites with the fastest turnovers (1,2 &3). An important fact to bear in mind is that rod capture is likely to select only the healthiest fish with the lowest burdens of *A. foliaceus*. For this reason, if increased stock turnover were to be used as a management strategy, either an alternative capture method such as seine netting, which is likely to target the most heavily infected fish should be used, or rod capture should be targeted at those times when high parasite burdens will not reduce capture rates, i.e. when the water is clear and cool.

3.4.2.3 Egg Laying Habits

Eggs of *A. foliaceus* were always found to be laid on firm clean surfaces. This was also observed by Shimura & Egusa (1980), and Hakalahti et al. (2004) in the case of *A. coregoni* and Gault et al. (2002) in *A. foliaceus*. Depth of egg laying appears to vary through the season. At the start of the year eggs were laid in the upper, possibly warmer layers of water, where development would be accelerated. As the season progresses, egg laying occurs in deeper water and in shaded locations, possibly to protect eggs from exposure to UV light, which Poly (1998) suggests may be the reason melanophores cover the ovaries of the female parasite. Garrett & Bennett (1995) found lake trout, which normally inhabit the surface layers, moved into deeper water when temperatures rose above 18°C, which may explain why egg laying occurred in deeper

water in the summer months. Alternatively it could act to slow development by placing them in cooler water, in order to disperse the parasite in time. Shimura & Egusa (1980), Mikheev *et al.* (2001) and Hakalahti *et al.* (2004) all observed *A. coregoni* to lay eggs in deeper water and in habitats sheltered from water flows. Bauer (1959), however, suggested *A. foliaceus* lays eggs in shallow water. Hakalahti *et al.* (2004) also found a preference for egg laying on dark substrates and suggests that habitat selection is an active process using visual cues and may reduce predation upon the juvenile parasite by predators. Interestingly, it was observed in the present study that *A. foliaceus* eggs were always found in high abundance in natural fish holding areas, i.e. features in the lake such as sunken boats and logs. This is biologically advantageous for two reasons:

a) It minimises the distance the parasite must travel to lay eggs.

b) As fish are abundant in these areas it would increase the chance of infection success, as the juvenile parasites would emerge in close proximity to potential hosts.

It may be that egg laying habitats are not actively selected, but simply reflect where host fish congregate. Gault *et al.* (2002) found higher numbers of *A. foliaceus* eggs were laid on horizontally suspended boards than on vertically suspended boards, this may be due to fish sheltering from predation under these surfaces. Hubert, Harris & Wesche (1994) found juvenile brown trout (*Salmo trutta*) in rivers conceal themselves

in sheltered habitats, as the fish grew, they used deeper waters with larger rocky substrates. Both bull charr (*Salvelinus confluentus*) and cutthroat trout (*Oncorhynchus clarki*) were found by Jakober, McMahon & Thurow (2000) to conceal themselves in the cover of large woody debris and boulder substrate crevices in deep river pools during the day. This habitat use by various trout species may explain the high levels of *A. foliaceus* egg laying activity around such features. Little published information is available on habitat use in lakes by rainbow trout. Shimura & Egusa (1980) noted that the majority of egg laying by *A. coregoni* occurred in the dark. This may give another explanation why eggs were found in shaded locations, as a change in light intensity caused by fish moving into these locations during the day, may trigger the parasite to leave its host to lay eggs. The information gained from this study on the depths and habitat used for egg laying would suggest that the risk factor 'drop in summer water level' identified in the cross sectional study (Chapter 2) is a chance association and unlikely to impact upon egg survival due to the eggs being laid deeper in the water column.

A final point of interest on the egg laying habits of *A. foliaceus* is that, for the first time, eggs have been recorded on the carapace of crayfish. This has potential significance as crayfish undergo overland migrations (Crocker & Bar 1968 and Cooper & Braswell 1995), which could allow them to act as a transmission vector for the parasite between land locked waters. No detailed information is available on the distances that crayfish travel overland, however Crocker & Bar (1968) state that it can be 'substantial'.

3.4.3 Summary and Areas for Further study

This study has identified that up to six cohorts of *A. foliaceus* can occur in a year depending on the management of the fishery. Three cohorts over-winter as eggs or hatched parasites, and go on to produce the subsequent first three cohorts of the next season. This strategy maximises the chance of the parasite persisting in the subsequent year by distributing the metanauplii over time and thus increasing the chance of the parasite infecting a host. Information on the timings of cohorts can be built into a management strategy for the parasite. The study showed that there can be a lack of understanding of the timing of interventions by fishery owners and has shown that the period over which interventions should be targeted can be optimised. Correlations have been identified between high burdens of *A. foliaceus* and high temperatures, low water clarity and slow stock turnover rates. The study also indicated that heavy infections by *A. foliaceus* do not of themselves necessarily cause the problem identified in chapter 2, of reduced catchability of fish, and that this may actually be attributed to a combination of a high abundance of *A. foliaceus*, low water clarity and high temperatures.

The current study has highlighted a number of areas which would be useful topics for further research. Laboratory and field studies are required to elucidate hatching times of the eggs of *A. foliaceus* in relation to temperature and other mechanisms behind hatching. It would also be of interest to establish how long eggs and hatched stages can over-winter, what their energy requirements are, and whether the hatched stages stop feeding and enter true diapause. Information on the role of potential reservoirs of

infection is needed since, as stated by Haydon et al. (2002), this is crucial in the control of an infection. *Argulus* spp. have a wide host range, but the role of alternative hosts in maintaining the parasite population on trout is not known. Do these alternate hosts merely aid the persistence of the parasite in 'hard times', or do they play an active role in causing the epizootics observed in some trout fisheries? The study has also shown the potential of connecting watercourses as a reservoir of infection. In addition to the potential for infected fish to move between waters, direct transmission of the free-swimming parasite may also occur. In any field study on fish disease, fish capture methods are critical to ensure, as far as possible, unbiased samples. The biases of different fish capture methods are well documented, however, to date no work has been published on their selectivity toward infected or uninfected fish within a wild population. This may lead the abundance of a disease causing agent to be overestimated within a population, or, possibly more seriously not detected. It may be that novel sampling methods will be required.

CHAPTER 4: STUDIES ON THE INFECTION PROCESS AND SURVIVAL OF *A. FOLIACEUS*

4.1 Introduction

In order to manipulate a parasite population there must be a good understanding of factors which affect it, such as those affecting the survival of the parasite within a host population and factors affecting the parasite's infection success. The cross-sectional study (chapter 2) identified several risk factors which may be significant in the abundance of *A. foliaceus*; rate of stock turnover, water clarity and a drop in the water level of the lake. Fast stock turnovers are likely to regulate the parasite population in an obvious way by the removal of parasites from the system along with their hosts. A drop in water level may affect survival of the parasite by killing its eggs by exposure to the air and subsequent desiccation; however data on the egg laying habits of *A. foliaceus* collected during the population study (chapter 3) would suggest that this is not the case and is likely to be a spurious risk factor. Water clarity, however, could potentially be involved in moderating the infection success and/or survival of *A. foliaceus* within a population of trout. When examined further through the longitudinal study, a significant correlation and a temporal association were established between high abundances of *A. foliaceus* and low water clarity in the weeks prior to the sample being taken. There is little information in the literature to suggest how and whether water clarity may influence an argulid population, however there is literature on the influence of water clarity on fish behaviour and feeding. This information has been

discussed in detail in chapter 3; however, in summary, it can be hypothesised that reduced water clarity may impact upon a population of *A. foliaceus* in two ways:

1) It may modify fish behaviour making them more sedentary and locating them in areas where they are likely to aquire greater levels of infection. Possible evidence in support of this was suggested by Poulin & Fitzgerald (1988) who found that although temperature had no direct effects upon the infection success of *A. canadensis* on sticklebacks, it had the indirect effect of causing the sticklebacks to move deeper in the water column where they were exposed to a greater number of *A. canadensis*. Mikheev *et al.* (1998, 2000) found that darkened conditions caused perch to adopt a sedentary behaviour. This increased the infection success of *A. foliaceus*, the behaviour of which also changed from that of a 'sit and wait' ambush predator during day light to an active search pattern in the dark. It may be that under turbid water conditions rainbow trout would also adopt a sedentary behaviour.

2) Reduced water clarity may inhibit the ability to feed of fish, such as trout, that rely primarily on vision. This may reduce the level of predation on the parasite by the fish and therefore allow the population to increase. Predation upon argulids has been observed in a variety of fish species [Herter, 1927, Chen, 1933, Bower-shore, 1940, Kiselev & Ivleva, 1953 (cited in Bauer, 1970) and Thomas, 1961].

Argulus spp. have been shown by several authors to show preferences for different host species (Wilson, 1902, Bower-Shore, 1940, Gurney, 1948, Fryer, 1968, Kabata, 1970, Kimura, 1970, Morrice, 1976, Mishra, 1991, Shafir & Oldewage, 1992). It is not clear however, whether these apparent differences are due to behavioural and morphological differences between hosts, or whether the parasite is unable to become attached to or survive on some hosts as effectively as others. To date only one paper on *Argulus* spp. has addressed this issue. Pasternak, Mikheev & Valtonen (2004) studied the growth and development of *A. coregoni* on a salmonid and cyprinid host. They found that both attachment and growth were greater on rainbow trout than on roach. No similar work is available on *A. foliaceus* and Pasternak *et al.* (2004) did not examine the overall survival of the parasite on these two hosts. It is important to obtain this information for hosts other than trout to examine the role that different host species may play in determining the abundance of *A. foliaceus* within a trout fishery. To further explore the influence of different hosts, an experiment was designed to study the infection success and survival of *A. foliaceus* on two host species noted in the literature as having differing susceptibility to infection: carp [associated with high infections (Bauer, 1970)] and roach [associated with low infections (Pasternak *et al.* 2000)].

Temperature is a factor that is well documented as important in influencing the abundance of a parasite within host populations. It often affects the rate of growth and development of a parasite, thus determining the length of time required for a generation to occur (Anderson, 1974). Data from the population study (chapter 3) showed that the time between cohorts of *A. foliaceus* was reduced as the temperature increased.

Although the effect of temperature on the growth of argulids is well documented (Kimura, 1970, Schluter, 1979, Shimura, 1983, Shafir & Oldewage, 1986, Pasternak *et al.* 2000, Gault *et al.* 2002, Hakalahti & Valtonen, 2003), little is known of its effect on the infection success and survival of *Argulus* spp. As mentoned earlier, Poulin and Fitzgerald (1988) showed temperature to indirectly affect the infection success of *A. canadensis* by causing their hosts to move into areas where the parasites were aggregated, but found no direct impact. To date no similar work has been done on *A. foliaceus*. Möller (1978) studied the effects of temperature on the off-host survival of *A. foliaceus*, but the survival of the parasite in relation to temperature when attached to the host is not known. Temperature was shown to affect the infection success and subsequent survival of *Lepeophtheirus salmonis* on salmon by Tucker *et al.* (2000), and to elucidate the effects of temperature on the survival and infection success of *A. foliaceus,* a survival experiment was conducted at a range of temperatures. A short-term trial was also carried out to examine the off host viability of the metanauplius at a fixed temperature, as Möller's (1978) work only studied the more developed stages of the parasite.

The distribution of *Argulus* spp. within a lake environment has been the subject of little study. Stammer (1959) suggested the parasite would congregate in areas into which the prevailing wind blew, and this has been shown to be the case with other parasites such as the digenean cercariae which cause swimmers itch [*Trichobilharzia stagnicolae, T. physellae* and *Gigantobilharzia* sp. (Leighton, Zervos & Webster, 2000)]. Poulin and Fitzgerald (1988) and Bower-shore (1940) both suggested *Argulus* spp. congregate

lower in the water column, whereas Bai (1981) working on *A. siamensis* and Mikheev *et al.* (2000) on *A. foliaceus* suggest they are phototactic and congregate in illuminated areas. There is no information on the parasite distribution and consequences for infection in a dynamic system such as a lake. To gain a greater understanding of the infection dynamics of *A. foliaceus* within a lake system it is necessary to have an understanding of the distribution of free swimming stages, and identify whether their distribution is patchy e.g. in bays receiving the prevailing wind, or whether they are dispersed throughout the system. In order to establish the locations in which fish become infected by *A. foliaceus*, a trial with cages of trout placed in marginal and open water habitats of a lake was carried out.

In summary the experiments in this chapter aimed to:

A) Determine the effect of light and dark conditions, different host species and temperature on the infection success and survival of *A. foliaceus*.

B) Develop an understanding of the distribution and infection process of *A. foliaceus* in a lake system.

4.2 Materials and Methods

4.2.1 Maintaining Laboratory Cultures and developing a Standard Challenge Method using adult *A. foliaceus* and *A. foliaceus* eggs:

Attempts were made to maintain populations of *A. foliaceus* in the laboratory and to develop a standard challenge method. Several preliminary trials were carried out to test the suitability of different methods. All laboratory studies described in this chapter were carried out at the Institute of Aquaculture, University of Stirling, Scotland.

4.2.1.1 Collection, transportation and storage of live parasites and eggs.

Trout from a stillwater fishery suffering from a heavy infection of *A. foliaceus* were captured by seine net as described in chapter 3. Heavily infected fish were selected and killed by a blow to the head. Fish were immediately placed into a bath of clean lake water. All stages of the parasite were removed by gently rubbing a hand over the submerged fish. Any parasites remaining on the fish were gently removed using forceps and placed in the water bath. This procedure was repeated until sufficient fish had been processed to obtain the required number of parasites, which were thenremoved from the bath by straining the water through a 1mm sieve. *Argulus* spp. were transferred into large polythene bags, a third filled with clean-carbon filtered mains water at the same temperature as the lake. Survival was low if the parasites were transported over long distances in lake water. Bags were inflated with oxygen and

sealed, and transported in a cool box with two ice packs. On arrival at the Institute of Aquaculture, the bags were removed from the cool boxes and left to stand in the appropriate study tank for up to an hour to allow the water temperature to equalise. Infection of fish was conducted as soon as the temperatures were equal. Thus, *A. foliaceus* were used for experimental infections within 9 hours of their removal from host fish.

Argulus foliaceus eggs were collected from the underside of boats kept on the lake. These were gently scraped off using a scalpel blade and placed into jars containing carbon filtered mains water. They were then placed in a cool box with ice packs until arrival at the Institute of Aquaculture. Eggs were then transferred into a 10 litre tank containing clean carbon-filtered mains water and stored at 9°C, under a 15L: 9D photoperiod until required. The tank was aerated gently via an air-stone.

4.2.1.2 Infection of fish with hatched stages of *A. foliaceus*

Infections were conducted in a 20L tank, with static carbon filtered mains water. A low water level of 15cm was used in an attempt to increase host-parasite contact. Water was maintained at the same temperature as the subsequent study tank. Fish were placed into the infection tank, and 15 parasites per fish added. Fish remained in the tank for 1-hour, after which they were removed with a hand net and allocated to a study tank. Water in the infection tank was strained through a 1mm sieve. Any parasites found in

the sieve were not used in the study. The mean number of parasites per fish was then calculated after accounting for those lice that did not attach.

4.2.1.3 Anaesthesia of fish and counting of parasites

A stock solution was made up of 50g of powdered benzocaine dissolved in 500ml of 100% ethanol. This was stored in a foil wrapped glass bottle with an air-tight lid in a cool dark place. 5ml of this stock solution was then mixed with every litre of carbon filtered mains water held at the same temperature as the tank housing the fish to be anaesthetised. Preliminary trials showed benzocaine to have no obvious effect on the viability of *A. foliaceus*. In all subsequent tank trials fish were placed individually into the anaesthetic bath and closely monitored until the respiration rate had slowed and the fish lost equilibrium. The fish was then removed, placed into a shallow tray of water and examined under a dissecting microscope to allow the parasite to be counted. All argulids were left *in situ* on the fish and once counting was complete the fish was returned with any free-swimming parasites to the study tank.

4.2.1.4 Experiment using flow through water systems.

Five rainbow trout of circa 70g were infected with adult *A. foliaceus* using the method described above, and allocated to a 10L tank. Carbon filtered mains water was passed through the tank at a rate of 0.5L/min. Outflow water was filtered through a 1mm mesh sieve to collect any parasites washed out of the system. Any live *Argulus* found in the

sieve were replaced into the study tank. The study was carried out using ambient water of 9°C and repeated at 15°C using a 75watt aquarium heater. Fish were fed on sinking trout pellet (EWOS crumb) on a maintenance ration of 1% biomass per day. Fish were observed *in situ* on a daily basis for the presence of the parasite. At weekly intervals, fish were anaesthetised and the parasites counted as described in section 4.2.1.3. Each trial was terminated when no parasites remained in the system.

4.2.1.5 Experiment using re-circulating water systems.

This experimental was as described above, except that water was re-circulated using an external Ehiem external canister filter with a flow rate of 0.7L/min, filled with activated charcoal. Gentle aeration was provided via an air-stone. The experiment was conducted at 10°C and repeated at 18°C.

4.2.1.6 Experiment using a static water system.

This experiment used a similar system to the re-circulating system but had no water flow. Gentle aeration was provided via an air-stone, and 15% daily water changes were made using carbon filtered mains water. This water was passed through a 20μm mesh and any parasites found were returned to the system. This trial was conducted at 18°C and used five, 70g rainbow trout, but was also repeated using only one 70g rainbow trout.

4.2.1.7 Infections using *A. foliaceus* eggs

In an attempt to optimise infection success, an experiment was conducted adding *A. foliaceus* eggs to the water with fish and allowing them to hatch naturally. Egg strings collected from the wild (see 4.2.1.1) were screened to determine their viability by observing them in water under a dissecting microscope. Only eyed eggs were selected for use in the study. Any eggs within a string that were not eyed were destroyed with a needle. Thirty eggs were then placed into a study tank containing static, aerated water, holding an individual 70g rainbow trout. Eggs were removed on a daily basis, and examined in a water-filled Petri dish under a dissecting microscope to note when hatching started. Once hatching was observed, eggs were no longer examined. The infection was then monitored as described previously (4.2.1.3).

4.2.2 Survival Studies

As none of these culture systems were considered successful at maintaining a parasite population, several experiments were designed to evaluate the experimental treatment in terms of the rate of *A. foliaceus* population decline under different conditions. These studies utilised the most successful culture system, which was a static tank holding an individual fish, and exposure to metanauplii. As static tanks are likely to have lower oxygen levels and higher levels of nitrogenous waste than flow through systems, juvenile carp were used in these trials as they are more tolerant of these conditions than trout (Alabaster & Lloyd, 1980). In all the survival studies, fish were examined using

the method described in section 4.2.1.3, 24-hours after the initial infection to determine infection success. At this point, water in the tanks was changed.

Experiments were designed that followed the survival of *A. foliaceus* over time in relation to factors considered likely to be important in the establishment of *A. foliaceus* populations, these were:

a) The effect of light & dark conditions to simulate the effects of increased turbidity, assuming that the effect of turbidity is solely to reduce light penetration.

b) The effect of different host species to investigate the role of reservoir hosts.

c) The impact of different temperatures on the infection success and survival of *A. foliaceus*.

In all three trials, fish were examined for *A. foliaceus* every seven days by anaesthetising them in benzocaine and inspecting them for *A. foliaceus* under a dissecting microscope; the water from the tanks was also screened. At each sampling point the number of *A. foliaceus* remaining in each tank was recorded. Data from these experiments was analysed by the use of Life Tables in the statistical package SPSS v.11 and factors compared both pair-wise between tanks and pooled using the Wilcoxon (Gehan) statistic.

4.2.2.1 The effect of light & dark conditions on the survival of *A. foliaceus* populations.

Six, 10L static tanks were used in this experiment. Three tanks were covered in black polythene and foil so that no light could penetrate and three were left uncovered and exposed to a simulated natural photoperiod (15L:9D). All tanks were held at a constant 16°C, using two 300w aquarium heaters and arranged in a random block design (figure 4.1). A single common carp, circa 7cm in length, was held in each tank. *A. foliaceus* eggs collected from the wild were placed in a beaker of carbon filtered mains water and held at 15°C in an incubator. Eggs were monitored daily until hatching occurred. One hundred and twenty metanauplii were removed from the beaker with a pipette and randomly allocated between 6 beakers. Each beaker was allocated to a study tank and the thirty freshly hatched *A. foliaceus* metanauplii in each added to the appropriate study tank on day 1 of the experiment. 80% water changes were made to each tank on a weekly basis, this water was filtered through a fine mesh and screened for the presence of *A. foliaceus*. Any *A. foliaceus* found were returned to their tank of origin. Fish were examined using the methods described in section 4.2.1.3. The experiment was terminated after 9 weeks. Throughout the experiment fish were fed on a maintenance diet of 1.5% body weight using trout pellet (EWOS crumb).

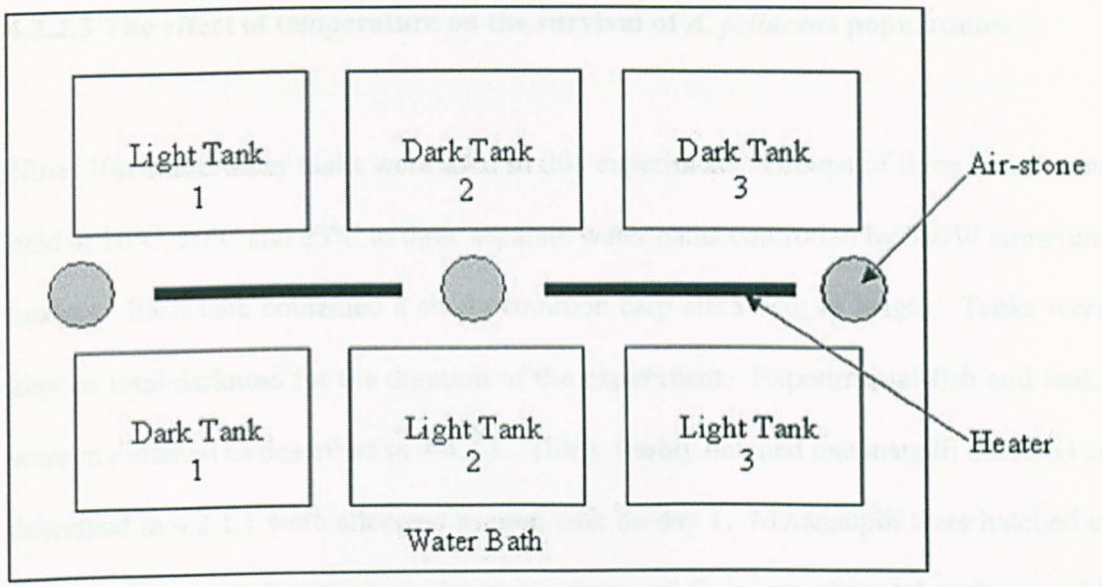


Figure 4.1. Experimental arrangement of tanks following a random block design, to study the effects of light and dark conditions on the survival of *A. foliaceus*.

4.2.2.2 The effect of different hosts on the survival of *A. foliaceus* populations.

Tanks and fish were maintained as in the light/dark trial (section 4.2.2.1), except that none of the tanks were shielded from light. Three tanks contained a single roach fry (*Rutilus rutilus*) circa 3cm in length and, three tanks held a single common carp (*Cyprinus carpio*) circa 7cm in length. Tanks were exposed to a 15L: 9D artificial light regime and held at a constant 15°C. Thirty metanauplii were added to each tank on day 1, following the method used in the light/dark trial. Fish were examined using the methods described in section 4.2.1.3.

4.2.2.3 The effect of temperature on the survival of *A. foliaceus* populations.

Nine, 10L static water tanks were used in this experiment. Groups of three tanks were held at 10°C, 20°C and 25°C in three separate water baths controlled by 300W aquarium heaters. Each tank contained a single common carp circa 7cm in length. Tanks were kept in total darkness for the duration of the experiment. Experimental fish and tanks were maintained as described in 4.2.2.1. Thirty freshly hatched metanauplii obtained as described in 4.2.1.1 were allocated to each tank on day 1. Metanauplii were hatched at 15°C and were acclimatised to the temperature of their experimental tank over 12 hours, by placing them in a beaker containing 500ml of water into the study tank. Fish were examined using the methods described in section 4.2.1.3. The experiment was terminated when no *A. foliaceus* remained in the system.

4.2.3 The Off Host Viability of Metanauplii

Eggs of *A. foliaceus* were placed in a beaker of carbon-filtered mains water in an incubator held at 12°C and monitored daily for hatching. On hatching, 30 metanauplii were randomly allocated between 3 beakers of carbon-filtered water. These beakers were then kept at 12°C until required for infection. Three 10L, static tanks containing 8L of carbon-filtered water were placed in a water bath at 12°C. A single carp of 7cm was held in each tank. All the metanauplii from a single beaker were added to tanks 1,2 and 3 on days 1, 3, & 6 post-hatching, respectively. Experimental fish and tanks were maintained as described in 4.2.2.1. Fish were examined for the number of attached *A.*

foliaceus on days 1 and 7 post-infection using the methods described in section 4.2.1.3. The proportion of *A. foliaceus* that attached to fish in each of the tanks at each day was compared using a chi-squared test. The experiment was terminated on day 10 post infection.

4.2.4 Local habitat effects on infection levels of *A. foliaceus* within a lake:

A cage trial was set up in a 9 acre trout lake (study site 2 from population study) with a heavy infection of *A. foliaceus* over the period 3rd-11th July 2002. Three $1m^3$ cages were placed along eastern margin of the lake in Northern, Central and Southern regions in water over 1m deep (Figure 4.2). Another three cages of the same size were paired with these, locating them in open water over 1m deep, parallel to the marginal cages. During the study there was a southerly wind and the water clarity was between 2.6 and 3 Secchi disc units (see chapter 3, section 3.2.2) throughout the lake.

Fifteen, 250g farmed rainbow trout not previously exposed to argulids were randomly allocated to each cage, using a random number generator to randomise the order of the numbers 1 to 90. The resulting numbers set was split into six groups and each was allocated to a cage. Ninety fish were netted from their holding raceways into a container. These were then caught individually and the order in which they were caught was used to determine which cage they went into by corresponding this to the numbers generated (de Clers, 1994). One week-post stocking all fish were removed individually from each cage, killed by concussion and placed into a plastic bag labelled

with the cage and fish number. The net used to capture fish from the cage was checked for *A. foliaceus* between fish by rinsing in a bucket of water and filtering the water through a 1mm mesh. Any found were placed into the bag with the appropriate fish.

Figure 4.2. Map of the study site 2, used to elucidate local habitat effects on infection levels of *A. foliaceus*.

Parasite counts were made by examining fish in a white tray with the aid of a hand lens. Parasite numbers were recorded and specimens of *A. foliaceus* from each cage were pooled for marginal and open water habitats and preserved in 70% ethanol for subsequent length frequency analysis, using the methods described in chapter 3 (sections 3.2.8). Comparisons of the population structure between the marginal and open-water habitats were based on the length frequency data and cohorts defined through fitting mixtures (chapter 3, section 3.2.9). Abundance of the parasite was compared between open water and marginal habitats and each region of the lake by a 2-way ANOVA, using the general linear model (GLM) function in SPSS v.11. Prevalence between open water and marginal habitats was compared using chi-squared tests, and the mean length distribution compared using bootstrap 2-sample t-tests with the statistical package Quantitative Parasitology 2 (QP2). The variance to mean ratio of the parasites abundance was also calculated in QP2 to show how the parasite was distributed through the host population.

4.3 Results

4.3.1 Maintaining laboratory cultures and developing a standard challenge method using hatched *A. foliaceus* and eggs:

The results of studies designed to maintain a laboratory population of *A. foliaceus* are summarised in table 4.1. The infection protocol was effective with 12-14 *A. foliaceus* attaching per fish from a possible 15. However, none of the systems were able to maintain an established population of *A. foliaceus,* with populations dieing out within 16 days where hatched parasites were used for the infection. Both re-circulating and flow through systems were found to be unsuitable as parasites were washed out of the system or trapped in the filters. Temperature appeared to have little effect.

The most effective system was a static system. High levels of predation of the parasite were observed in the tank containing five fish. When the number of hosts was reduced to one fish, survival time increased, although predation was still observed. The longest survival time was found in the static tank in which eggs were placed with the host. Hatching began 5 days after the start of the experiment. In this tank parasites remained in the system for 26 days post hatching and one female *A. foliaceus* reached sexual maturity and laid a small number of eggs.

Table 4.1. Survival of *A. foliaceus* in different tank systems

Method	Mean number of *A. foliaceus* per fish	Survival Time (Days)	Possible explanation for die out
Flow Through 9°C	14	7	Parasites washed out of the system
Flow Through 15°C	12	9	Parasites washed out of the system
Re-circulating 10°C	12	6	Parasites trapped in filtration system
Re-circulating 18°C	13	7	Parasites trapped in filtration system
Static infected with adult *A. foliaceus* – 5 fish	14	12	Parasites eaten by host
Static infected with adult *A. foliaceus* – 1 Fish	13	16	Parasites eaten by host
Static infected with eggs of *A. foliaceus*	30 Eggs	26 (from hatching starting).	Parasites eaten by host

4.3.2 Survival Studies

4.3.2.1 The effect of light & dark conditions on the survival of *A. foliaceus* populations.

The initial infection under both light and dark conditions was very successful with all metanauplii attaching to a host within a day. Numbers dropped rapidly within a week

to between 40-60% of the initial infection level, with the median survival of the parasite was between 10.78 and 17.50 days in all tanks apart from the light tank 1 (figure 4.1), which had a median survival time of over 63 days.

Comparing the pooled data between the light and dark groups suggested that under light conditions the parasite survived significantly longer (Table 4.2). When the data set is broken down into pairwise comparison it is clear than this relationship was only present in the first and third pairs of tanks. In the second pair of tanks the relationship was reversed with the parasite surviving longer under dark conditions than light, the difference was not, however, statistically significant.

Table 4.2. Summary of Life Table data comparing survival of *A. foliaceus* on *C. carpio* under light and dark conditions.

Data Set	Median Survival Time (days): Light	Median Survival Time (days): Dark	Wilcoxon (Gehan) Statistic	Degrees of Freedom	Significance (Probability)
Pooled	17.50	12.74	12.684	1	0.0004
Pair 1	63.00+	10.78	28.385	1	0.0000
Pair 2	15.96	17.50	1.776	1	0.1827
Pair 3	14.42	12.81	0.324	1	0.5691

4.3.2.2 The effect of different hosts on the survival of *A. foliaceus* populations.

The initial infection was very successful on both host species, with all metanauplii attaching to their hosts within a day. Numbers dropped rapidly with a median survival time of between 11.20 and 12.18 days in all tanks (Table 4.3). No significant differences in survival were observed in either the pairwise comparisons or pooled data. However, in all cases median survival was slightly less on carp.

Table 4.3. Summary of Life Table data comparing survival of *A. foliaceus* different host species: *C. carpio* and *R. rutilus*.

Data Set	Median Survival Time (days): Roach	Median Survival Time (days): Carp	Wilcoxon (Gehan) Statistic	Degrees of Freedom	Significance (Probability)
Pooled	11.83	11.41	1.220	1	0.2693
Pair 1	11.83	11.62	0.001	1	0.9699
Pair 2	11.62	11.41	0.208	1	0.6485
Pair 3	12.18	11.20	1.888	1	0.1694

4.2.2.3 The effect of temperature on the survival of *A. foliaceus* populations.

There was no significant difference in the survival of *A. foliaceus* populations at 10°C and 20°C (Figure 4.3, Table 4.4); however, survival was significantly longer ($p<0.05$) at both of these temperatures than at 25°C (Table 4.4). Although in real time the parasite

populations held at 10°C and 20°C survived for approximately the same period, those parasites held at 20°C had developed much further than those at 10°C. Using the growth equations developed in chapter 3 (section 3.3.3) daily growth rates for each population were calculated, showing that at 20°C, growth was approximately four times faster than at 10°C and the parasite was therefore four times larger at the median survival time (Table 4.5). This data also shows that although the 25°C population only survived for a short time, the parasite actually reached a larger size than in the 10°C population by the median survival time.

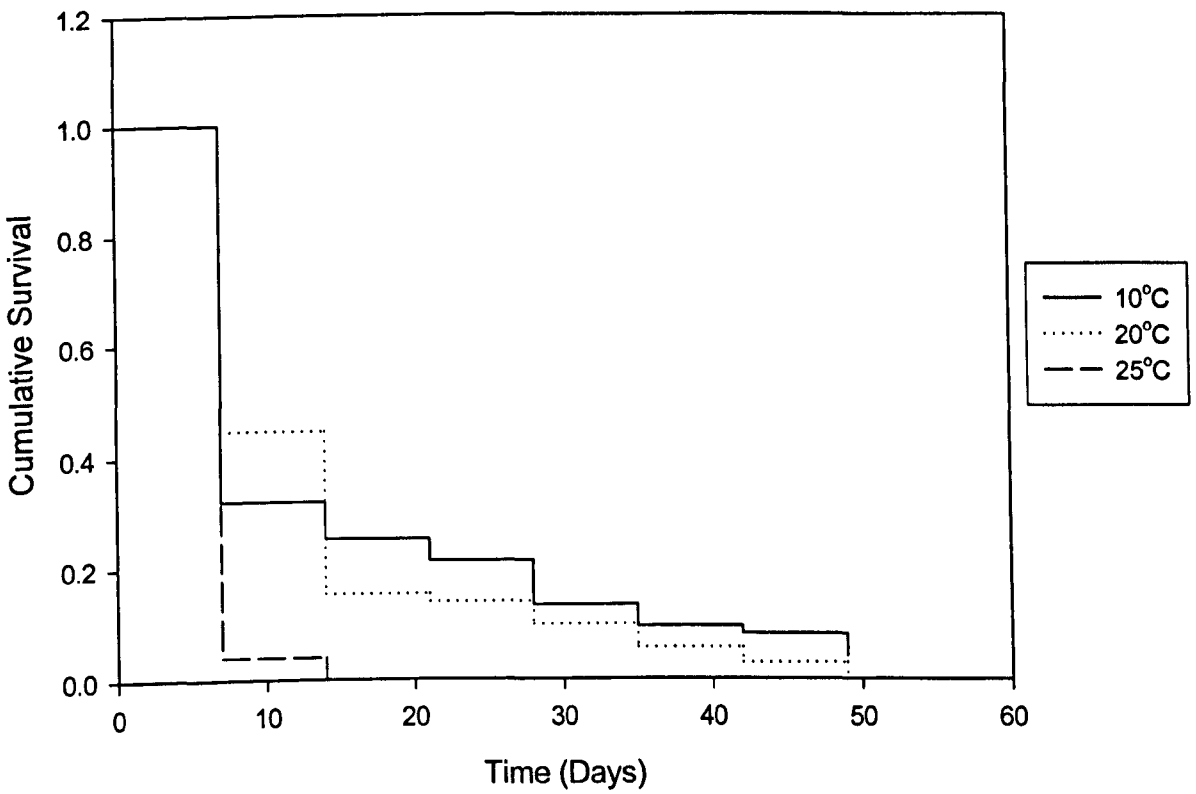


Figure 4.3. The survival of *A. foliaceus* populations under different temperature regimes.

Table 4.4. Comparison of survival times of *A. foliaceus* populations held under different temperature regimes, based on life-table data.

Data Set	Wilcoxon (Gehan) Statistic Comparing Real Time	Degrees of Freedom	Significance (Probability)
10°C vs. 20°C	0.764	1	0.3820
10°C vs. 25°C	20.980	1	0.0000
20°C vs. 25°C	34.692	1	0.0000
Overall Comparison	32.389	2	0.0000

Table 4.5. Median survival times and growth rates of *A. foliaceus* held under different temperature regimes.

Temperature (°C)	Median Survival Time (Days)	Growth per day (microns)	Size (microns) at median survival time (assuming parasite hatches at 800 microns)
10	12.18	22.1	1069
20	13.30	81.7	1887
25	3.64	125.7	1258

4.3.3. The off-host viability of metanauplii

Table 4.6 shows the percentage of metanauplii from each of the three challenge groups (1, 3 and 6 days post-hatching), that had successfully attached one day post-infection. Also shown is the percentage of these surviving at day 10 post,-infection.

Table 4.6. Infection success and subsequent survival of *A. foliaceus* metanauplii held off a host for different time periods prior to infection.

Group: Number of days post hatching	Percentage of metanauplii attached 1 day post-infection	Percentage of attached metanauplii surviving to 10 days post-infection
1	80	67
3	27	25
6	0	N/A

The infection success of the parasite dropped markedly the longer the parasite remained without a host after hatching. Infection success was high (80%) if the metanauplii were exposed to a host one day after hatching. The subsequent survival of these attached parasites was also high with 67% of those infecting their host surviving for a further 10 days on the host. In the group of metanauplii not exposed to a host until 3 days post hatching the infection success dropped to 27% and only 25% of these survived the subsequent ten days on their host. None of the final group of parasites, not exposed to

a host until six days post-hatching, were capable of infecting their host fish, even though the parasites were alive at this time.

4.3.4. Local habitat effects on infection levels of *A. foliaceus* within a lake:

During the experiment the water temperature averaged 19.5°C and the water clarity 2.8 S.D.U. Data from the population study (chapter 3) on the level of infection in the lake showed 100% prevalence and an abundance of 184 *A. foliaceus* per fish during July 2002. Table 4.7 gives a summary of population data collected during the cage trial. Although prevalence was slightly higher in the open water environment, no significant differences were observed between the open water and marginal habitats. In both environments the variance to mean ratio was low suggesting a normal distribution of the parasite throughout the population (Wilson *et al.* 2002). Significant differences were observed between the mean size of the parasites found in the two habitats, with the mean length significantly shorter in the open water cages at 3.3mm compared with 3.8mm. Length frequency analysis of the samples revealed two cohorts of the parasite in the open water habitat, averaging 2.1 and 4.9 mm in length (Figure 4.4). In the marginal habitat only one cohort was present averaging 4.8mm.

Table 4.7. Comparison of *A. foliaceus* populations in caged rainbow trout held in open water and marginal habitats in July 2002.

	Margins	Open Water	Significance
Prevalence	89.7%	94.6%	P=0.647
Variance/Mean Ratio	2.25	2.40	-
Average length (microns)	3816	3314	P=0.006

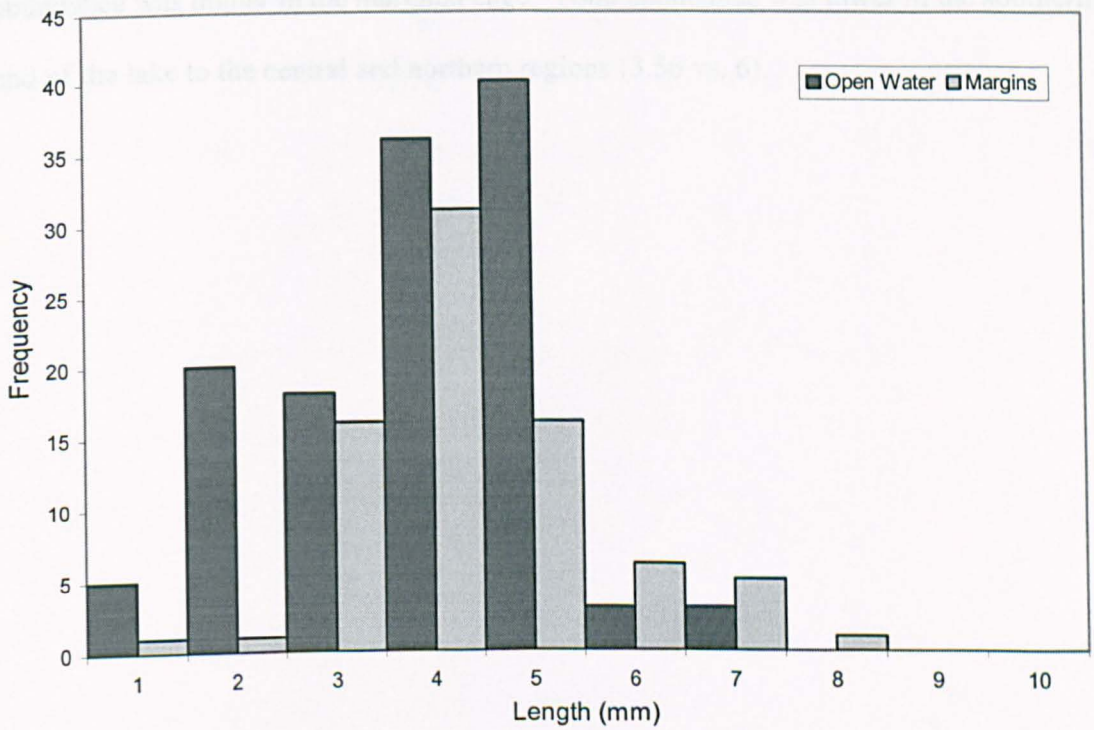
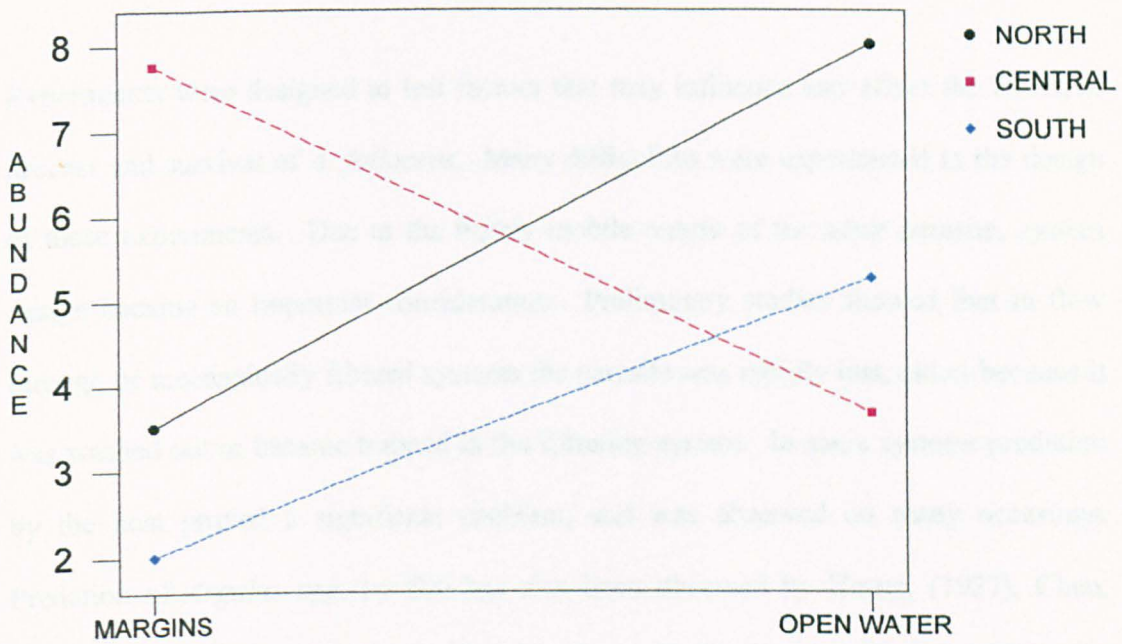


Figure 4.4. Length/frequency distribution of *A. foliaceus* in caged rainbow trout populations held in open water and marginal habitats in July 2002.

Variance in *A. foliaceus* abundance data between lake locations and open water and marginal habitats was homogeneous (Levene's test, F=1.56, df1=5, df2=60, P=0.19). 2-way ANOVA showed very strong and statistically significant interaction effect between habitat and lake region. This demonstrated that the relationship between habitat and abundance changed in different locations within the lake. Tukey's multiple comparisons test revealed that significant differences occurred between marginal and open water habitats in each separate region, but the direction of this relationship varied between regions (figure 4.5). In both the northern and southern regions the abundance was highest in the open water cages, however, in the central region of the lake abundance was higher in the marginal cage. Total abundance was lower in the southern end of the lake to the central and northern regions (3.56 vs. 6).

Table 4.8. ANOVA table showing the relationship between the abundance of *A. foliaceus* on caged rainbow trout in marginal and open water habitats in different locations in a lake.

Source	Type III Sum of Squares	d.f.	Mean Square	F	P
Corrected Model	309.04	5	61.81	8.52	0.000
Intercept	1577.38	1	1577.38	217.53	0.000
Location (N,C,S)	**67.06**	**2**	**33.53**	**4.62**	**0.014**
Habitat (M, OW)	**23.30**	**1**	**23.30**	**3.21**	**0.078**
Location*Habitat	**220.26**	**2**	**110.13**	**15.19**	**0.000**
Error	435.08	60	7.25		
Total	2238.00	66			
Corrected Total	744.12	65			



Margin, Central	P<0.05				
Margin, South	ns	P<0.05			
Open water, North	P<0.05	ns	ns		
Open water, Central	ns	P<0.05	ns	ns	
Open water, South	P<0.05	ns	P<0.05	ns	P<0.05
	Margin, North	Margin, Central	Margin, South	Open water, North	Open water, Central

Figure 4.5. Interaction plot showing the abundance of *A. foliaceus* on caged rainbow trout populations held in open water and marginal habitats in different locations in a lake in July 2002. Table shows where statistically significant differences lie according to Tukey's pairwise comparisons (ns= not significant).

4.4. Discussion

Experiments were designed to test factors that may influence and affect the infection process and survival of *A. foliaceus*. Many difficulties were experienced in the design of these experiments. Due to the highly mobile nature of the adult parasite, system design became an important consideration. Preliminary studies showed that in flow through or mechanically filtered systems the parasite was rapidly lost, either because it was washed out or became trapped in the filtration system. In static systems predation by the host proved a significant problem, and was observed on many occasions. Predation of *Argulus* spp. by fish has also been observed by Herter, (1927), Chen, (1933), Bower-shore, (1940), Kiselev & Ivleva, [1953 (cited in Bauer, 1970)] and Thomas, (1961). One possible method of reducing the level of predation would have been to keep the experimental fish fed to satiation at all times. However, in static tank systems this would lead to unacceptable levels of nitrogenous waste in the water and compromise the welfare of the experimental animals. A more suitable option was to reduce the number of hosts in a tank to one. Although this had some success and the survival time of the parasite was increased, predation was still significant. To overcome this problem, survival studies were designed to follow the rate of decline in the parasite population under different conditions over time.

4.4.1. The effect of light & dark conditions on the survival of *A. foliaceus* populations.

Results of the preliminary trials suggested that, under certain conditions, predation upon the parasite by the host may be substantial, and poses the question whether it could be sufficient to regulate the parasite population. Some success was achieved by Treasurer (1993) in regulating sea lice populations in caged salmon using wrasse (Labridae). Bower-Shore (1940) and Bark (2000) suggest that roach may predate upon *Argulus* spp. at a level sufficient to regulate the parasite, however, no controlled studies have been conducted, and no quantitative data is available.

During the cross-sectional study (chapter 2), water clarity was identified as a potential risk factor in determining whether a fishery was likely to suffer a problem infection, i.e. turbid waters were at greater risk. The longitudinal study (chapter 3) identified a significant correlation between water clarity and the abundance of *A. foliaceus* in the subsequent month, showing that, as the water clarity decreased, the burden increased. There are two possible hypotheses to explain this:

a) Increased turbidity changes the host parasite interaction, increasing the infection success.

b) The reduction in water clarity reduces the level of predation on the parasite by the fish host.

The light/dark survival study was designed to test these hypotheses, but found no differences either in the initial infection success or the survival of the parasite over time under either set of conditions. Mikheev, Mikheev, Pasternak & Valtonen (2000) demonstrated differences in the infection success of advanced stages of the parasite on perch under light and dark conditions. They found that under darkened conditions perch suffered heavier infections of *A. foliaceus* than perch kept in illuminated conditions. They showed this to correspond with the sedentary behaviour exhibited by perch in the dark, and the parasite adopting an active swimming pattern under darkened conditions. They also demonstrated that this pattern varies between different host species with the opposite occurring in roach, which became more heavily infected under illuminated conditions. This was attributed to the bright colouration of the fish attracting the parasite.

In this study no differences were observed between the initial infection success under light and dark conditions. There are several possible explanations for this. Firstly, the tanks used for the experiment were fairly small and therefore likely to lead to high infection levels due to the high chance of random host parasite contact. Secondly, the parasites used in the trial were at the metanauplius stage as opposed to the more developed stages used by Mikheev *et al.* (2000) and may have a different infection behaviour from the advanced stages. Finally, the survival studies were conducted using carp as a host. It is possible that the host/parasite interaction under light and dark conditions does not alter in terms of success using this host species.

There was also no observed difference in the survival of the parasite over time under these conditions. Suggesting that water clarity/darkened conditions have little influence on the level of predation on the parasite. It would be worthwhile repeating the study using trout if a system that would maintain both the host and parasite could be developed. Trout are highly visual feeders and the level of visibility is therefore more likely to influence the level of predation, whereas carp rely more on chemosensory and tactile cues during feeding, so that water clarity is likely to have less effect (Barrett, Grossman & Rossenfield, 1992).

4.4.2. The effect of different hosts on the survival of *A. foliaceus* populations

Several studies have shown that argulids show a preference for certain host species (Wilson, 1902, Bower-Shore, 1940, Gurney, 1948, Fryer, 1968, Kabata, 1970, Kimura, 1970, Morrice, 1976, Mishra, 1991, Shafir & Oldewage, 1992). What has not been definitively shown is whether this preference is due to host selection or whether the parasite cannot attach to or survive on, certain hosts better than others. This study was designed to determine the infection success and survival over time of *A. foliaceus* on two different host species; carp and roach. Keeping host populations separate allowed the elimination of host selection through behavioural differences by the fish. No differences were found between the initial infection success of the parasite and survival over time between these species. Useful information could be obtained by repeating the trial using mixed populations of roach and carp to examine whether there are any differences in host behaviour. As in the previous trial, the study was limited by small

tank sizes that may have increased both the infection rate and the level of predation by the host. Survival may also vary should the host fish shoal. Bower-Shore (1940) noted that predation by juvenile roach upon *A. foliaceus* was high, and even suggested the fish as a possible form of biological control. In this study there were no significant differences in the level of predation on the parasite by single roach and carp, however, repeating the trial using shoals of each species might yield a different result.

4.4.3. The effect of temperature on the survival of *A. foliaceus* populations.

Infection success of *A. foliaceus* was similar at 10, 20 and 25°C, however significant differences in the survival of the parasite were observed under these regimes. In terms of real time, no difference in survival was noted between the 10 and 20°C groups, but survival was significantly shorter at 25°C than in both the other groups. Under all three regimes thee was an initial infection success of 100%, but the population decreased rapidly over the first seven days of the experiment. After this the mortality rate slowed in the 10 and 20°C tanks, with the populations declining steadily until the experiment was terminated on day 49. In the 25°C tanks, however, the entire population had died out by day 14. The median survival time of this group was 3.4 days which is similar to the findings of Moller (1978) for *A. foliaceus* who observed the median survival at 25°C to be about four days. However, work by Schluter (1979) suggests that *A. foliaceus* can continue to survive and grow at temperatures up to 28°C. Median survival in the current study was slightly longer at 20°C than at 10°C (13.3 days compared to 12.18). This is in contrast to Moller's (1978) findings that median

survival was approximately 8 days longer at 10°C (circa 13 days) than at 20°C. A possible explanation for this is that in Moller's trial the parasite was not kept on a host, but instead fed at weekly intervals. It is possible that at temperatures of 20°C the parasite processed nutrients rapidly and a weekly feeding regime was insufficient for the parasite to survive.

Using the growth equations developed in the population study (chapter 3), it was possible to predict growth rates for all three groups. At 10°C, growth was very slow, and when comparing the size reached by the median survival time in each group, even the 25°C group, which had a median survival time of only 3.64 days had reached a larger size. By the median survival time, specimens in the 20°C group was substantially larger than in the other groups and, by the end of the trial on day 49, a remaining adult specimen measuring 4.9mm was found. This is close to the estimate of 4.8mm predicted by the growth model, and much larger that the estimate of 1.9mm for the 10°C group at the same time point. In conclusion it would appear that there is a greater likelihood of the parasite reaching adulthood at higher temperatures. However, further study is required to understand the impact of temperature on the overall population success as, at lower temperatures, it is possible that the parasite may produce a greater number of viable eggs than at higher temperatures to compensate for the reduced likelihood of reaching adulthood. The number of eggs produced by sea lice (*Lepeophtheirus salmonis*) is higher at lower temperatures (Ritchie, *et al.*, 1993), but infection success and survival of the parasite is better at warmer temperatures (Tucker, *et al.*, 2000). Further studies are required to determine whether similar strategies occur

in argulids. Shafir & Van As (1986) found that the hatching success of *A. japonicus* eggs increased with temperature up to 25°C, however, no studies have determined the influence of temperature on fecundity and number of eggs laid. This information is critical to predict the reproductive success of cohorts, and therefore an important aid in managing parasite populations.

4.4.4. The off host survival and viability of *A. foliaceus* metanauplii

The results of these experiments suggest that the metanauplii of *A. foliaceus* require to attach to a host within a few days of hatching, and do not appear to have sufficient energy reserves upon hatching to allow them to survive for more than a few days without a host. This is unlike the parasitic copepods (*Lepeophtheirus* spp. & *Caligus* spp.) which hatch as a less advanced, non-feeding, nauplius stage, which allows for dispersal of the parasite. This however, is likely to be unnecessary in the case of argulids due to their lack of host specificity, the relatively small habitats in which they live and the egg laying strategy which they appear to adopt. Results of the egg laying studies detailed in chapter 3 suggest that the eggs of *A. foliaceus* are laid in areas where fish naturally congregate. This would mean the parasite is likely to hatch in close proximity to a host and the chances of infection are high, thus negating the need for an extensive dispersal period.

The study also suggests that the longer it takes the metanauplius to infect a fish the lower the chances of subsequent survival on the host. This may be a result of the

parasite having used up too greater a proportion of its energy reserves, possibly preventing further moulting even though it may have successfully attached to a host and be able to feed. This has been shown to occur in *Lepeophtheirus salmonis* (Tucker, 1998). Further investigations are required to establish the affect of temperature on the survival and settlement of *A. foliaceus*.

4.4.5 Local habitat effects on infection levels of *A. foliaceus* within a lake.

The strong interaction effect observed in the abundance of *A. foliaceus* between habitat and lake region suggests that the parasite has a patchy distribution within a lake. This patchiness may be determined by areas used for egg laying by the parasite, by patchiness in the distribution of fish hosts in the lake or a combination of the two. Studies on the locations of egg laying (section 3.3.5, page 145) suggest that the latter is likely as eggs were located in natural fish holding areas. This would not only increased the number of eggs in particular regions but would also increase the number of hatched stages due to the high densities of hosts. Stammer (1959) observed *A. foliaceus* congregated in areas into which prevailing wind blew. Leighton *et al.* (2000) also demonstrated the same relationship between wind direction and the distribution of the digenean cercariae of *Trichobilharzia stagnicolae*, *T. physellae* and *Gigantobilharzia* sp.. Although no clear reasons for the variation in infection levels in the study cages was observed between habitat and region the overall infection levels were higher at the northern and central regions of the lake into which the prevailing wind blew compared to the southern region. This may affect the infection level by causing free-swimming

stages of the parasite to congregate in these regions. Wind may also affect the distribution of *A. foliaceus* indirectly by influencing the distribution of the fish. The fishery owner had observed that fish capture rates were always higher in areas of the lake that had received the wind for several days, hypothesising that fish moved into these areas as prey items were driven there by the wind. If fish densities increased in the northern and central area as a result of the prevailing wind, a corresponding increase in number of parasites present in these regions would also be expected.

In both open water and marginal habitats the variance to mean ratio was low, suggesting there was no aggregation of the parasite population between hosts and the parasite was normally distributed. This is unusual amongst parasite populations as it is well documented that they are normally follow an aggregated distribution with most hosts having low numbers or no parasites and a few having a high number (Anderson & May, 1978). Poulin & Fitzgerald (1988) observed this in *A. canadensis* in stickleback populations kept under laboratory conditions. They found that fish already infected with one specimen were more likely to become infected than fish harbouring no infection, and suggested this may have been due to altered swimming behaviour by infected fish or by chemical cues released by infected fish or attached argulids. In the current trial it is possible that aggregation did not occur as the swimming behaviour of the fish may not have been affected by the infection due to their confinement and to the relatively large fish size compared to the low infection level. Also, due to the high density of fish within a cage compared to the lake it is likely that any chemical cues released by the host or parasite would distribute rapidly throughout the cage making it

unlikely aggregations would occur through this means. Other mechanisms responsible for parasite aggregation such as size, age, genetic strain and sex (Wilson *et al.*, 2001) and differences in exposure time were, unlikely to occur, as fish in the caged populations were stocked at the same time point and homogeneous, being all female, of the same age and strain and of similar size. The sample of lake fish taken during July (12 days after termination of the cage trial) as part of the population study (chapter 3) showed a greater degree of aggregation in the parasite population, with a variance: mean ratio of 4.73. This would be expected as the lake population varied in size, age, strain, species (rainbow and brown trout) and length of exposure (i.e. time in the lake).

An interesting finding of the study was a statistically significant difference in the mean size of the parasites found in the open water and marginal habitats. The mean length of *A. foliaceus* on the open water fish was smaller than that of the fish in the margins. At the time of the experiment length frequency analysis from the caged populations detected two cohorts averaging 2.1mm and 4.9mm in length. This was similar to the length frequency distribution of the parasite in the lake population taken 12 days later, which detected two cohorts averaging 2.6mm and 5.4mm. The smaller mean size of the parasite on the open water fish was due to infection by a greater proportion of parasites from the smaller cohort. Egg laying data from the longitudinal study showed that *A. foliaceus* lays its eggs in deep water, especially as the lake temperature increases. This would mean a greater proportion of eggs would probably be laid towards the deeper centre of the lake. Thus, fish in the marginal cages might not receive the same level of exposure to hatching stages as those in the open water. The infection on cage fish in

the lake margins may be a result of direct fish to fish transmission, due to lake fish sheltering amongst marginal cover (possibly around the cages), and the larger stages of the parasite which leave their hosts more regularly than the smaller stages (Kimura, 1970).

4.5 Summary

These trials have successfully explored a number of factors affecting the survival and infection success of *A. foliaceus*. Preliminary trials showed *A. foliaceus* to be difficult to maintain on trout in the laboratory for a variety of reasons, but predominantly predation by the host. This limited the experimental designs that could be used, and led to the development of trials that followed survival of populations of *A. foliaceus* over time in relation to a variety of factors. No differences in infection success or survival were observed under light and dark conditions, or on two different host species. Survival was significantly lower at 25°C than at lower temperatures, however, further investigations are required establish whether the same result would be obtained if the period of acclimatisation to this temperature was extended prior to infection. No significant differences in infection or survival were observed at 10°C and 20°C, but parasites in the 20°C population reached a more advance developmental state by the time of population extinction. Further investigation is required to establish the effect of temperature on the off host survival of *A. foliaceus* metanauplii which, from this study, appear to remain viable for between 3 and 6 days post-hatching at 12°C.

CHAPTER 5: SUMMARY AND CONCLUSIONS

This project arose from the emergence of a problem experienced by the UK stillwater trout fishery industry that was perceived to be caused by *Argulus* spp.. Prior to this study only a handful of papers had been written documenting the impact of *Argulus* spp. within lake systems (Knight, 1996, Northcott, *et al.* 1997, Gault, *et al.* 2002), and no work had been published on ways in which the parasite might be controlled in these situations. An increasing number of reports of problem infections had been received by both the Environment Agency of England and Wales and the University of Stirling's Institute of Aquaculture. However, no information was available to explain why the number of reports were increasing, what the nature of the problem was, or its real extent and severity.

This study was carried out within the broad aims of the sponsors, which were as follows:

a) To review of the current perception of the extent and severity of *Argulus* spp. infections in UK stillwater trout fisheries, and identification of the methods currently employed for controlling these infections.

b) To review and increase the knowledge of the biology and ecology of *Argulus* spp. in relation to these systems.

c) To assess the prospects for novel control and management strategies to reduce economic loss.

In order to meet these aims the study was conducted in a series of phases. Based on the available information on *Argulus* spp. a questionnaire was developed that subsequently formed the basis of a cross-sectional study of stillwater trout fisheries. This study was used to assess the nature of the problem and identify risk factors associated with problem infections. Information gained as a result of this study was subsequently used in a field based longitudinal study of the population ecology of the parasite, supported by a series of laboratory experiments. The success, limitations and findings of these components are discussed below.

5.1 Cross-sectional Study

This study successfully determined the nature of the problems caused by *Argulus* spp. in stillwater trout fisheries. It established that a substantial proportion of the fisheries visited had suffered a problem infection, but also determined that the presence of *Argulus* spp. was not necessarily a problem. *A. foliaceus* was the species responsible for all infections, with a single exception where *A. coregoni* was found. The study established that there was a lack of practical control measures, but successfully identified several risk factors associated with problem infections that might be manipulated in order reduce the impact of the parasite.

The study has provided useful information and was successful considering its limited sample size and lack of prior knowledge about the parasite in the UK. Although time consuming and labour intensive, an interview-based survey provided a greater response rate and quality of data than could have been gained by telephone or postal based

alternatives. However, in order to reduce recall bias the survey had to be conducted over a short time scale, thereby limiting the number of fisheries that could be visited. Several risk factors were, however, successfully identified and subsequent investigation established a link between these and problem infections, even though the small sample size restricted the number of variables that could be included in the logistic regression models. Many hypotheses can be developed to explain how the risk factors that were identified may act, and the study therefore provided a valuable foundation on which to base further study. Without the risk factors identified through this study the subsequent population study and experiments would have lacked focus.

The lack of prior knowledge on the parasite reinforced the need for a study to establish its status in the UK, but also led to several difficulties in conducting the survey. A case definition could not be defined prior to the survey and had to be determined as part of it. The case definition was defined prior to analysing the dataset so as to prevent bias that might occur through judging the data prior to arriving at a definition. Ideally, the data would have been analysed by someone other than the data collector. Due to the retrospective nature of the study it was not possible to validate that a case had occurred (i.e. a problem infection). The nature of the perceived problem also made this difficult, as it was not quantifiable and not necessarily associated with the presence of the parasite, meaning there were no records on which to base a validation. Fisheries currently do not have to register with any authorities and are not obliged to report *Argulus* spp. infections to any authority. As no licensed treatments are currently available for the control of *Argulus* spp. in the UK, infections are rarely reported to

veterinarians. The Environment Agency do keep some records of *Argulus* spp. infections that have either been reported to them or identified through routine health checks. However there were no records on the level of infection and whether it had caused a problem within individual waters. Therefore, due to a lack of time and resources, it was not feasible within this study to determine a case definition prior to data collection. In any subsequent studies of this nature an outcome variable that can be quantified and validated should be found. This may not actually be related to the parasite itself, but the perceived problem. For example, rod capture rates in August (when the parasite is perceived to cause the greatest problems) could be taken as the outcome variable, and the abundance of the parasite in this month used as independent variable. This would establish the impact of the parasite on capture rates in relation to other factors. It would also allow more quantitative data on factors such as the level of macrophyte cover or the biomass of fish species to be collected, which was not possible during the current survey due to its retrospective nature.

The survey of the distribution of *Argulus* spp. has shown the parasite to be widespread throughout England and Wales, and updated our knowledge of the distribution of the introduced species, *A. japonicus*. The survey was unable to determine whether the parasite had spread within the UK over recent years, or routes by which the parasite was transmitted between sites. An attempt was made to address the problem by asking fisheries about their suppliers in order to see if infected sites had common suppliers. It became apparent however, that many suppliers are often used by individual fisheries and, with no information available on the time taken from the introduction of the

parasite to the development of a problem infection, it was not possible to identify suppliers which might be introducing infections. The survey did however identify crayfish as a possible transmission vector for the parasite, and further screening of other potential vectors and watercourses feeding each site might identify other potential sources of infection. However, current legislation on the movement of fish and screening for disease does not allow this information to be elucidated. *Argulus* spp. are not notifiable, it does not have to be recorded as part of the remit of Environment Agency or CEFAS health checks (the two regulatory bodies responsible for fish movements in England and Wales) and infections are only sometimes recorded as a matter of interest and the species involved is often not identified. Environment Agency data showed *Argulus* spp. to be widely distributed throughout England and Wales but no information was available for Scotland and it was not possible to track changes in parasite distribution. There is a current perception that *Argulus* spp. is spreading north into Scotland (Northcott, *et al.* 1997 and pers. comms. with industry representatives). This study provided no evidence to support this hypothesis, and an intensive survey to determine the current distribution of the parasite is required.

To identify the sources of infection for a site a prospective study of uninfected waters would be required, following supply sources and other routes of introduction. Such a survey would be time consuming and expensive, and was outwith the scope of this project. This survey does, however, highlight gaps in the current legislation governing fish health, demonstrating that, at present, the information collected cannot be used to track and monitor native pathogens and their occurrence.

5.2 Population ecology

This study has increased our knowledge of both the life-cycle and the population ecology of *A. foliaceus* in stillwater trout fisheries. It has also elucidated how the risk factors that were identified as part of the cross-sectional study may interact with the *A. foliaceus* population. The study successfully applied novel techniques for discriminating cohorts present in the parasite population through length frequency analysis. This analysis allowed growth, recruitment and mortality for each cohort to be defined, and would have provided even greater information if narrower sampling intervals and larger sample sizes had been possible. This technique could be extended into other parasite systems where distinct life stages are not readily discernable and with sufficient data points it could be used to develop life table data with which to compare populations.

The study established a significant correlation between water clarity, temperature and the abundance of *A. foliaceus* in the subsequent month. No significant correlations were found between rate of stock turnover and the abundance of the parasite, however the parasite was observed to die out in waters with fast stock turnovers. Water clarity was also found to be correlated with the rate of stock turnover, however, the parasite's abundance was not. This is interesting as the parasite is perceived to reduce capture rates. The majority of the variability in rates of stock turnover was found to be due to site differences, and likely to be attributed to the amount of fishing pressure. Although this study would suggest the parasite is not necessary responsible for reducing the

number of fish caught, further investigation is required to understand the average catch per angler in relation to the parasite's abundance. No evidence was found to suggest a causal association between a drop in the lake water level and reduced burdens of *A. foliaceus*. It was hypothesised that a drop in water level may expose the eggs of the parasite, causing them to die thereby reducing any subsequent recruitment. Studies on the egg laying habits of the parasite showed this to be unlikely as the parasite was found to lay its eggs in deeper water, especially during the summer months when the water level was most likely to drop.

In terms of the parasite life cycle the study found that up to three cohorts over-winter, two as eggs which hatch in April and May in the subsequent year, and one as hatched stages the eggs of which hatch in the following June. These give rise to three subsequent generations that emerge in July, August and September. This temporal spacing of cohorts makes it difficult to see how control of the parasite could be achieved through targeting any one-time point. If fishing pressure is sufficient over the winter months the over-wintering hatched stages of the parasite will be removed from the host population. This however, leaves the two cohorts of over-wintering eggs to hatch in the spring. These cohorts need to be targeted, either through destroying the eggs over the winter months (possibly through draining and drying the fishery) or by managing the fishery in such a way as to remove the juvenile stages of the parasites from these two cohorts before they have the opportunity to lay eggs. Targeting these three over-wintering cohorts should reduce recruitment during the summer months when the parasite is perceived to cause problems.

The information gain from this study has been valuable in forming the basis of a series of management strategies, despite constraints due to the effort and biases involved in fish capture, which was time consuming, costly in terms of man-power and often produced relatively small sample sizes. This restricted the amount of fish level data that could be used to explain variations in parasite numbers between fish. It was hoped that the effect of fish size and species on the parasite burden could be determined, but insufficient numbers of different species or sizes of fish were obtained to allow meaningful comparisons of this kind. Sample sizes were also small in comparison to the total trout population of each lake and, therefore, caution must be taken when interpreting prevalence and intensity data as fluctuations may be attributed to sampling variation. Small sample sizes also reduce the likelihood of detecting the parasite when it is only present in low numbers, resulting in errors in documenting its presence or absence from the population. Sampling such extensive aquatic systems for pathogens is difficult and many of the sampling methodologies suggested for disease sampling in terrestrial (Thrusfield, 2003) or intensively managed aquatic systems (Cameron, 2002) are not feasible or applicable to extensive fisheries. Fish capture methods are selective in terms of the species, size or swimming speed of the fish they target, so they will also only select pathogens occurring on that particular subset of the host population. For the purpose of this study, seine netting was used to sample fish. Depending on the mesh size used, this is perceived to show little bias in terms of the species and size of fish that it targets (Lagler, 1970). It is however, likely to be most effective against slower fish and therefore the most heavily infected, possibly leading to inflated estimates of prevalence and parasite burden. In practice this method of capture not only provided

higher estimates of parasite burden than rod capture but was also found to be relatively selective towards trout. This prevented a comparison of the life-cycle of the parasite between host species in the same system and an assessment of the role of other fish species as reservoir hosts. The experimental studies and literature show that many fish species can act as hosts for the parasite, however it was impossible to determine what proportion of the *A. foliaceus* population is harboured by these alternative hosts. This information would be important in the development of mathematical models to describe the population dynamics of *A. foliaceus*. Investigations into the bias occurring when sampling parasite populations through fish capture methods are needed, and protocols need to be developed for the most effective and least biased methods for conducting surveys of pathogens in extensive aquatic systems.

In both the population and cross-sectional studies the investigator was reliant on the fishery owner to provide reliable data on stocking, fish capture and water clarity. The quality of this data was variable between owners and, as a result, could lead to bias in the data sets between sites. The investigator was also reliant on the fishery owner's estimations of the standing stock of fish present in the lake, as neither mark-recapture nor catch depletion methods of estimating population size were feasible.

5.3 Experimental Studies

Experimental studies provide the most robust way of validating the findings of the two observational studies documented above. The experiments conducted produced

interesting findings in relation to the effect of temperature and different host species on the infection success and survival of *A. foliaceus*. This study, as much of the literature suggests, showed that other fish species can act as a reservoir host for the parasite. Unfortunately difficulties were encountered in maintaining parasite populations on trout in the laboratory, resulting in experiments being conducted on carp. Although this was unlikely to affect the results of the temperature trials, it was likely to influence the trial of survival under light and dark conditions where predation on the parasite by the host was an issue. This trial was designed to simulate the effect of low water clarity on the survival and infection success of the parasite. No significant differences were observed suggesting this is not a causal risk factor. The field data collected during the longitudinal study does however suggest a plausible biological link and the experiment should repeated on trout, which differ greatly in their behaviour from carp, before definitive conclusions are drawn.

5.4 Future Studies

Further experimental work needs to be conducted to confirm the nature of the relationship between the risk factors identified and the ecology of the parasite. To do this, an effective system for maintaining the parasite on rainbow trout in tank systems must be developed. This would allow the parasite to be studied under a variety of conditions and allow further information to be gathered on the effect of water clarity on the infection success and survival of the parasite.

Further epidemiological studies are required, specifically designed to determine routes by which fisheries become infected. On a small scale it may be possible to conduct a prospective cohort study, following a handful of uninfected waters over time in relation to factors likely to be responsible for transmitting the parasite. Such studies are time consuming and expensive, and in most cases unlikely to be feasible. It is also unlikely that the required numbers of suitable sites would be available at the start of any such study. One possible alternative approach would be to use a form of time until event analysis, such as Cox regression (survival analysis). This allows sites to enter the study at different time points as long as they meet relevant entry criteria. These could then be followed through time until a defined problem infection (which would be classed as the event) was observed. Sites not developing a problem infection by the end of the study could then be censored from the population.

The effect of possible interventions to control *Argulus* spp. must be assessed in the field. Although it may be possible to gain an indication of the effect of an intervention through laboratory trials, it is necessary to establish its effects in the field where there are many interacting factors. Ideally a series of controlled trials would be conducted to subject a group of infected waters to an intervention and use another group as a control and compare the effect. To do this a quantifiable response must be identified to allow comparisons to be made, e.g. an estimate of parasite abundance at a particular time or the rate of stock turnover. Such studies are time consuming, expensive and difficult to control and there are problems obtaining sufficient sample sizes. A more feasible (although more hypothetical) option is to develop mathematical models that describe

the population dynamics of *Argulus* spp. in relation to environmental variables and risk factors. Such models could be used to create simulations to identify the effect an intervention may have on the parasite population. The development of such models would rely on good data sets relating to parasite population dynamics and laboratory studies to quantify the effect of factors of interest. Similar models could also be developed to describe the effect of argulids and other variables on the rate of stock turnover, as this is a major concern for fishery owners.

CHAPTER 6: RECOMMENDATIONS FOR THE CONTROL AND MANAGEMENT OF *A. FOLIACEUS* INFECTIONS IN UK STILLWATER TROUT FISHERIES

A potential form of control of *Argulus* spp. has been developed by Gault *et al.* (2002). This method involves the collection of eggs of *Argulus* spp. on boards suspended in the lake and removing them from the system before the eggs can hatch. This is based on a similar principle described in Bauer *et al.* (1970) who describe the removal of argulid eggs from farm ponds either by collecting them on submerged doors or on bundles of floating sticks and removing them prior to hatching. However, no controlled trials have been carried out to show the impact such a measure would have in such extensive water bodies, such as those used in this study with so many potential egg laying habitats.

This project has led to an increased understanding of the life-cycle of *A. foliaceus* and how some risk factors may influence parasite population dynamics. This information can be used to identify potentially vulnerable points in its life cycle and suggest strategies that may reduce the parasite burden during the summer months when the parasite is perceived as a problem. The data also suggest that the parasite is not the sole cause of the problems observed and it may therefore be possible to alleviate the problem without actually reducing the parasite burden. From the information gained through the project it is possible to make the following recommendations to reduce the impact of the parasite in a stillwater trout fishery. This was the main aim of the study sponsors.

1) Introduce a screening and monitoring programme:

Every fishery is different and the exact timings and success of cohorts of *Argulus* spp. will vary between waters. None of the fisheries visited during the project had a regular screening programme in place. Regular screening of fish will allow a fishery owner to identify potential problems and take action before they occur by implementing interventions at optimal time points. By monitoring the parasite population in relation to the risk factors identified by this project and other factors fishery owners consider important, and keeping records of this data, a fishery owner will be able to gain a greater understanding of how the parasite population dynamics in their site are influenced. It is important that a dedicated screening programme is set up and that fishery owners do not just rely on visual inspections of angler caught fish as this is likely to lead to misleading conclusions.

2) If possible trickle stocking is better than batch stocking:

Currently two methods of stocking fisheries are used; trickle and batch stocking. Batch stocking involves stocking large numbers of fish at one time thus increasing the population size markedly. These fish are then caught over a period of months until the population is reduced to a certain level. The result of this is that fish remain in the lake for a long period of time thus increasing their exposure to infection. The high numbers of fish present after a stocking are also likely to increase the infection success of the parasite. If batch stocking is used it should be conducted so that lows in the fish

population correspond to periods when the parasite hatches. Trickle stocking involves replacing small numbers of fish on a very regular basis, often weekly. This keeps the standing stock of the lake relatively constant and reduces the amount of time that fish spend in a lake. This also has the advantage that, should a heavy infection level be experienced there are still likely to be clean, catchable fish present in the fishery due to the regular stocking, allowing the fishery to remain open. One of the fisheries surveyed during the cross-sectional study changed its stocking policy from batch to trickle stocking after an outbreak of *Argulus* spp.. This was perceived to substantially reduce the problem in subsequent years. The disadvantage with trickle stocking is that unless fish are farmed on site there is a delivery cost with every batch of fish introduced. This additional cost is often prohibitive to smaller fisheries that stock very low numbers of fish each week.

3) Avoid stocking in April and May and/or speed up stock turnover during this period:

Spring is a critical period in the life-cycle of *A. foliaceus* in UK trout fisheries, and its success during this period is likely to determine its abundance in the summer. Cohorts of the parasite emerge at the end of April and May. If the infection success of these cohorts can be disrupted, then the population size in subsequent months is likely to be reduced. Thus, a reduction in the number of hosts in the fishery at these times is likely to reduce infection success. This would be especially important in waters that employ a batch stocking policy, as adding large numbers of fish during this period would allow

them to become infected by the parasite and remain in the lake long enough to lay large numbers of eggs. Many of the fisheries visited throughout the study that used batch stocking, held their first stocking of the year between March and May. This would actually be the optimum time to hold a fallow period if this was possible for a fishery.

An alternative to this strategy is to carry out batch stocking at this time, but to increase stock turnover so that the parasites are removed before they reach a size where they are capable of laying eggs, thus reducing recruitment later in the season. This strategy has two advantages; a) at this time of year infected fish can still be rod caught as the confounding effects of low water clarity and high water temperatures are not yet combining with the parasite burden to reduce capture rates. If control through this method is left until later in the year, alternative methods of capture would be necessary as rod capture would be unlikely to target heavily infected fish. b) The lower temperatures observed at these time compared to the summer, give a larger window of opportunity to target the parasite due to its slower development times. Methods of increasing stock turnover during these periods will vary between fisheries but might include events such as a fishing competition, club or corporate day, which would remove large numbers of infected fish from the system. It may also be possible to increase turnover to some extent by stopping catch and release, which would prevent infected fish from being returned. However stopping this often popular practice may have the opposite effect, by reducing the number of anglers that would otherwise attend the fishery. In the warmer months it may be possible to target heavily infected fish

through seine netting, or by removing fish from cool water inlets, where observations during the study showed heavily infected fish to congregate.

4) Do not hold a closed season over the winter months:

Some fisheries hold a closed season over the winter months, often from October until March. In waters infected with *A. foliaceus* this might allow the parasite to over-winter as hatched stages which then lay eggs early in the spring once the water temperature rises above 8°C. By allowing fishing to continue over the winter months many of the infected fish are likely to be removed thus reducing egg laying in the spring and consequent recruitment later in the season.

5) Interventions should be used in spring to target hatched stages of the parasite and winter to target the eggs:

Although at present there are no licensed treatments to target the hatched stages of *Argulus* spp. in the UK, future treatments or treatments prescribed by a veterinarian under the cascade system could be used in April/May to reduce subsequent recruitment. This applies whether the intervention is a treatment applied to the watercourse or to the fish in the form of a vaccine or in-feed treatment. Eggs of the parasite should be targeted towards the end of winter when two cohorts are present ready to "kick-start" the population in the spring. At present the only legitimate way of targeting the eggs is through draining, drying and, if possible, liming the fishery. If this approach is taken, it

is necessary to drain the fishery completely as the project would suggest that the majority of eggs are laid towards the bottom of the lake. During the population study two of the sites conducted interventions during August when the burden of A. *foliaceus* was high. Although these interventions successfully reduced the abundance of the parasite in that month, it did not affect subsequent cohorts as there was no impact on eggs laid by earlier generations.

6) Increase water clarity:

Algal blooms have some potential for management, but water clarity less so. There are several commercial products that claim to break down algal blooms and there is much grey literature suggesting that dredging lakes and the use of barley straw will also help. If possible, lakes should have a fast turnover of water, although care must be taken that the water received is clean and low in nutrients, the use filter beds may be effective. Water from fish farms, septic tanks or stagnant ditches or ponds should be avoided. Removal of cages of fish if present may also reduce the amount of organic loading in the water. Coarse fish, especially cyprinids, are associated with turbid water due to their feeding behaviour (Lougheed, Crosbie, Chow-Fraser, 1998, Schrage & Downing, 2004), so their removal may be of benefit. Care should be taken that if water clarity is increased macrophyte growth does not become excessive, which can cause difficulties to angler. Several fishery owners also associated high levels of macrophyte cover with problem argulid infections. This was not identified as a risk factor, however, it was difficult to assess accurately with the methods used and is worthy of further study.

7) Removal of alternate hosts:

This was a factor that was unable to be effectively studied during this project, and the true impact of alternate host species is still unknown. Although some authors suggest certain fish species, such as roach, may predate upon the parasite (Bower-Shore, 1940, Bark, 2000), there is no evidence to show this to be effective at regulating the parasite population. It is however likely that fish species other that trout may act as a reservoir for the infection, allowing the parasite to persist if a trout population is not available, or aiding its reproductive success in the presence of a trout population. Removal of alternate hosts will eliminate this reservoir of infection and reduce stocking density. This is likely to make control of the parasite through management of trout stocks more effective.

Conclusions

This project has identified the need for methods with which to control *Argulus* spp. infections within the UK. The management strategies developed as a result of the project provide not only a chemical free alternative to the control of the parasite, but has also identified key points in the life-cycle of the parasite at which chemotheraputants should be targeted in order to minimise their use. Efforts should now be made to assess the efficacy of the management strategies suggested, and identify ways in which they can be improved.

REFERENCES

Ahne, W (1985) *Argulus foliaceus* L. and *Piscicola geometra* L. as mechanical vectors of spring viraemia of carp virus (SVCV). *Journal of fish diseases* 8 (2): 241-242.

Alabaster & Lloyd (1980) Water *Quality Criteria for Freshwater Fish.* Butterworths, London pp 297.

Anderson, R.M. (1974). Population dynamics of the cestode *Caryophyllaeus laticeps* (Pallas, 1781) in the bream (*Abramis brama* L.). *Journal of Animal Ecology* 43: 305-321.

Anderson, R.M. (1976) Seasonal variation in the population dynamics *of Caryophyllaeus laticeps. Parasitology* 72:281-305.

Anderson, R.M. & May (1978) Regulation and stability of host-parasite population interactions. I. Regulatory process. *Journal of Animal Ecology* 47: 219-247.

Anon. (1998) *"Look out for Freshwater Lice"* – information leaflet. Co-sponsored by The Freshwater Fisheries Laboratory and East of Scotland Water.

Anon. (2001) *The World of Copepods; Copepod Species Taxonomy Result.* National Museum of Natural History – Department of Invertebrate Zoology. http://www.nmnh.si.edu/cgi-bin/wdb/cope/cope_taxa/query (Last accessed: 19/10/2004).

Anon (2003). *Designing and using questionnaires: Getting the most out of your surveys. University of New South Wales www.comed.unsw.edu.au/cgpis/publicPHCRelD/ND%20-20Questionnaire%20design%workbook.pdf (last accessed 13/06/03).*

Atkinson, A. (1998) Life cycle strategies of epipelagic copepods in the southern ocean. *Journal of Marine Systems* 15:289-311.

Avenant-Oldewage, A; Swanepoel, JH. (1993). The male reproductive-system and mechanism of sperm transfer in *Argulus japonicus* (Crustacea; Branchiura). *Journal of Morphology* 215(1); 51-63

Bai, AS. (1981) Photic effects on embryonation and phototactic responses by larvae of *Argulus siamensis. Proceedings of the Indian Acadamy of Science,* 90(5); 513-517.

Bark, S. 2000. *Argulus. Fish Farmer* 23(3): 40.

Barrett, J. C., Grossman, G. D. & Rosenfield, J (1992) Turbidity-induced changes in the reactive distance of rainbow trout. *Transactions of the American Fisheries Society* 121: 437-443.

Bauer, O. N. (1970). The Ecology of Parasites of Freshwater Fish. In *Parasites of FreshwaterFish and the Biological Basis of their Control. Bulletin of the State Scientific Research Institue of Lake and Fisheries,* Vol. XLIX, pp. 3-207. Translated from Russian: Israel Program for Scientific Translations Jerusalem, 1962.

Bayley & Herendeen (2000) The Efficiency of a Seine net. *Transactions of the American Fisheries Society* 129: 901-923.

Bazal, K; Lucky, Z; Dyk, V (1969). Localization of fish-lice and leeches on carps during the autumn fishing. *Acta Veterinaria* 38; 533-544.

Bebak, J., Baumgarten, M & Smith, G. (1997). Risk factors for bacterial gill disease in young rainbow trout (*Oncorrhychus mykiss*) in North America. *Preventative Veterinary Medicine.* 32:23-34.

Begon, M. Mortimer, M. & Thompson, D.J. (1996) *Population Ecology, A unified study of animals and plants.* Blackwell Science, third edition pp.247.

Bower-Shore, C. (1940). An Investigation of the Common Fish Louse, *Argulus foliaceus* (Linn). *Parasitology* 32; 361-371.

Bron, Sommerville, Wootten & Rae (1993) Fallowing of marine Atlantic salmon, Salmo salar L., farms as a method for the control of sea lice, *Lepeophtheirus salmonis* (Kroyer, 1837). *Journal of Fish Diseases* 16(5): 487-93.

Buckley, B.R.; Morrice, C.P. (1976). Some observations on host selectivity and the preferred location of *Argulus foliaceus* L. on four fish species. *Fisheries Management* 7(2), 34-37.

Burgess, T. F. (2001). *A general introduction to the design of questionnaires for survey research. University of Leeds www.leeds.ac.uk/iss/documentation/top/top2.pdf (last accessed 13/06/03).*

Bush, A. O., Lafferty, K. D., Lotz, J. M. & Shostak, A. W. (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* 83(4): 575-583.

Campbell, AD. (1971). The Occurrence of *Argulus* (Crustacea: Branchiura) in Scotland. *Journal of Fish Biology.* 3; 145-146.

Cameron, A. (2002) Survey toolbox for aquatic animal diseases, A practical manual and software package. *ACIAR Monograph.* No.94, 375pp.

Cassie, R. M. (1954). Some uses of probability paper in the analysis of size frequency distributions. *Australian Journal of Marine and Freshwater Research.* 5: 513-522.

Chen, T (1933). A study on the methods of Prevention and Treatment of Fish Lice in Pond Culture. *Lignan Science Journal* 12 (2); 241-244.

Claus, C. (1875) Uber die Entwicklung, Organisation und systematische Stellung der Arguliden. *Zeitschr. F. wiss. Zool.* 25; 217-284.

Cooper, J.E. & Braswell, A.L. (1995) Observations on North Carolina Crayfishes (Decapoda: Cambaridae). *Brimleyana* 22: 87-132.

Countryside-alliance (2002) The Real Rural Agenda.http://www.countryside-alliance.org/fmd/010323fish.htm Last accessed: 25/4/2002.

Crocker, D.W. & Bar, D.W (1968) *Handbook of the Crayfishes of Ontario.* University of Ontario Press, Toronto 158pp.

des Clers, S. (1994) Sampling to detect infections and estimate prevalence in aquaculture. Pisces Press, Stirling.

Du, J. (2002) Combined Algorithms for Constrained Estimation of Finite Mixtures Distribution with Grouped Data and Conditional Data. *MSc Thesis , McMaster University, Hamilton, Ontario, Canada.*

Dunn, P. (2002). 2000 survey of Trout Production in England and Wales. Trout News 33:5-13.

Environment Agency (2001) *National Trout and Grayling Fisheries Strategy.* External Report.

Evans, DW; Matthews, MA (2000). First record of *Argulus foliaceus* on the European eel in the British Isles. *Journal of Fish Biology* 57 (2): 529-530.

Friendly, M. (2000) *Visualizing Categorical Data.* SAS Institute, Cary, NC.

Fryer, G (1968) The Parasitic Crustacea of African Freshwater fishes; their Biology and Distribution. *Journal of Zoology.* 156; 45-95.

Fryer, G. (1982). *The Parasitic Copepoda and Branchiura of British Freshwater Fishes: A Handbook and Key.* Scientific Publications of the Freshwater Biological Association, Ambleside 46: 87 pp.

Galarowicz, T; Cochran, PA (1991) Response by the parasitic crustacean *Argulus japonicus* to host chemical cues. *Journal of Freshwater Ecology* 6(4): 455-456.

Garret, J. W. & Bennett, D. H. (1995) Seasonal movements of adult brown trout relative to temperature in a coolwater reservoir. *North American Journal of Fisheries Management* 15: 480-487

Garson (2002). Logistic Regression, North Carolina State University. *www2.chass.ncsu.edu/garson/pa765/logistic.htm (last accessed17/04/04)*

Gault, N. F. S., Kilpatrick D.J. and Stewart M.T. (2002) Biological Control of the Fish Louse in a Rainbow Trout Fishery. *Journal of Fish Biology* 60; 226-237.

Gurney, R. (1948) the British Species of Fish-Louse of the Genus *Argulus.Proceedings of the Zoological Society of London.* 118 (3); 553-558.

Hakalahti, T. & Valtonen, E.T. (2003) Population Structure and Recruitment of the Ectoparasite *Argulus coregoni* Thorell (Crustacea: Branchiura) on a Fish Farm. *Parasitology* 127; 79-85.

Hakalahti, T., Bandilla, M., & Valtonen, E.T. (2003) Host susceptibility and immunity in the transmission of the ectoparasite *Argulus coregoni* (Crustacea: Branchiura). *Proceedings of 11th International Conference of the EAFP* P-33.

Hakalahti, T. Pasternak, A.F. & Valtonen, E.T. (2004) Seasonal dynamics of egg laying and egg-laying strategy of the ectoparasite *Argulus coregoni* (Crustacea: Branchiura). *Parasitology* 128: 655-660.

Hakalahti, T. Hakkinen, H. & Valtonen E.T. (2004) Ectoparasitic *Argulus coregoni* (Crustacea: Branchiura) hedge their bets – studies on egg hatching dynamics. *OIKOS* 107:295-302.

Harding, J. P. (1949) the use of probability paper for the graphical analysis of polymodal frequency distributions. *Journal of the Marine Biological Association, UK.* 28: 141-153.

Haydon, D. T., Cleaveland, S., Taylor, L.H. & Laurenson M.K. (2002) Identifying Reservoirs of Infection: A conceptual and Practical Challenge. *Emerging Infectious Diseases.* 8 (12):1468-1473.

Herter, K. (1927) A study of the Reaction Physiology of the Carp Louse (*Argulus foliaceus* L.). *Z. vergl. Physiol.* 5,283-370.

Hill, A.B. (1965) The Environment and Disease: Association or Causation? *Proceedings of the Royal Society of Medcine* 58: 295-300.

Hindle, FRS. (1948) Notes on the Treatment of Fish Infected with *Argulus. Proceedings of the Zoological Society of London.* 119; 79-81.

Hoffman, G. L.(1977). *Argulus:* A Branchiuran Parasite of Freshwater Fishes. *U.S Fish and Wildlife Service, Fish Disease Leaflet 49.*

Hubert, W.A., Harris D. D. & Wesche T. A. (1994). Diurnal shifts in use of summer habitat by age-0 brown trout in a regulated mountain stream. *Hydrobiologica* 284: 147-156.

Hudson, Dobson & Newborn (1998) Prevention of population cycles by parasite removal. *Science* 282:2256-2258.

Jafri, SIH & Ahmed, SS (1994). Some observations on mortality in major carps due to fish lice and their chemical control. *Pakistan Journal of Zoology* 26(3): 274-276.

Jakober, M. J., McMahon T. E., & Thurow R.F. (2000). Diel Habitat Partitioning by Bull Charr and Cutthroat Trout During Fall and Winter in Rocky Mountain Streams. *Environmental Biology of Fishes* 59 (1): 79-89

Jarp, J., Tangen, K., Djupvik, H. O. & Tveit A. M. (1993). Risk factors for infection with *Aeromonas salmonicida* subsp. *salmonicida* in Norwegian freshwater hatcheries. *Diseases of Aquatic Organisms* 17: 81-86.

Jarp, J., Gjevre, A. G. Olsen, A. B. & Bruheim, T. (1995) Risk factors for furunculosis, infectious pancreatic necrosis and mortality in post smolt of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases.* 18: 67-78.

Kabata, Z. (1970). *Diseases of Fish, Book 1; Crustacea as Enemies of Fishes* (Eds, Snieszko, SF; Axelrod, HR). T.F.H publications, U.S.A.

Kennedy, C.R. (1971) The effect of temperature upon the establishment and survival of the cestode *Caryophyllaeus laticeps* in orfe, *Leuciscus idus. Parasitology.* 63: 59-66.

Kimura, S. (1970). Notes on the reproduction of water lice (*Argulus japonicus* Thiele). *Bulletin of the Freshwater Fisheries Research Laboratory.* 20; 109-126.

Knight, P (1996). Trends and profitability of game fisheries. *Trout News.* 23; 14-19.

Kollatsch, D (1959). Untersuchgen uber die Biologie und Okologie der Karpfenlaus (*Argulus foliaceus* L.). *Zoologische Beitrage.* 5; 1-36

Lagler, K.F. (1970) Capture, Sampling and Examination of Fishes. In: *Methods for Assessment of Fish Production in Fresh waters. IBP Handbook No. 3.* Ed. Ricker, W.E. Second Printing pp7-45.

LaMarre, E & Cochran, PA (1992). Lack of host species selection by the exotic parasitic crustacean, *Argulus japonicus. Journal of freshwater ecology* 7(1): 77-80.

Leighton, B. J., Zervos, S., & Webster, J. M., (2000) Ecological factors in schistosome transmission, and an environmentally benign method for controlling snails in a recreational lake with a record of schistosome dermatitis. *Parasitology International* 49: 9-17.

Leung, P., Tran, L. T. & Fast, A. W. (2000). A logistic regression of risk factors for disease occurrence on Asian shrimp farms. *Diseases of Aquatic Organisms.* 41: 65-76.

Lougheed, V. L, Crosbie, B., & Chow-Fraser, P. (1998). Predictions on the effect of common carp (*Cyprinus carpio*) exclusion on water quality, zooplankton, and submergent macrophytes in a Great Lakes wetland. *Canadian Journal of Fisheries and Aquatic Science* 55(5): 1189-1197.

Lutsch, E; Avenant-Oldewage, A (1995). The ultrastructure of the newly hatched *Argulus japonicus* Thiele, 1900 larvae (Branchiura). *Crustaceana* 68(3): 329-340.

Macdonald, P. D. M. & Pitcher, T. J. (1979) Age-Groups from Size-Frequency Data: A Versatile and Efficient Method of Analyzing Distribution Mixtures. *Journal of the Fisheries Research Board of Canada* 36: 987-1001.

Macdonald, P.D.M. (1991). Practical aspects of fitting mixture distributions to data, *1991 Proceedings of the Biopharmaceutical Section,* American Statistical Association, 78-83.

Madsen, N. (1964) The Anatomy of *A. foliaceus* Linne. *Lunds Universitets, Arsskrift.* 59:1-31.

Martin, M. (1932). On the morphology and classification of *Argulus* (Crustacea). *Proceedings of the Zoological Society of London.* pp 771-806.

Meehean, OL. (1940) A review of the Parasitic Crustacea of the Genus *Argulus* in the Collections of the United States National Museum. *Proceedings of the U.S. National Museum.* 88; 459-522.

Menezes, J; Ramos, MA; Pereira, TG & Moreira da Silva, A (1990). Rainbow trout culture failure in a small lake as a result of massive parasitosis related to careless fish introductions. *Aquaculture* 89(2): 123-126.

Mikheev, VN; Valtonen, ET. & Rintamaeki-Kinnunen, P (1998). Host searching in *Argulus foliaceus* L. (Crustacea: Branchiura): the role of vision and selectivity. Parasitology, vol. 116, no. 5, pp. 425-430.

Mikheev, VN; Mikheev, AV; Pasternak, AF & Valtonen, ET (2000) Light-mediated host searching strategies in a fish ectoparasite, *Argulus foliaceus* L. (Crustacea: Branchiura). Parasitology, vol. 120, no. 4, pp. 409-416

Mikheev, VN., Pasternak, AF & Valtonen, ET (2004) Tuning host specificity during the ontogeny of a fish ectoparasite: behavioural responses to host-induced cues. *Parasitology Research.* 92(3):220-4.

Mikheev VN, Pasternak AF, Valtonen ET, Lankinen Y. (2001) Spatial distribution and hatching of overwintered eggs of a fish ectoparasite, *Argulus coregoni* (Crustacea: Branchiura). *Diseases of Aquatic Organisms.* 12;46(2):123-8

Mishra, BK (1991). *Observations on the susceptibility of the hybrid, Labeo rohita x Catla catla to Argulus siamensis Wilson infection in composite culture ponds.* National Symposium On New Horizons In Freshwater Aquaculture, 23-25 January Proceedings 1991. pp. 184-185

Moller (1978) The effects of Salinity and temperature on the development and survival of fish parasites.

Molnar, K; Szekely, C. (1998). Occurrence of Skrjabillanid nematodes in fishes of Hungary and in the intermediate host, *Argulus foliaceus* L. *Acta Veterinaria Hungarica* 46(4); 451-463.

Moravec, F; Vidal-Martinez, V & Aguirre-Macedo, L. (1999). Branchiurids (*Argulus*) as intermediate hosts of the Daniconematid nematode *Mexiconema cichlasomae*. *Folia Parasitologica* 46 (1); 79.

Motulsky, H. (1995). *Intuitive Biostatistics.* Oxford University Press.

Northcott, SJ. (1997). The Scientific angle. In: *Atlantic Salmon Trust, Progress Report,* December 1997; 38-40.

Northcott, SJ. (1998). Licebergs in Scottish Waters? In, *Salmon, Trout and Sea Trout Magazine, February 1998;* 46-48.

Northcott, SJ, Lyndon, AR & Campbell, AD (1997). An Outbreak of Freshwater Fish Lice, *Argulus foliaceus* L., Seriously Affecting a Scottish Stillwater Fishery. *Fisheries Management and Ecology* 4: 73-75.

Northcott, SJ; Walker, AF (1996). The best laid plans of lice and men. *Atlantic Salmon Trust, Progress Report,* December,1996; 28-29.

Northcott, SJ; Walker, AF (1997). Is a southern problem heading north? *Fish, Magazine of the Institute of Fisheries management;* 11.

Okland, KA (1985). Fish lice *Argulus* - morphology, biology and records from Norway. *Fauna* 38(2): 53-59.

Oppenheim, A. N. (1992). *Questionnaire design, interviewing and attitude measurement.* London: St Martins Press.

Pallant, J. (2001). *SPSS Survival Manual.* Buckingham: Open University Press.

Pasternak, AF; Mikheev, VN; Valtonen, ET (2000). Life history characteristics of *Argulus foliaceus* L. (Crustacea: Branchiura) populations in Central Finland. *Annales Zoologici Fennici*, 37(1): 25-35.

Perkins S.E., Cattadori, I. M., Tagliapietra, V., Rizzoli, A. P. & Hudson, P.J. (2003) Empirical evidence for key hosts in the persistence of tick-borne disease. *International Journal of Parasitology.* 33: 909-917.

Pfeil-Putzien,C. (1978). Experimental transmission of spring viraemia of carp through carp lice (*Argulus foliaceus*). *Zentralbl. Veterinarmed.*, B, 25(4), 319-323.

Pfeil-Putzien,C.; Baath,C. (1978). Isolation of the Rhabdovirus carpio from carp and carp lice (*Argulus foliaceus*). *Berl. Munch. Tierarztl. Wochenschr*, 91(22), 445-447.

Poly, WJ (1998). New state, host, and distribution records of the fish ectoparasite, *Argulus* (Branchiura), from Illinois (USA) *Crustaceana*, 71(1): 1-8.

Post, G. (1987) *Textbook of Fish Health.* Revised & Expanded. T.F.H Publications Canada.

Poulin, R & FitzGerald, GJ (1987). The potential of parasitism in the structuring of a salt marsh stickleback community. *Canadian Journal Zoolology* 65(11): 2793-2798.

Poulin, R & FitzGerald, GJ (1988). Water temperature, vertical distribution, and risk of ectoparasitism in juvenile sticklebacks. *Canadian Journal Zoolology* 66(9): 2002-2005.

Poulin, R & FitzGerald, GJ (1989a). A possible explanation for the aggregated distribution of *Argulus canadensis* Wilson, 1916 (Crustacea: Branchiura) on juvenile sticklebacks (Gasterosteidae). *Journal of Parasitology* 75(1): 58-60.

Poulin, R & FitzGerald, GJ (1989b). Male-biased sex ratio in *Argulus* canadensis Wilson, 1916 (Crustacea: Branchiura) ectoparasitic on sticklebacks. *Canadian Journal Zoolology* 67(8): 2078-2080.

Poulin, R & FitzGerald, GJ (1989c). Risk of parasitism and microhabitat selection in juvenile sticklebacks. *Canadian Journal Zoolology* 67(1):14-18.

Poulin, R & FitzGerald, GJ (1989d). Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). *Behavioral Ecology and Sociobiology* 24(4): 251-255.

Powell, J. (2000) *Epidemiological Methods 2000: Third Year seminar Course.* University of Birmingham, Department of Public Health and Epidemiology.

Prabhavathy, G; Sreenivasen, S. (1976). Occurrence of *Argulus japonicus* in Brood Fish Ponds in Tamilnadu. *Journal of the Inland Fisheries Society India.* 8; 131-133.

Rahman, MM. (1995). Some aspects of the biology of a freshwater fish parasite, *Argulus foliaceus* (L.) (Argulidae, Branchiura, Crustacea). *Bangladesh Journal of Zoology.* 23(1); 77-86.

Rahman, MM. (1996). Effects of a freshwater fish parasite, *Argulus foliaceus* Linn. infection on common carp, *Cyprinus carpio* Linn. *Bangladesh Journal of Zoology* 24(1); 57-63.

Rasbash, J. Browne, W. Goldstein, H. Yang, M. Plewis, I. Healy, M. Woodhouse, G. Draper, D. Langford, I. & Lewis, T. (2002) *A User's Guide to MlwiN.* Centre for Multilevel Modelling, Institute of Education, University of London.

Ritchie, G. Mordue, A. J., Pike A.W. & Rae G. H. (1993) *The reproductive output of Lepeophtheirus salmonis adult females in relation to seasonal variability of temperature and photoperiod.* In: Pathogens of wild and farmed fish: Sea lice. ed. Boxshall & Defaye. Ellis Horwood, London.

Rizvi, S. H. (1969). Studies on the Structure of the Sucker and the seasonal Incidence of *Argulus* spp. *foliaceus* on some Freshwater Fishes. *Crustaceana* 17; 200-206.

Rowe, D. K. (1984) Factors affecting the foods and feeding patterns of lake-dwelling rainbow trout (*Salmo gairdnerii*) in the North Island of New Zealand. *New Zealand Journal of Marine and Freshwater Research* 18: 129-141.

Rozsa, L., Reiczigel, J., & Majoros, G. 2000. Quantifying parasites in samples of hosts. *Journal of Parasitology, 86, 228-232.*

Ruane, N; McCarthy, TK & Reilly, P (1995). Antibody response to crustacean ectoparasites in rainbow trout, Oncorhynchus mykiss (Walbaum), immunized with *Argulus foliaceus* L. antigen extract. *Journal of fish diseases.* 18(6): 529-537.

Rushton-Mellor, SK (1992). Discovery of the fish louse, *Argulus japonicus* Thiele (Crustacea: Branchiura), in Britain. *Aquaculture and Fisheries Managent* 23(2): 269-271.

Rushton-Mellor, SK & Boxshall, GA (1994) The developmental sequence of *Argulus foliaceus* (Crustacea: Branchiura) *Journal of Natural History* 28(4): 763-785.

Schluter, U. (1978). Observations about Host-attacking by the common Fish Louse *Argulus foliaceus* L. (Crustacea, Branchiura). *Zoologischer Anzeiger* 200; 85-91.

Schluter,U. (1979). The effect of temperature on growth and moulting cycle of *Argulus foliaceus* (L.) (Branchiura). *Crustaceana*, 37(1), 100,

Schrage L. J. & Downing, J. A (2004). Pathways of Increased Water Clarity After Fish Removal from Ventura Marsh; a Shallow, Eutrophic Wetland. *Hydrobiologia.* 511 (1-3): 215-231.

Shafir, A & van As, JG (1986). Laying, development and hatching of eggs of the fish ectoparasite *Argulus japonicus* (Crustacea: Branchiura). *Journal of Zoology.* (A) 210(3): 401-414.

Shafir, A & Oldewage, WH (1992). Dynamics of a fish ectoparasite population: Opportunistic parasitism in *Argulus japonicus* (Branchiura). *Crustaceana*, 62(1): 50-64.

Shimura,S. (1981). The larval development of *Argulus coregoni* Thorell (Crustacea: Branchiura) *Journal of Natural History*, 15(2), 331-348.

Shimura, S (1983a). SEM observation on the mouth tube and pre-oral sting of *Argulus coregoni* Thorell and *Argulus japonicus* Thiele (Crustacea: Branchiura). *Fish Pathology, Tokyo.*, 18(3):151-156.

Shimura, S (1983b). Seasonal occurrence, sex ratio and site preference of *Argulus coregoni* Thorell (Crustacea: Branchiura) parasitic on cultured freshwater salmonids in Japan. *Parasitology*, 86(3): 537-552.

Shimura,S. & Egusa,S. (1980). Some ecological notes on the egg deposition of *Argulus coregoni* Thorell (Crustacea, Branchiura). *Fish Pathology, Tokyo*, 15(1), 43-47.

Shimura, S; Inoue, K (1984). Toxic effects of extract from the mouth-parts of *Argulus coregoni* Thorell (Crustacea: Branchiura). *Bull. Jap. Soc. Sci. Fish.* 50(4): 729.

Shimura, S; Inoue, K; Kudo, M; Egusa, S (1983). Studies on effects of parasitism of *Argulus* coregoni (Crustacea: Branchiura) on furunculosis of *Oncorhynchus masou* (Salmonidae). *Fish Pathology*,18(1): 37-40.

Simpson, D. & Mawle, G. (2001) *Public Attitudes to Angling*. Environment Agency Technical Report W2-060.

Singhal, RN; Jeet, S; Davies, RW (1986) Chemotherapy of six ectoparasitic diseases of cultured fish. *Aquaculture*, 54(3): 165-171.

Singhal, RN; Jeet, S; Davies, RW (1990) The effects of argulosis-saprolehniasis on the growth and production of Cyprinus carpoi. *Hydrobiologia*, 202(1-2): 27-31.

Stammer, HJ.(1959) Beitrage zur morphologie, biologie und bekampfung der karpenlaus. *Zeitschrift fuer Parasitenkinde* 19; 135-208.

Stuart-Smith, R.D., Richardson, A. M. M & White, R.W.G (2004). Increasing turbidity significantly alters the diet of brown trout: a multi-year longitudinal study. *Journal of Fish Biology*. 65(2): 367.

Thomas, MM (1961). Observations on the Habits and Post-Embryonic Development of a Parasitic Branchiuran *Argulus puthenveliensis* Ramakrishna. *Journal of the Marine Biological Association of India*. 3(1&2); 75-86.

Thrusfield, M (2003). *Veterinary Epidemiology*. 2nd Edition, Blackwell Science, Oxford.

Tokioka, T. (1936). Larval development and metamorphosis of *Argulus japonicus*. *Memoirs of the college of Science Kyoto Imperial University*, Series B 7(1) article 4; 93-114.

Tolonen, K.T., Hämäläinen, H., Holopainen, I.J., Mikkonen, K. & Karjalainen, J. (2003). Body size and substrate association of littoral insects in relation to vegetation structure. *Hydrobiologia*. 499 (1-3): 179-190.

Tonguthai, K. (1997) Control of freshwater fish parasites: a Southeast Asian perspective. *International Journal of Parasitology* 27(10):1185-91

Treasurer, J. W. (1993) Management of sea lice (Calagidae) with wrasse (Labridae) on Atlantic salmon (*Salmo salar* L.) farms. *In: Pathogens of wild and farmed fish:sea lice. Ed. Boxshall, G.A. & Defaye, D.* Ellis Horwood, Chichester, UK.

Tu, J.V. (1996). Advantages and Disadvantages of Using Artificial Neural Networks verses Logistic Regression for Predicting Medical Outcomes. *Journal of Clinical Epidemiology*. 49 (11): 1225-1231.

Tucker, C. S. (1998) Larval settlement and epidemiology of *Lepeophtheirus salmonis* Kroyer, 1837 (Copepoda; Caligidae). *Ph.D Thesis, University of Stirling*.

Tucker, C. S. Sommerville, C. & Wootten, R. (2000) The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Kroyer, 1837) on Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 23(5): 1365-2761.

Tucker, C. S. Sommerville, C. & Wootten, R. (2002) Does size really matter? Effects of fish surface area on settlement and initial survival of *Lepeophtheirus salmonis*, an ectoparasite of Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 49: 145-152.

Vagsholm, I., & Djupvik, H. O. (1998a). Risk factors for skin lesion in Atlantic salmon, Salmo salar L. *Journal of Fish Diseases* 21: 449-453.

Vagsholm, I., & Djupvik, H. O. (1998b) Risk factors for spinal deformaties in Atlantic salmon, Salmo salar L. *Journal of Fish Diseases* 21: 449-453.

Vagsholm, I., & Djupvik, H. O. (1999) Risk factors for abdominal adhesions in Atlantic salmon, Salmo salar L. *Journal of Fish Diseases* 21: 449-453.

Williams, C. (1997) *Investigation of Diflubenzuron in the control of Argulus foliaceus L. in carp, Cyprinius carpio L. in relation to management stratagies. MSc Thesis, Applied fish biology.* University of Plymouth.

Wilson, C. (1902). *North American Parasitic Copepods of the Family Argulidae, with a Bibliography of the Group and a Systematic Review of all Known Species.* Proc. U.S. Nat. Mues. Vol. XXV –No. 1302; 635-783.

Wilson K, Bjornstad, O.N. Dobson, A.P. Merler, S., Poglayen, G, Randolph, S.E., Read, A.F.& Skorping, A. (2001) *Heterogeneities in macroparasite infections: patterns and processes.* In: The ecology of wildlife diseases. Oxford University Press. pp197.

APPENDIX 1

Date

Address
Of
Fishery

Dear .....

I am currently undertaking a research project at the Institute of Aquaculture, University of Stirling, under the supervision of Prof. C. Sommerville and Dr R. Wootten. The project is designed to establish factors associated with infections of fish lice (*Argulus* spp.) in stillwater trout fisheries throughout the UK, with a view to developing effective management and prevention strategies. Fish lice pose a serious threat to trout fisheries and as a result the project is being funded by various organisations within the trout industry, including the ASGFM, ASSF, BTA, S&TA & BTFRA as well as the Environment Agency.

I am writing to ask for your assistance with the project. I am currently conducting interviews concerning trout fisheries and their management, and would greatly appreciate it if you could find the time to participate in an interview. If it is convenient it would also be useful to collect some water samples, basic physical measurements and if possible examine some fish. The interview itself takes approximately 45 minutes and is carried out under total confidentiality. All fisheries will be assigned a code that will be known only to myself. No other party (including the sponsors) will be able to relate any data back to a fishery without the specific permission of the fishery.

In order to gain the comparisons necessary to identify risk factors associated with lice infections, I am just as interested in fisheries that have not been infected with lice as those with a history of lice infections. At some point over the next couple of weeks I will contact you by telephone to establish whether you are able to assist in the project. Should you have any questions or require any further information in the mean time, please do not hesitate to contact me on 01786 473171 ext7928 (University of Stirling) or nght1@stir.ac.uk (e-mail).

Your help in this project is vital to its success.

Yours sincerely

Nick Taylor

APPENDIX 2

***Argulus* spp. questionnaire:**

DATE:

1) Location of fishery – NGR Postcode County
2) How many trout fishing lakes are there at the fishery?
3) What are their surface areas?

(Select study lake from this data)

Section 1 - Presence of *Argulus* in the year 2000

4) Was this lake in use for trout fishing in the year 2000? Y N (if no select another lake).
5) Have you seen *Argulus* in this lake this year? - Y N
6) Were *Argulus* present in the year 2000? - Y N if no go to question 10

All of the following questions refer to data from year 2000.

7) Were any other fish health problems in this lake in 2000? Y N if yes which
8) Were there any other problems in the lake e.g. pollution, water quality, low DO ?
   Y N if yes give details
9) When numbers of *Argulus* reached their highest in the year 2000 did you note any of the following:

	Yes	No
Were *Argulus* noticed on more than 1 in 5 rod caught fish?		
Sores/Lesions/blood spots on fish?		
Fungus present on fish?		
Cessation in or reduced catchability compared to normal for that time of year?		
Tight shoaling of fish?		
Unusual swimming behaviour?		
Abnormally high levels of jumping?		
Abnormally high levels of flashing?		

Abnormally high levels of fish mortality?

Eye problems?

Dropsy/blotted, distended abdomen?

Pop eye?

Fin rot?

Leeches?

On average how may lice were seen on fish? <10 10's 100's 1000's

Do you keep records of the number of fish mortalities? (if yes is it possible to view these?)

Were there any other signs? If yes give details.

10) What do you perceive to be the main problems caused by *Argulus* spp.?

11) Were the levels of *Argulus* present last year sufficient to cause the problems associated with *Argulus*, that you described in the previous section (Question 2)?

Section 2 – The fishery

12) What is the land surrounding the fishery used for? A) Woodland B) Arable C) Pasture D) Other – Details – Ask about treatments on neighbouring land.

13) What is the fisheries primary water source: A) Spring B) River/Stream C) Static D) Other – Details

14) What is the fisheries secondary water source: A) None B) Spring C) River/Stream D) Static E) Other – Details

15) Is the systems geology primarily: A) Chalk B) Gravel C) Clay D) Other – Details

16) What was the average number of anglers per month throughout the year in 2000? (include months the fishery was closed in)

17) In which month were numbers of anglers highest in the year 2000?

18) In which month were numbers of anglers lowest in the year 2000?

19) Who supplied you with fish in the year 2000?

Supplier	Species	Number/proportion

Section 3 - The lake details from the year 2000 (all subsequent questions refer to the selected lake, not to the fishery as a whole)

20) Is the lake inter-connected with other lakes? Y N

21) Is this lake a) Spring fed b) river/stream fed c) fed by an adjoining lake d)static e)other - details

22) Is the lakes substrate primarily a) Chalk b) Gravel c) Clay d) Silt e) Boulders f) Other

23) For how long has the lake been used for trout fishing?

24) Has it been in constant use since then Y N if No give details.

25) What is the average depth of the lake?

26) What is the maximum depth of the lake?

27) What is the surface area of the lake?

28) What is the water holding capacity of the lake?

29) In summer what is the average rate at which water is discharged from this lake? (Static=0)

30) Can this be adjusted? Y N

31) During the summer of 2000 by approximately how much (if at all) did the lakes water level drop? [(sign. drop = Y or N) recalculate lake volume from this & calc sign. change in volume = Y or N] (Is it drying that has an effect by killing eggs or does it have an effect by crowding fish?)

32) Has the lake ever suffer from flooding that causes it to merge with another water-body? Y N if yes give details of the waterbody and the year(s).

33) Do trees surround: A) ≤¼ B) >¼ to ½ C) >½ to ¾ D) >¾ of the lake?

34) Do reeds surround: A) ≤¼ B) >¼ to ½ C) >½ to ¾ D) >¾ of the lake?

35) Did much of the lake remain shaded throughout the day? Y N

36) Was additional aeration provided in the summer months? Y N

37) Is the lake highly exposed to the wind? Y N

38) What proportion of the lake bed was covered by weed when it reached its maximum: A) ≤¼ B) >¼ to ½ C) >½ to ¾ D) >¾

39) What proportion of the lake surface was covered by emergent weed when it was at its maximum: A) ≤¼ B) >¼ to ½ C) >½ to ¾ D) >¾

40) Did the lake experience an algal bloom? Y N

41) Does the lake receive large numbers of fallen leaves from surrounding trees? Y N

42) What was the maximum number of waterfowl present in this lake at any time of the year?
(divide by the lakes acreage and feed this value into the analysis).

43) Were frogs, newts, toads or tadpoles noted in this lake in the year 2000? Y N

44) Were water voles, mink, rats or otters present in this lake in the year 2000? Y N

45) Were piscivorous birds present in this lake in the year 2000? Y N

46) Can the lake be drained? A) No B) Partially C) Fully

47) Was the lake drained and were *Argulus* present in the following years?

Year	No	Partially	Fully	Argulus present
2000				
1999				
1998				
1997				

Section 4 – Lake management and fish in the year 2000

48) What species of fish were present in this lake?

Rainbow trout Y N

Brown Trout Y N

Other salmonid's (list) Y N

Coarse fish (list) Y N

Minor species e.g. Gudgeon, minnows, sticklebacks, bleak etc. (list) Y N

49) If *Argulus* was present did certain fish species seem more susceptible to infection?

Y N DK if yes please give details.

50) What is the average size of trout stocked? Rainbow: Brown:
Other:

51) What is the largest size of trout stocked? Rainbow: Brown:
Other:

52) What is the smallest size of trout stocked? Rainbow: Brown:
Other:

53) Were certain sizes of trout more susceptible to infection by *Argulus* than others?

Y N DK if yes please give details.

54) What was the date of you first stocking in 2000?

55) What was the date of you last stocking in 1999?

56) What do you estimate the residence time/turnover of trout to be?

57) Did freshly stocked fish appear to be equally infected by *Argulus* as older fish?
Y N DK if yes please give details.

58) How many trout were present at any time? Were trout a) mixed sex
b) all female c) triploid

59) Were coarse fish stocked last year? Y N

60) What was the overall stocking density taking both salmonids and coarse fish into account?

61) How often did stocking of trout occur?

62) How many trout were stocked at a time?

63) What was the total number of trout stocked in the year 2000?

64) What was the total number of trout mortalities in the year 2000?

65) What was the total number of unexplained losses trout in the year 2000?

66) What was the average number of trout caught per month?

67) Were trout brought in small and grown on before stocking? Y N

68) Were stock cages used on the lake to hold fish Y N if no proceed directly to Q75

69) What species of fish did the cages hold (list)?

70) How many cages were there?

71) For how many months in the year were the cages in use?

72) On average what was the stocking density in these cages?

73) Are caged fish given supplementary feed?

74) Were these fish taken into account in the overall stocking density of the lake? Y N

75) Were fish treated in anyway before stocking? Y N if yes give details.

76) Were fish held elsewhere before stocking either for quarantine or holding? Y N if yes did these fish suffer from *Argulus* infections.

77) Did the fishery allow catch and return/release for trout Y N if yes give details

78) Was any method trout fishing allowed? Y N

79) For how many months of the year was the lake open to anglers? (Which months)

80) If open for less than 12 months was this due to the lake be closed due to *Argulus* infections? Y N

81) Was there any supplementary feeding carried out for lake fish? Y N

82) Was coarse fishing allowed on the lake? Y N

83) If yes was ground baiting allowed? Y N

84) Were disinfectant baths provided for anglers to dip nets, waders etc? Y N

Section 5 – Interventions in the year 2000

85) Was anything used in an attempt to reduce the numbers of *Argulus*? Y N

86) In year 2000 did you do any of the following?

Fallow period/Delayed stocking		Y	N If yes how long for?
Draining		Y	N
Draining and Drying	Y	N	
Draining and freezing	Y	N	
Draining and Liming	Y	N	If yes, what was the rate applied?
Chemical/chemotheraputants?		Y	N include chemicals for weed removal!
Remove weed	Y	N	
Other (eg Barley straw)		Y	N If yes please give details?

87) Was this intervention successful? Y N

88) Did this intervention need repeating? Y N If yes, how many times in the year was it repeated?

89) Were all lakes at the fishery affected equally by *Argulus* (give details)?

Section 6 – History of *Argulus* spp.

90) Have *Argulus* ever been present in this lake? Y N if no please go straight to section 7

91) In which months did *Argulus* appear to be present (list)?

92) In which month were the numbers of *Argulus* lowest?

93) In which month were the numbers of *Argulus* highest?

94) Have *Argulus* always affected this lake? Y N if yes please go straight to section 96

95) In which year did *Argulus* first occur?

96) Have *Argulus* occurred every year since they first appeared? Y N if yes please go straight to question 96

97) In which years did *Argulus* not occur?

98) In years that *Argulus* did not occur and using the above questions as a guide can you think of anything that was different about this lake?

99) Have you ever estimated the total financial cost to your fishery caused by *Argulus* infections? Please give details.

100) Are there any other observations about *Argulus* and outbreaks that you would like to mention?

Section 7 – 2001

101) Has the fishery had to close this year? Y N if Yes for how long?

102) Have there been lower numbers of anglers than would normally be expected for this part of the year?

Y N if yes give details

103) If yes to either questions 98 or 99 has this caused you to change your normal stocking practice (e.g. frequency, amount stock, fish size date of first stocking)?

Y N if yes give details