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Argulus spp. infections in UK stillwater trout fisheries

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Professor Mike Depledge   Head of Science

EXECUTIVE SUMMARY

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. has been reported to cause mortality in stillwater trout fisheries, but little data is available.

This study was designed with four primary objectives:

1) To review the current perception of the extent and severity of *Argulus* spp. infections in UK stillwater trout fisheries.
2) To assess the prospects for novel control and management strategies to reduce economic losses to fisheries and illegal pesticide use.
3) To review the biology and ecology of *Argulus* spp..
4) To review the current treatments and management practices used to avoid or minimise *Argulus* spp. problems.

A cross-sectional, questionnaire-based study of 70 randomly selected stillwater trout fisheries showed that *Argulus* spp. infections may cause an economic loss through a reduction in the number of anglers attending a fishery. *Argulus* spp. infections 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 found to be widely distributed throughout the UK, although problem infections appeared to be 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 risk factors associated with problem *Argulus* spp. infections. Presence of an algal bloom/high turbidity from other causes, shallow lakes and slow stock turnovers were all associated with an increased risk of a problem infection.

A longitudinal study of the population ecology of *Argulus* spp. in 5 trout fisheries of varying management intensity was conducted from May 2002 to May 2003 to identify correlations between risk factors and changes in the populations of *Argulus* spp. 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.

High numbers of *Argulus* spp. are associated with reduced catch rates. However, it appears from the study that reduced capture only occur when *Argulus* spp. infections

combine with high water temperatures and algal blooms, and that high numbers of *Argulus* spp. alone may not affect the catch.

Abundance of *Argulus* spp. is greatest towards the end of summer and drops to low levels over winter. The first cohort of the season hatches towards the end of April, from eggs that have over-wintered once the water temperature is higher than 10°C. This cohort becomes adult and starts laying eggs in June. The over-wintering of hatched stages of *Argulus* spp. 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 June. A cohort then hatches every month until September, giving a total of 5 cohorts in a year. The August cohort lays eggs that over-winter, and the September cohort over-winters as hatched stages.

The study has led to the formulation of a number of management recommendations for trout fisheries that may be effective in reducing argulid populations:

1) If possible trickle stocking is better than batch stocking as this will reduce the length of time a fish spends in a lake, thus increasing stock turnover rates.

2) If possible avoid stocking April/May and speed up stock turnover during this period as this is when the first cohort of *Argulus* spp. hatches. By keeping stock low in these months it will reduce the likelihood of a parasite finding a host. Increasing stock turnover during this period will remove parasites before they are able to lay eggs.

3) Killing hatched stages of the parasite over winter will reduce the number of eggs in the subsequent year. This suggests that no closed season over the winter period will be beneficial as fish capture over this period will reduce the *Argulus* spp. population (success will be dependent on the number of reservoir hosts present).

4) Draining and drying/liming the fisheries over-winter will kill many of the over wintering eggs.

5) If the trout population is fished out over winter, a closed season from the middle of April until the end of May will act as a fallow period just as the first cohort of *Argulus* spp. is hatching (success dependent on the number of reservoir hosts).

6) Targeting interventions early in the year before the parasite lays eggs, and towards the end of the summer, will help prevent over-wintering of eggs.

7) Increased water clarity may reduce the infection success and survival of *Argulus* spp.. It may be feasible to increase water clarity through the use of barley straw, a clean water source and removal of cyprinids.

8) Removal of alternate hosts to reduce the reservoir of infection.

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1 INTRODUCTION

Argulidae are branchiuran crustaceans, the majority of which are ectoparasites of fish. They are found distributed throughout the world (Fryer 1968, Kabata 1970, Post 1987, & Rushton-Mellor 1992). In the genus *Argulus* 143 species 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*, *A. japonicus* and *A. coregoni*. These species are also found throughout much of Europe and many other parts of the world (Post 1987). A marine species*, A. arcassonensis,* has also been recorded around the UK coast (Rushton-Mellor 1992), but little information is available on this species.

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 12mm in length (Gurney 1948). The parasite hatches as a stage known as a metanauplius (Figure 1.1b) and then undergoes a number of moults until it becomes adult (11 in the case of *A. foliaceus*), after which it can continue to grow. *Argulus* spp. reproduces sexually, the female leaving its host to lay eggs on a firm substrate. Temperature is responsible for controlling the rate at which eggs and hatched stages of the parasite develop: the higher the temperature, the faster the development. In temperate climates this makes *Argulus* spp. seasonal in nature, being most abundant during the summer and lowest in the winter, (Shimura 1983, Pasternak, Mikheev, Valtonen 2000, Gault, Kilpatrick, Stewart 2002, Hakalahti, Valtonen, 2003).

Argulus spp. attach to their host by means of hooks in the case of juveniles and suckers in the more advanced stages. *Argulus* spp. are believed to feed predominantly on blood, through a mouth tube. To increase blood flow at the site of feeding *Argulus* spp. deliver a toxin that is possibly a form of anti-coagulant, by means of a pre-oral stylet (Shimura & Inoue 1984). Menezes *et al.* (1990) associated the feeding activity of large numbers of *Argulus* spp. 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, Shimura, Inoue, Kudo & Egusa 1983, Singhal, Jeet & Davies 1990, Rahman 1996, Molnar & Szekely 1998 and Moravec, Vidal-Martinez & Aguirre-Macedo 1999).

Argulus spp. have been reported to cause problems in fisheries and fish farms throughout the world, as well as in the UK. Many of the early records of *Argulus* spp. in Europe refer to it as a problem in carp farming, with much of the German literature calling it the 'Carp Louse' (Herter 1927, Kollatsch 1959, Schluter 1978 and Stammer 1959). In the UK, however, *Argulus* spp. are known to cause mortality in stillwater trout fisheries [(Knight 1996, Northcott, Lyndon & Campbell 1997), personal communications with representatives of the trout industry)], where it is regarded as a relatively recent problem. Interestingly, the first known record of *Argulus* spp. by Baldner 1666 (cited by Wilson 1902) describes it as predominantly affecting trout. A comprehensive review of the biology and ecology of the freshwater species present in the UK has been submitted to the Environment Agency as part of this study.

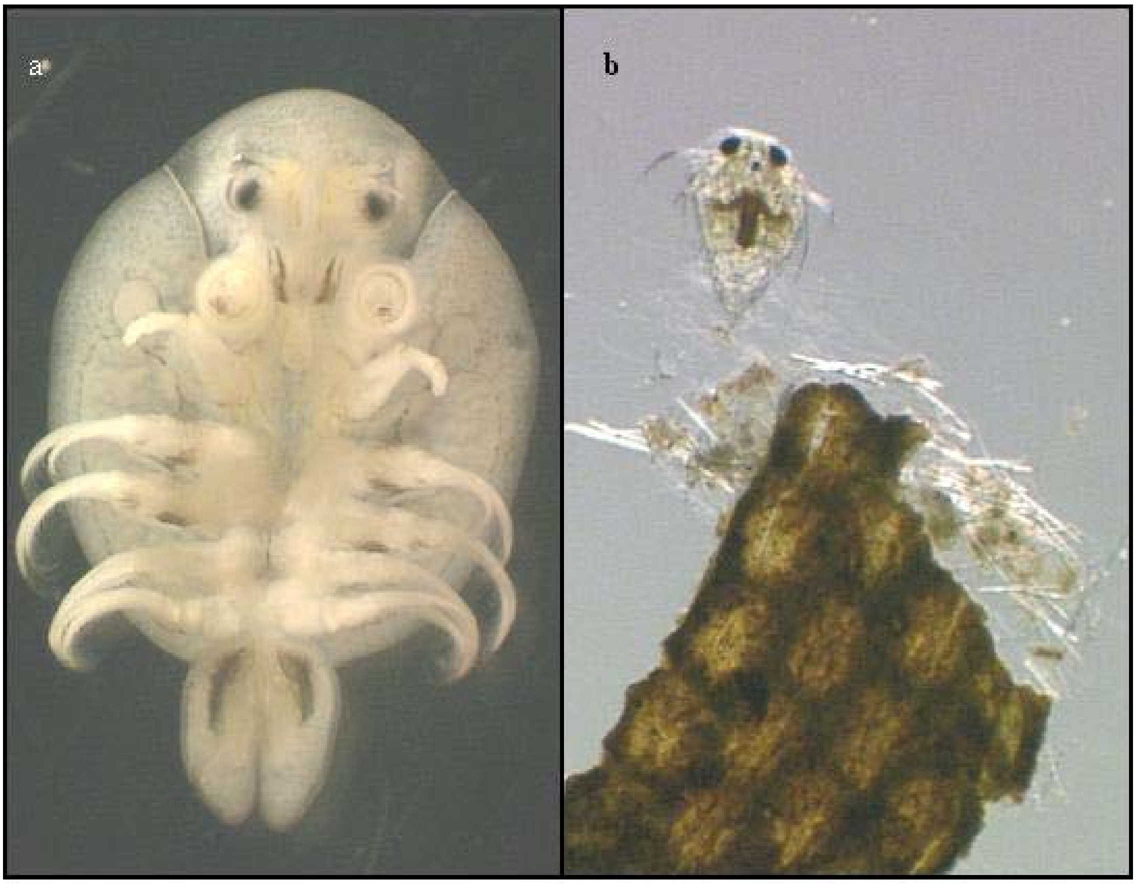


Figure 1.1 a) Ventral view of an adult male specimen of *A. foliaceus*.
b) *A. foliaceus* metanauplius freshly hatched from an egg string.

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 could 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 430000 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 and Environment Agency 2001). The vast majority of trout waters rely on supply by the trout farming industry and hauliers to obtain their stock. In the year 2000, 121 farms in England and Wales produced 3427 tonnes of trout for restocking and growing, 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.

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. There were also no known effective management strategies or licensed chemical treatments with which to control *Argulus* spp.. This has raised concerns within the Environment Agency that illegal pesticides were being used to control the parasite, and identified the need for the development of effective management practices.

With this information in mind a three-year research study was designed with four primary objectives:

1) To review the current perception of the extent and severity of *Argulus* spp. infections in UK stillwater trout fisheries.
2) To assess the prospects for novel control and management strategies to reduce economic losses to fisheries and illegal pesticide use.
3) To review the biology and ecology of *Argulus* spp..
4) To review the current treatments and management practices used to avoid or minimise *Argulus* spp. problems.

In order to establish the nature, extent and severity of the *Argulus* spp. problem, current attempts at control, and to identify risk factors associated with *Argulus* spp. infections a cross-section of trout fisheries was randomly selected and surveyed.

Risk factors are variables that are statistically associated with an outcome variable, in this case the presence of a problem *Argulus* spp. infection. If risk factors can be identified and a biological link between them and the outcome established, it may be possible to influence the likelihood of a problem infection occurring through a change in management.

To understand more about the way in which risk factors were acting and how they interacted with the biology of *Argulus* spp., a population study was conducted in five stillwater trout fisheries. This was supported by a series of laboratory trials.

The results of this study have been used to formulate a series of recommendations for the management of *Argulus* spp. in stillwater trout fisheries.

2 MATERIALS AND METHODS

2.1 Cross-Sectional Study of UK Stillwater Trout Fisheries

The study was conducted from May 2001 to December 2001. The survey was retrospective with the majority of data collected referring to the situation in year 2000. All data were collected by the author in the form of site-based interviews and sampling.

2.1.1 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 the commercial fisheries as the majority advertise in fishing magazines, whch 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 final list was comprised of 406 fisheries distributed throughout the UK, which the fishery guide had stratified into 11 regions. To ensure fisheries from throughout the UK were represented in the study, 30% of the waters in 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 for study. 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. At sites where more than one lake was present, it was necessary to select one of them at random so as to reduce the interview time. This was done by arranging the lakes in size order and numbering them 1 to n. A dice was then used to select the study lake.

A standardized letter stating the purpose of the project and the funding bodies involved was drawn up and sent to each of the selected fisheries. To increase response rates, the letter highlighted the fact that all data obtained would be treated as confidential and would not be released without written permission. 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 and an interview was conducted with the fishery owner using a standardized questionnaire (see appendix 1). Questions in the questionnaire were identified through discussions with trout industry representatives, and information in the literature on the biology and ecology of *Argulus* spp. and the management of fisheries. In broad terms the questionnaire covered aspects of the fisheries including: location & geography, physical parameters relating to the study lake, management practices and disease history. Once complete, the questionnaire was commented on by the project review panel and field-tested at nine fisheries. This allowed problems to be identified and corrections made before the questionnaire was used in the survey.

Where possible, fish from the survey sites were examined for the presence of *Argulus* spp. If *Argulus* spp. were found, specimens were collected and preserved to be speciated in the laboratory. At sites where *Argulus* spp. had been noted but were not found during the visit, a vial of alcohol and a return 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 during 2001.

For the purpose of enhancing our knowledge of the distribution of *Argulus* spp. in the UK, Environment Agency databases containing records of the presence of *Argulus* spp. in stillwaters (including non-trout waters) in England and Wales dating back to 1996 were screened and locations of the occurrence of *Argulus* spp. noted. This data, along with confirmed records of *Argulus* spp. obtained as part of the cross-sectional survey, together with records obtained through reviewing the literature on *Argulus* spp.*,* were used to plot distribution maps of the parasite within the UK.

2.1.2 Data collation and analysis

A database was created in Epi-info 2000 to collate all of the survey data. This package has been designed by the Centre for Disease Control (CDC), USA, specifically for the collation and analysis of epidemiological survey data. All descriptive statistics and graphs were produced in Excel 2000, Epi-Info 2002 or Sigma Plot. An Excel spreadsheet was used to collate co-ordinates of both study sites and sites identified as infected with *Argulus* spp. from Environment Agency records. This data took the form of longitude and latitude of each site and was plotted on to an outline of the UK using Epi-info's G.I.S. program Epi-map.

Risk factors were identified based on the dichotomous outcome "Presence of problem *Argulus* spp. infection in year 2000 = Yes/No" (see page 16 for the definition of Problem *Argulus* spp.). This outcome was chosen over presence of the parasite, as presence alone does not necessarily result in a problem to a fishery. Problem infections were defined once the survey had been completed, and were based on the perceived problem. An infection was only defined as a problem if the fishery owner had answered "yes" to questions referring to specific clinical signs identified through literature searches and discussions with industry representatives, prior to being asked the nature of the problem caused by *Argulus* spp. It was not possible to determine the sensitivity or specificity of this method of screening due to constraints in terms of time and manpower. Problem infections are not normally reported to government organisations or vets, so that no independent retrospective information on infection status was available. Also, problem infections normally last only a few months making it impossible to screen a sufficient number of sites during a prospective study. Due to the obvious nature of the parasite and the simplicity of the clinical signs used it was felt the results of the survey would be sufficiently accurate. The outcome was based on the following signs:

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

Additional signs not associated with *Argulus* spp. infections, and unlikely to be observed as a result of *Argulus* spp. infection, were also included in the questionnaire in order to identify fishery owners who were giving the answers that they perceived the interviewer wanted to hear. Fishery owners answering yes to all of the clinical signs presented to them were removed from the survey.

Due to the large number of potential risk factors and the relatively small sample size it was necessary to screen the data set in order to reject some variables. Using the case definition as an outcome, potential risk factors were first screened individually using 2x2 tables and univariable logistic regression models. All variables significant at $p \leq 0.25$ were retained for subsequent multivariable analysis.

Retained variables were used to build multivariable logistic regression models. Such models identify and adjust for confounding and interactions that may occur between variables. If left unadjusted, biased point estimates of the strength of association between a variable and the outcome will occur. Models were built using a forward stepwise approach in SPSS, with factors significant at $p \leq 0.05$ retained in the model. No more than 1 variable per 5 cases of a problem infection were subjected to this analysis, as recommended by Motulsky (1995).

2.2 Longitudinal Study On The Population Ecology Of *Argulus* spp.

A longitudinal study of 5 UK stillwater trout fisheries involving monthly visits was conducted between May 2002 and May 2003. The object of this study was to increase our understanding of the biology and ecology of *Argulus* spp. in UK trout fisheries, identify periods during which an intervention could be targeted, and establish a link between risk factors identified in the cross-sectional study and the parasites' biology. Sites with different rates of stock turnover (fast to slow) and varying levels of management (intensive to non-intensive) were selected for the study in order to establish correlations between stocking policies and parasite population dynamics. Table 2.1 summarises the characteristics of each site. These are presented throughout this report in order of their rate of stock turn-over relative to one another (fastest first). For the purpose of this study, stock turnover was defined as the time taken to remove and replace the entire standing stock of trout in a lake. Due to the invasive and disruptive nature of the study it was necessary to select waters where the managers would give their full cooperation throughout the year. Selected sites were identified from those visited during the year 2001 cross-sectional study; thus the history of the sites with respect to *Argulus* spp. infections was already known. Due to the sensitive nature of the data collected during the study, sites were promised confidentiality.

Table 2.1 Details of study sites selected for May 2002 to May 2003 longitudinal study of *Argulus* spp. population dynamics in UK Stillwater trout fisheries.

Site Number	Size (Acres)	Rate of Stock Turnover	Location
1	1	V. Fast (<3 weeks)	S. Wales
2	9	Fast	Gloucestershire
3	22	Fast	Gloucestershire
4	38	Slow/Intermediate	Staffordshire
5	1.5	Slow (>52 weeks)	Wiltshire

2.2.1 Temperature data

Two Gemini data loggers were deployed into the deepest part of each of the study lakes and set to take a temperature reading at 3-hour intervals. Loggers were attached to a rope secured to an anchor weight and a buoy set in the deepest part of the lake. One sensor was set 30cm under the water surface, the other 30cm above the lakebed. Before deployment, all the sensors used were calibrated against one another to make sure they were recording within 0.1°C of each other. As lake depths were different and surface water temperatures prone to high levels of variation a combined mean of the monthly surface and bottom temperatures was calculated to compare temperatures between each lake.

2.2.2 Turbidity

Each site was issued with a Secchi disk and a record sheet. The discs were made from a 15 x 15cm white plastic square drilled through the centre. A 3.5m string knotted at 30cm intervals was passed through the string and tied to a weight to allow the disk to sink. Secchi disks were lowered into the water until they just disappeared from view; at this point the depth to which it had sunk was taken as the turbidity reading. Readings were taken at the same point in the lake each time.

2.2.3 *Argulus* spp. egg laying data collection

Adult female *Argulus* spp. leaves the host to lay eggs on a firm substrate. Several studies have shown that *Argulus* spp. will lay eggs on an artificial substrate that can subsequently be removed (Bauer 1959, Shimura 1983 and Gault, Kilpatrick & Stewart 2002). To assess egg-laying activity, foam-PVC boards were placed in each of the study lakes. Each board was grey in colour, 30x30cm in size and drilled top and bottom. Boards were threaded onto a rope set to the lake depth at 30cm intervals, 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.

Boards were examined each month and egg strings counted and removed with a knife. The number of egg strings on each board was recorded. After inspection each board was scrubbed clean and replaced in the same location. The average number of egg strings found on each site visit was calculated and graphed in Excel 2000.

2.2.4 Stock data

Monthly stock turnover was defined as the number of fish removed and replaced each month, and in all cases but one was assumed to be equal to the number of fish stocked between visits. The number of fish stocked is normally calculated based on the number of fish removed plus an adjustment for mortality, predation and poaching calculated by the fishery owner. Site 5 was the only exception as there was no stock turnover other than the fish that were sampled by the author, the *Argulus* spp. removed and the fish returned clean. In this case the number of fish sampled was used as the rate of stock turnover. This data was graphed in Excel 2000.

2.2.5 Fish sampling

Fish capture method is a difficult issue not only in terms of actually catching fish, but also in obtaining a representative sample. Rod capture is likely to sample only the healthiest fish that are feeding and bait choice is likely to determine the species that will be caught; it also requires a substantial time effort to catch large numbers of fish (Lagler 1970). Given that one of the clinical signs associated with *Argulus* spp. infection is reduced feed uptake, and the number of fish required each month, rod capture was deemed an unsuitable method.

Seine netting avoids the problem of species specificity in catches but it may select the slowest or sickest fish (Bayley & Herendeen 2000). Although not a perfect method, given the time, manpower and resources available it was felt to be the most suitable for the study.

A 30m long by 2m deep seine net was used on the two smallest waters, and a 25m long by 3.5m deep net on the three larger lakes. Both nets had a 1.5cm mesh size. Netting was conducted by shooting the net across a suitable bay.

Table 2.2 shows the numbers of netted rainbow trout sampled on each site visit. The target sample size was 30 trout; this was often not possible to achieve due to constraints of time and manpower.

Table 2.2 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	6	5	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

2.2.6 Parasite sampling

All trout caught were anaesthetised in benzocaine. Parasites were removed from fish 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. The water in which the fish were anaesthetised was filtered through a 0.7mm mesh and checked for *Argulus* spp. Where no parasites were found, the fish was recorded as being 'clean'. Post-inspection, fish were then allowed to recover fully in clean water before being returned to their lake of origin.

2.2.7 Analysis of parasite data

The parasites in each vial from each site visit were counted, and the prevalence of infection and mean abundance calculated. Prevalence of infection is defined as the percentage of fish infected with *Argulus* spp. The mean abundance is the average number of *Argulus* spp. found on each fish in a sample including uninfected fish.

2.2.8 Length frequency / size distribution analysis

Image analysis was used to accurately measure the length of *Argulus* spp. specimens. This data was important to understand how the structure of the parasite populations varied between sites and sampling time points. Although Rushton-Mellor & Boxshall (1996) described 11 different moults to adulthood in the development of *A. foliaceus*, examination of specimens in the study showed that the often subtle differences between moults would make this method of assessment of development too time consuming to gain the necessary information on population structure. To overcome this the parasite length was used as a measure of maturity. As the parasite continues moulting and growing once adulthood is reached, Rushton-Mellor & Boxshall's (1996) estimate of 4.6mm was used as the length at which *Argulus* spp. were deemed adult.

Length data of *Argulus* spp. collected from each site visit were converted into length frequency data in Excel 2000 using the histogram function. Data was divided into 200μm wide bins ranging from 0 to 8000μm and then imported into the program Length Frequency Distribution Analysis (LFDA) 5.0 (Kirkwood, Aukland & Zara 2001) in which it was plotted.

2.3 Experimental Studies on the Survival of *Argulus* spp.

The cross-sectional study identified some potential risk factors that required further investigation to establish a causal link between them. Attempts were made to develop a standard method of challenging fish with *A. foliaceus* to allow experiments to be conducted. These attempts demonstrated that maintaining a laboratory population of *A. foliaceus* for long periods of time was difficult as populations died out within two weeks. Therefore, a series of experiments was designed that followed the survival of *A. foliaceus* populations over time in relation to a number of different factors. Preliminary studies showed static tank systems (no filtration or water flow) to be most successful at maintaining *A. foliaceus* populations. Due to their tolerance of such conditions, it was decided to use carp (*Cyprinus carpio*), as opposed to trout, for the purpose of these trials.

The factors studied were chosen in relation to those identified in the cross-sectional study as possibly significant; these were the effect of 'light & dark' conditions (chosen to simulate the effects of increased turbidity), and the effect of different host species to investigate the role of reservoir hosts. Also of interest was the impact of different temperatures on the 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. Benzocaine was used as laboratory trials showed it to have no adverse effects on *A. foliaceus*. 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 and factors compared using the log-rank function.

2.3.1 The effect of light & dark conditions on the survival of *Argulus* spp. populations.

Six, 10L static tanks were used in this experiment. Three replicate tanks were covered in black polythene and aluminium foil so that no light could penetrate and three were left uncovered and exposed to a 15L:9D artificial light regime. All tanks were held at a constant 16ºC. A single common carp, circa 7cm in length, was held in each tank. Thirty freshly hatched *A. foliaceus* metanauplii were added to each tank on day 1 of the trial. 80% water changes were made to each tank on a weekly basis; this water was filtered through a fine mesh and checked for the presence of *A. foliaceus*. Any *A. foliaceus* found were returned to their tank of origin. The experiment was terminated after 9 weeks.

2.3.2 The survival of *Argulus* spp. on different hosts

Six, 10L static tanks were used in this experiment. Three tanks contained a single roach fry (*Rutilus rutilus*) circa 3cm in length, and three tanks a single common carp circa 7cm in length. Tanks were exposed to a 15L:9D artificial light regime and held at a constant 12ºC. Thirty freshly hatched *A. foliaceus* metanauplii were added to each tank on day 1. The experiment was terminated after 7 weeks.

2.3.3 The effect of temperature on the survival of *Argulus* spp. populations

Nine, 10L static tanks were used in this experiment. Groups of three tanks were held at 10ºC, 20ºC and 25ºC. Each tank contained single common carp circa 7cm in length. Tanks were kept in total darkness for the duration of the experiment. Thirty freshly hatched *A. foliaceus* metanauplii were added to each tank on the first day of the trial. These metanauplii were hatched at 15ºC and were acclimatised to the temperature of their experimental tank over 12 hours. The experiment was terminated after 7 weeks.

3 RESULTS

3.1 Cross-Sectional Study

3.1.1 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%) were deemed to have provided reliable data suitable for further analysis. The following results relate only to those 69 sites.

61% of sites interviewed had heard of *Argulus* spp. and were aware of some of the problems it caused. 42% of sites stated that the presence of *Argulus* spp. had been noted at least once during the history of the fishery; 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 reduced catchability and affected the condition/aesthetic appeal of the fish, which showed reduced weight and scale loss along with the presence of the parasite. The reduced catchability and aesthetic appeal of fish were perceived to lead to an economic loss through a reduction in the number of anglers attending the fishery. Based on this definition, 25% of the study waters had suffered 'Problem *Argulus* spp.' infections in year 2000. Some study sites contained other lakes that suffered problem infections where the study lake did not. This meant that an additional 4% of sites had suffered a problem infection in year 2000, resulting in 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.

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,* whereas specimens from the other fishery were identified as *A. coregoni*.

3.1.2 Distribution of *Argulus* spp. in UK fisheries

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 of the survey did confirm its presence in these areas.

Environment Agency data sets relating to all stillwater fishery types, 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 in these regions). The distribution of *Argulus* spp. as determined by this survey, confirmed reports and Environment Agency records are summarised in Figure 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. This data would suggest that *A. japonicus* has a similar distribution to other *Argulus* spp. species.

Environment Agency data was also plotted separately for each year to see if there was any indication whether *Argulus* spp. has been spreading through the country in recent years. The data showed *Argulus* spp. to be widely distributed since 1996, suggesting it has not spread in recent years, and as a result, the separate annual maps have not been presented here.

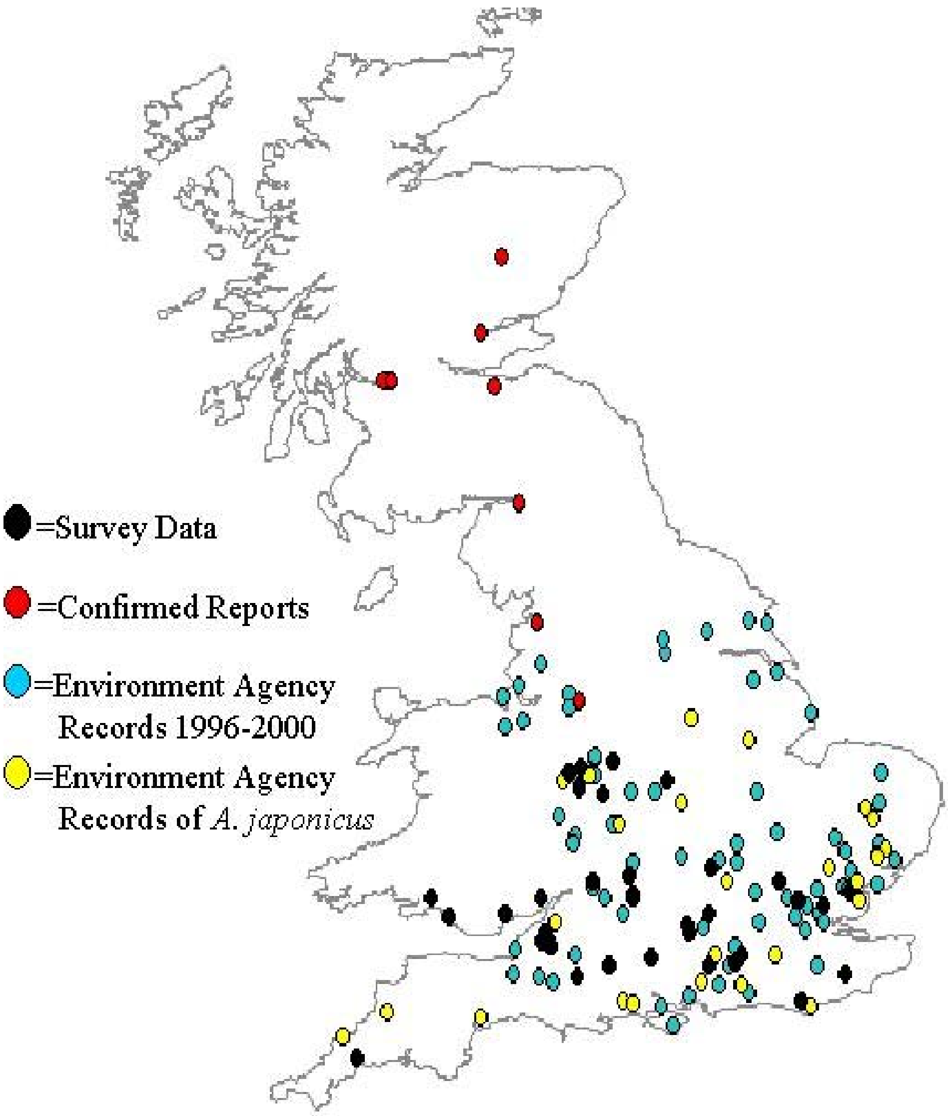


Figure 3.1 The distribution of *Argulus* spp. in UK stillwaters from cross-sectional survey data, confirmed reports and Environment Agency records from 1996 to 2000.

Due to the low proportion of sites surveyed in each study region, prevalence estimates for each region would be relatively meaningless. Instead, regions were amalgamated into a northern, central and southern region by combining the 4 most northerly, 4 central and 3 southern regions (as the latter contained the most sites). This gave almost equal numbers of sites between regions: North = 21, Central = 25 and Southern = 23. This method of division was chosen, as it is unbiased.

Tables 3.1, 3.2 and 3.3 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 north. *Argulus* spp. is most common in the central regions, with infected sites outnumbering uninfected sites.

Table 3.1. The number of stillwater trout fisheries surveyed by region, noting the presence of *Argulus* spp. at any time during its history (numbers in parenthesis refer to the equivalent percentages).

	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 3.2. The number of stillwater trout fisheries surveyed by region noting the presence of *Argulus* spp. in year 2000 (numbers in parenthesis refer to the equivalent percentages).

	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 3.3. The number of stillwater trout fisheries surveyed suffering 'Problem *Argulus* spp.' infections in year 2000 (numbers in parenthesis refer to the equivalent percentages).

	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

3.1.3 Perception of fish susceptible to infection by *Argulus* spp.

Data in this section is based on the observations and perceptions of the fishery owners surveyed. Of the infected survey sites with more than one fish species present, nine felt 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 after 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 appear to suffer the most from infection by *Argulus* spp. The majority (11) of infected sites felt that all sizes of trout were equally susceptible to infection. Of the remaining infected sites, four felt large fish were more susceptible, two thought 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 thought fresh stocked and seven did not know.

3.1.4 Current attempts at the control of *Argulus* spp.

All of the infected sites surveyed stated the need for an effective, legal method of controlling *Argulus* spp. infections. The most commonly used treatment was the organophosphate, Diptrex 80. Five sites admitted to having used this 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 at killing 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 trickle as opposed to batch stocking.

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.

3.1.5 Determination of risk factors

Univariable analysis reduced the number of potential candidate variables to 20, four of which were significant at $p<0.05$. These were: presence of an algal bloom, presence of substantial coarse fish populations, presence of crayfish and turbidity of less than 1 metre. All of these factors are significantly associated with an increased likelihood of suffering from problem *Argulus* spp. Of the 20 candidate variables 9 were rejected on the grounds that the data available was either unrepresentative of the sample or were obviously co-linear, e.g. mean & maximum lake depth which increase with one another. Presence of crayfish and turbidity of less than 1 metre were two of the candidate variables excluded from further analysis on the grounds that the data obtained for these variables was not representative of the entire data set.

Logistic regression models were built using a forward stepwise approach (both automated and manually) based on the 11 remaining candidate variables, using the statistical program, SPSS.

After adjusting for confounding and the effects of regional variations three risk factors were identified from the logistic regression (Table 3.4). The presence of an algal bloom is associated with an increased likelihood of suffering a problem *Argulus* spp. infection. Fast stock turnovers and deep lakes were associated with a reduced risk of a problem infection. It is possible that some of the factors could be caused by the presence of *Argulus* spp. For example, slow stock turn over could be attributed to reduced feeding of fish caused by the presence of *Argulus* spp. Thus, removal or implementation of one of these factors will not necessarily affect the *Argulus* spp. population.

Table 3.4 Risk factors identified for the presence of problem *Argulus* spp. infections. Risk factors obtained from logistic regression models developed from data obtained in a cross-sectional study of stillwater trout fisheries in the UK.

Variable	Risk or Preventative factor
Presence of Algal Bloom/Turbidity	Increased Risk
Fast Stock Turnover	Preventative
Increased lake depth	Preventative

3.2 Longitudinal Study

3.2.1 Abundance

A comparison of the abundance of *Argulus* spp. on seine netted rainbow trout in all five of the study lakes over the study period is shown in Figure 3.2. The figure shows a high level of variation in the abundance of the parasite between sites. General trends can, however, be seen. In all sites the abundance of *Argulus* spp. was low in the spring, then increased until it peaked at the end of summer. The parasites' abundance then dropped to very low levels over the winter months before increasing once again in the spring

Site 1 started the sampling year (May) with an extremely low abundance of *Argulus* spp., and remained low until increasing in August. Abundance peaked in September at which point the lake was netted and stocked with clean fish. There was a subsequent rapid drop in numbers to almost zero in October. Abundance was zero from November until April when low numbers were again present. By May there was a 4-fold increase in the parasite abundance.

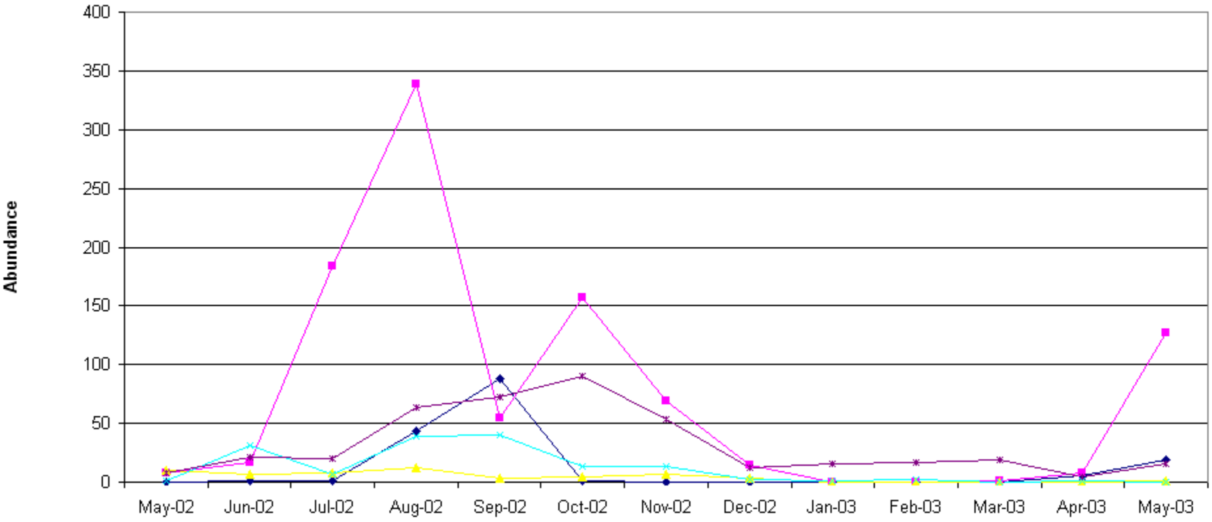
In site 2 there are also low numbers of *Argulus* spp. in May, but there was then an exponential increase until numbers peaked in August. At this point the abundance was over five times higher than in any other site. Due to the high parasite burden, an intervention was implemented leading to a substantial drop in parasite abundance in the September sample. Parasite numbers increased significantly by October before dropping steadily until the parasite population has almost disappeared by January.

There appeared to be no recruitment over the winter months until April when numbers began to increase once more. By May 2003 the abundance of *Argulus* spp. had increased further to over six times that of any of the other sites.

The abundance of *Argulus* spp. in site 3 was very low from May to November. Over winter the abundance dropped to near zero and remained at this level for the rest of the study.

In site 4 parasite numbers appeared to fluctuate throughout the year, but small sample sizes mean that few of these fluctuations were statistically significant. Abundance increased between May and June before decreasing again in July. There was then a further rise in August and September before numbers dropped by 75% in October. The parasite persisted in low numbers over the winter and unlike the other sites did not appear to increase in April or May.

In common with the other sites, abundance in site 5 was low in May. Numbers then increased and reach a peak in October (at least one month later than the other sites). Over the subsequent two months there was a steady drop until numbers remained at approximately 17% of their previous level until April when there is a significant drop, before numbers increased again in 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	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

Month

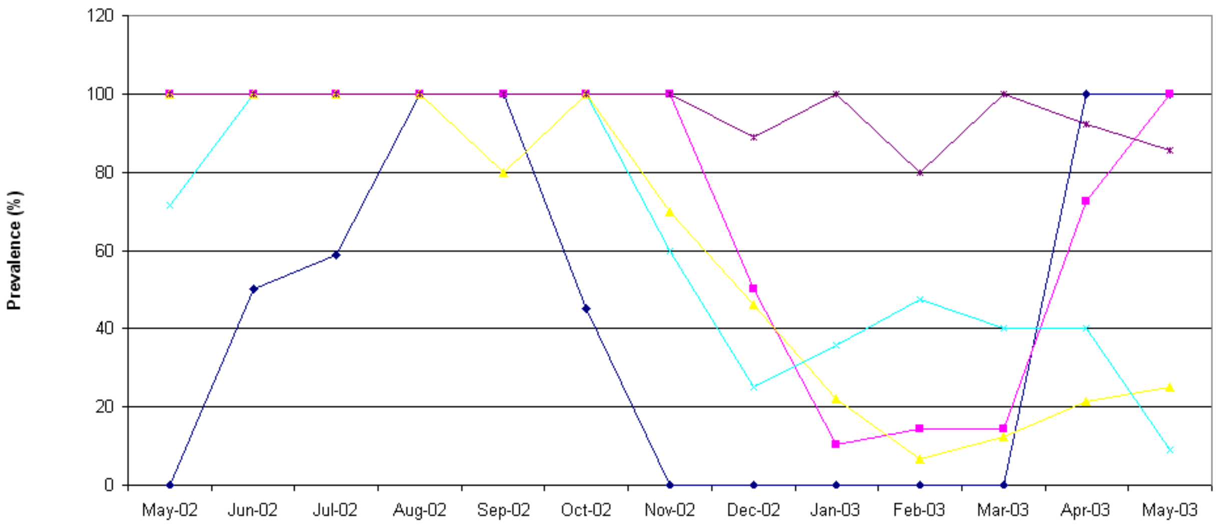
Figure 3.2 The monthly abundance of *A. foliaceus* on rainbow trout in 5 UK stillwater trout fisheries from May 2002 to May 2004.

3.2.2 Prevalence

The prevalence of the parasite throughout the year is shown in figure 3.3. Sites 2,3 and 5 started the year with 100% prevalence; site 4 also started with a high prevalence of 71.4%. Site 1 had 0% prevalence in May, which then increased reaching 100% in August. After September prevalences dropped and reached 0% in November, the parasite was absent until April 2003 at which time it returned to 100%.
Prevalence in site 2 remained at 100% until November. Over the next two months the level dropped to between 10 and 15% and remained constant until it increased in April before reaching 100% in May 2003.

Prevalence in sites 3 and 4 remained at 100% until October after which the prevalence dropped. Small sample sizes make the prevalence in site 4 difficult to interpret but in site 3, prevalence reached its lowest point in February before slowly increasing again. Prevalence in site 3 only reached 25% by May 2003 and also remained low in site 4.

The prevalence in site 5 remained at 100% until December from when it fluctuated between 80 and 100% until the end of the study. The difference between the fluctuations was not statistically significant at the 5% level.



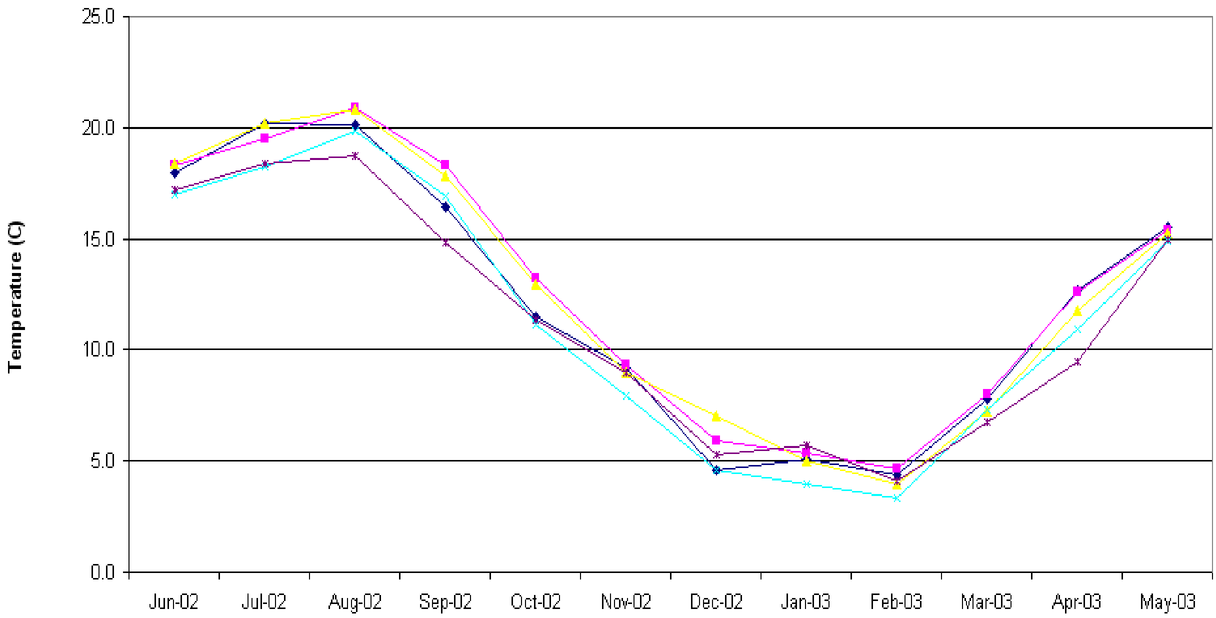
	May-02	Jun-02	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Site 1	0	50	58.88	100	100	45	0	0	0	0	0	100	100
Site 2	100	100	100	100	100	100	100	50	10.5	14.3	14.3	72.7	100
Site 3	100	100	100	100	80	100	70	46.1	22.2	6.7	12.5	21.4	25
Site 4	71.4	100	100	100	100	100	60	25	35.7	47.6	40	40	9.09
Site 5	100	100	100	100	100	100	100	88.9	100	80	100	92.3	85.7

Month

Figure 3.3 The monthly prevalence of *A. foliaceus* on rainbow trout in 5 UK stillwater trout fisheries from May 2002 to May 2004.

3.2.3 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 follow very similar profiles over the year. In all lakes the temperature peaked in August at 18-21°C and was at it lowest in February at 3-5°C. The greatest level of variability was seen in September between sites 2 and 5 where there was a difference of 3.5°C. Variability was very low throughout the winter but lowest in March, with only a 1.2°C difference between sites 2 and 5.



	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

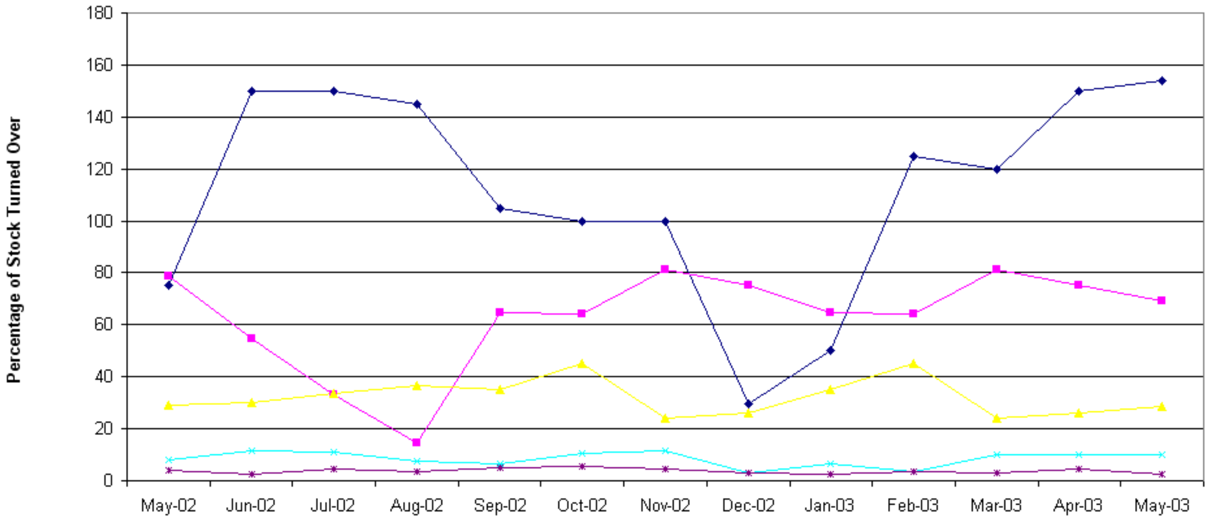
Month

Figure 3.4 The temperature profiles of the 5 sites studied in the May 2002 to May 2003 longitudinal study of populations of A. foliaceus

3.2.4 Stock turnover

The percentage of stock turnover differs greatly between all 5 sites. Figure 3.5 shows the percentage of the lakes standing stock of trout that was removed and replaced each month. On the whole, turnover is highest in site 1 and decreases with an increase in site number. Turnover fluctuates greatly in site 1 throughout the year (probably due to the small size of the lake). In this lake, turnover was highest from June to August and lowest in the winter months, reaching a low in December.

Stock turnover is fairly constant in site 2, except for July and August when it drops substantially, corresponding to a peak in temperature and abundance of *Argulus* spp.. Stock turnover is also constant and low in sites 3, 4 and 5, although it is substantially higher in site 3 than in 4 and 5. In site 5, stock turnover is actually due to the investigator removing fish each month and returning them after parasites were removed. This lake is normally stocked once a year, in April and the fish remain in the lake year round until they are caught and removed.



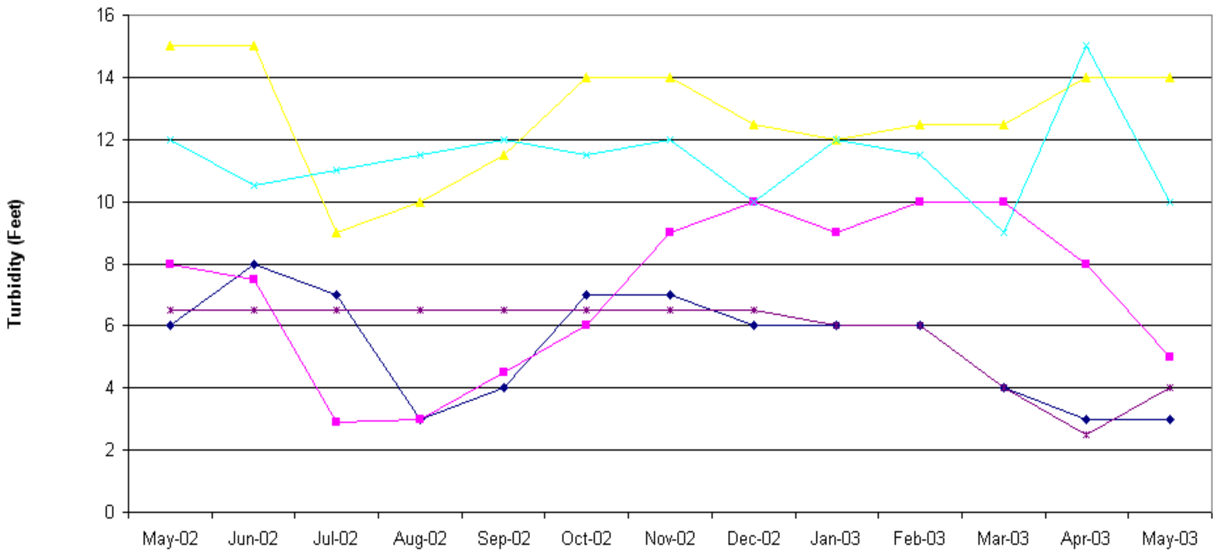
	May-02	Jun-02	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03
Site 1	75	150	150	145	105	100	100	29.5	50	125	120	150	154
Site 2	78.7	54.7	33.1	14.3	64.8	64.0	81.1	75.0	64.8	64.0	81.1	75.0	69.2
Site 3	29.3	29.9	33.4	36.7	35.3	45.1	24.0	26.3	35.3	45.1	24.0	26.3	28.5
Site 4	8.2	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

Month

Figure 3.5 The percentage of stock turn over each month between the 5 sites studied in the May 2002 to May 2003 longitudinal study of populations of *A. foliaceus*.

3.2.5. Turbidity

Turbidity in sites 1 and 2 shows a clear seasonal pattern with water clarity being poor in the summer (corresponding to peaks in temperature and the abundance of *Argulus* spp.), and highest in the winter (Figure 3.6). The two sites that suffered the lowest burdens of *Argulus* spp., sites 3 and 4, remained the clearest throughout the year with turbidity fluctuating between 10 and 15 feet. Site 5 had a turbidity reading that remained at 6.5 feet throughout the study, except for the final three months of the study reaching a low of 2.5 feet in April 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	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

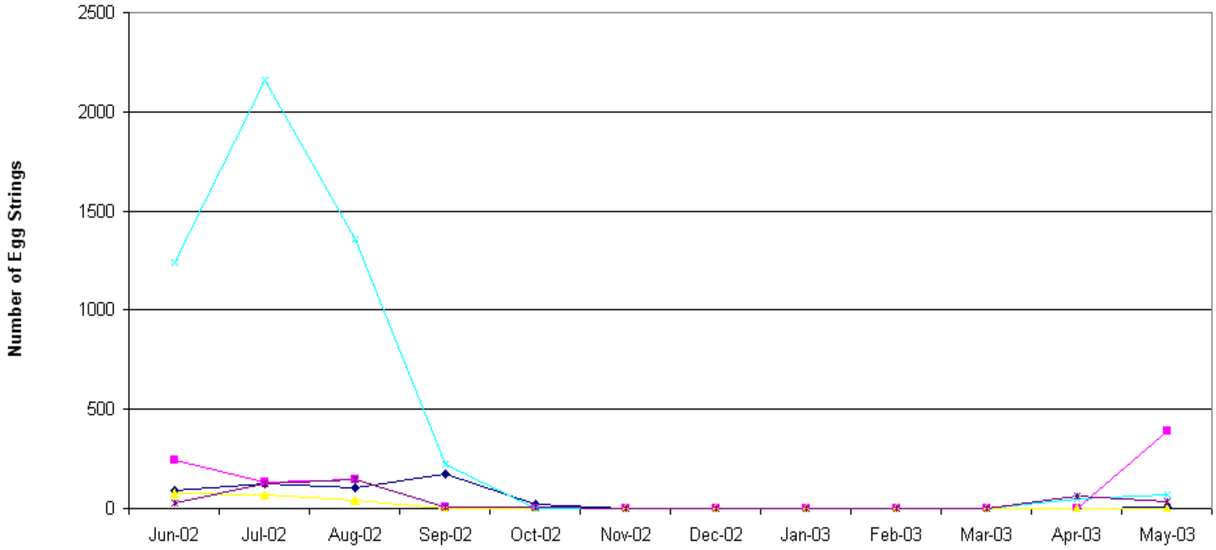
Month

Figure 3.6 The turbidity profiles of the 5 sites studied in the May 2002 to May 2003 longitudinal study of populations of *A. foliaceus* .

3.2.6. Egg laying

In 2002 egg laying by *Argulus* spp. peaked in September in site 1, June in sites 2 and 3, and August in sites 4 and 5 (Figure 3.7). Egg laying stopped in site 3 in September, continued for an extra month in sites 2 and 4, and an additional two months (until October) in sites 1 and 5. 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. The first laying was noted in April in sites 4 and 5. These were the two sites with the slowest stock turnovers in which hatched *Argulus* spp. had over-wintered. Sites, 1, 2 and 3 had the fastest stock turnovers and hatched *Argulus* spp. did not over-winter. In these three lakes egg laying did not occur until May.



	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

Month

Figure 3.7 The egg laying activity of *A. foliaceus* in the 5 stillwater trout fisheries studied in the May 2002 to May 2003 longitudinal study.

3.2.7 Length-frequency data

Length-frequency data shows similarities in the recruitment and development pattern of *Argulus* spp. in the five study sites (Figure 3.8). In site 1 *Argulus* spp. were found in May 2002 and only low numbers in June and July. The size of the parasites found in these months varied greatly, however insufficient data was available to determine the number of cohorts present. By August one distinct cohort was present, in which the parasites were approximately 75% developed. This cohort either emerged from eggs that hatched just after the previous site visit and had grown rapidly to this size or was the result of immigration of parasites from an adjoining lake.

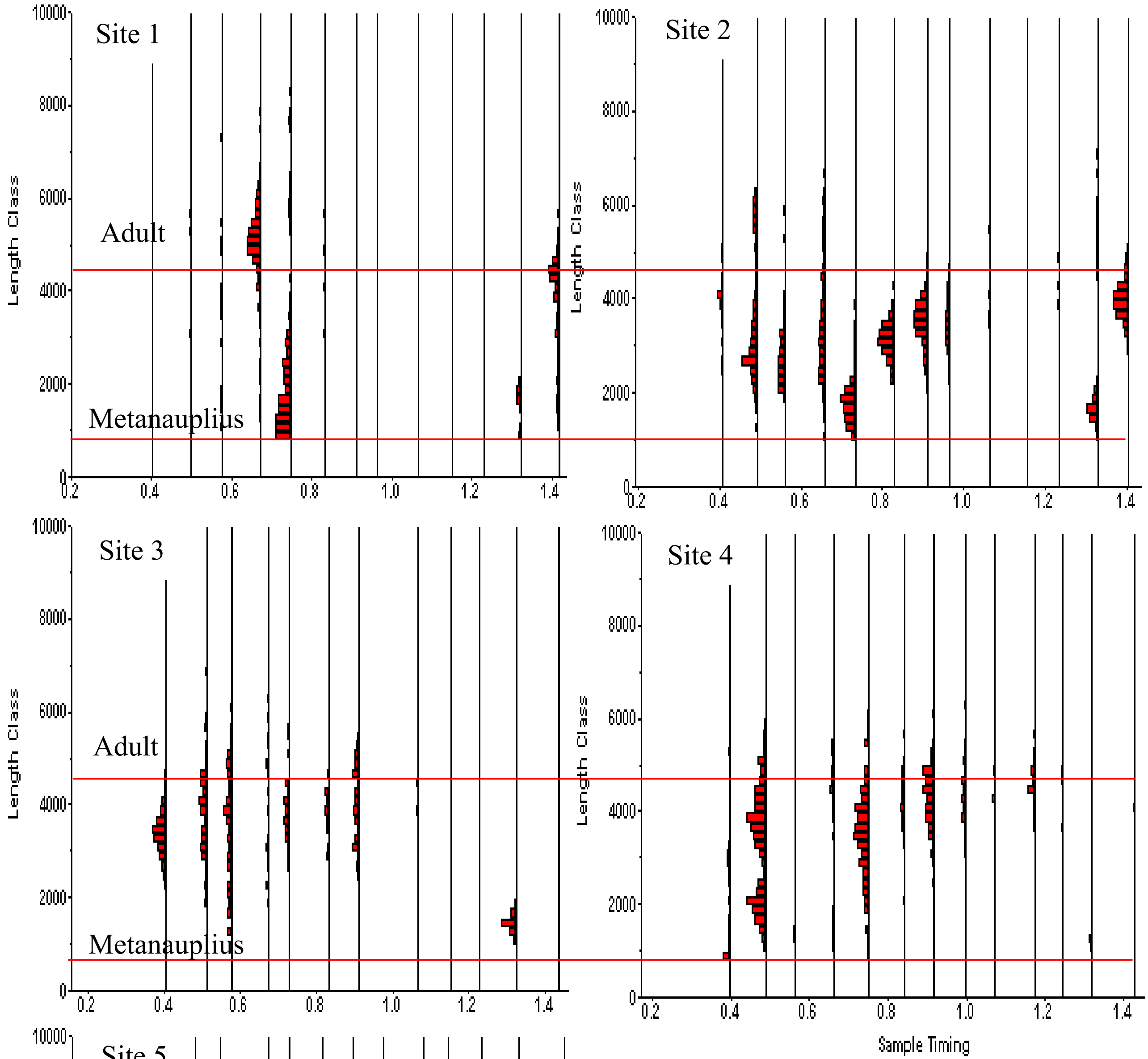
By September there was substantial recruitment of metanauplii into the system producing a second distinct cohort. The positively skewed shape of the cohort suggests the sample was taken less than halfway through a period of recruitment, and the width of the cohort would suggest a relatively long period of recruitment.

Numbers of *Argulus* spp. reduced dramatically in October in site 1, but it is clear that the September cohort had grown substantially by this time. No recruitment was seen through the winter months and the parasite apparently died out by November. April 2003 shows the start of recruitment for the year and the growth of the parasite can be followed into May 2003 when a second cohort was also starting to emerge.

In site 2 *Argulus* spp. were present from May 2002 to December, with only very low numbers persisting through the winter. In May 2002 one cohort approximately halfway through development is present. By June this is almost fully-grown and a second cohort is present. The first cohort is likely to have come from eggs that have over-wintered, and the second from eggs laid in the spring by adult parasites that survived the winter. A third cohort emerges in July, probably from eggs laid by the first cohort of the year. There is more recruitment in August when a wide range of size classes can be found. By September there are no large stages of the parasite present, possibly as a result of a intervention by the fishery owner. There is, however, a large amount of recruitment, producing a fifth and final cohort for the year. Although the cohort has almost died out by January, it is possible to follow it through the winter and a clear seasonal growth pattern can be seen. By April 2003 this cohort has reached adulthood, and the first new cohort of the year has started to emerge, the growth of which can be followed into May 2003.

Site 3 shows a similar pattern of development to site 2. A single cohort is present in May 2002, which is at the mid-point of development. Recruitment occurs in June and July, but there is insufficient data to determine distinct cohorts. In contrast to site 2 there does not appear to be any recruitment in August or September. The parasite dies out in the final few months of winter. Recruitment is seen for the first time again in April 2003, but this cohort could not be detected in the following month.

Development and recruitment in site 4 is very similar to that of site 2. Unlike site 2, where an intervention was used at the start of September there is no die out of the larger stages of the parasite in September. Only low numbers of the parasite were detected in the winter of 2002 and spring of 2003. Site 5 follows the same pattern, only this time substantial numbers of the parasite survive the winter. These follow a seasonal growth curve and reach adulthood in March 2003. By April these adult parasites have disappeared from the sample and recruitment is just beginning. In May 2003 there are two distinct cohorts. The first is probably a result of eggs that have over-wintered and hatched, the second from eggs laid in the spring by the over-wintered adults.



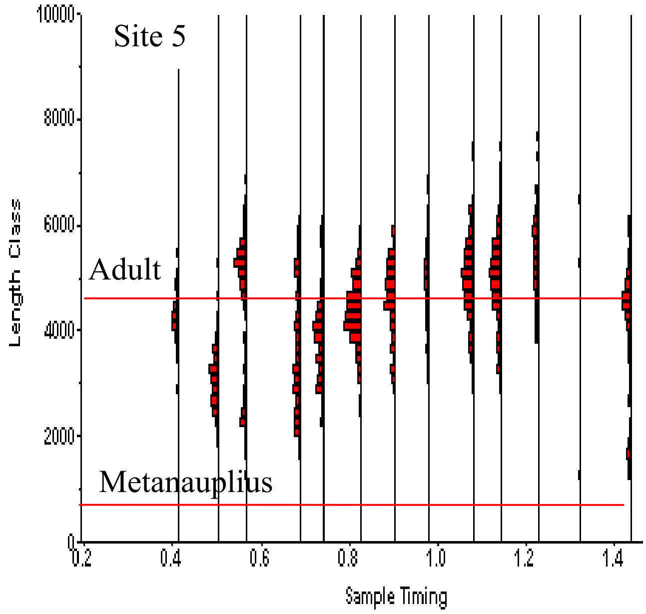


Figure 3.8 Length-frequency distribution of *A. foliaceus* in the 5 sites studied during the May 2002 to May 2003 longitudinal study of UK Stillwater trout fisheries.
Length class refers to µm.

Sample timings are precise and are equal to the number of days into year when the sample was taken divided by 365.

0.4 = 26th May 02	1.0 = 31st Dec 03
0.6 = 7th July 02	1.2 = 14th Mar 03
0.8 = 28th Sept 02	1.4 = 26th May 03

3.3. Experimental Studies on the Survival of *Argulus* spp.

3.3.1 The effect of light & dark conditions on the survival of *Argulus* spp. populations.

Figure 3.9 summarises the results from this study. The initial infection under both light and dark conditions was very successful but dropped rapidly within a week to between 40-60% of the initial infection level. Although Fig. 3.9 suggests that *A. foliaceus* survived longer under light conditions, there was no significant difference ($p>0.05$) in survival between the light and dark regimes after accounting for tank effect, with survival in two of the three pairs of tanks being longer in the dark tanks.

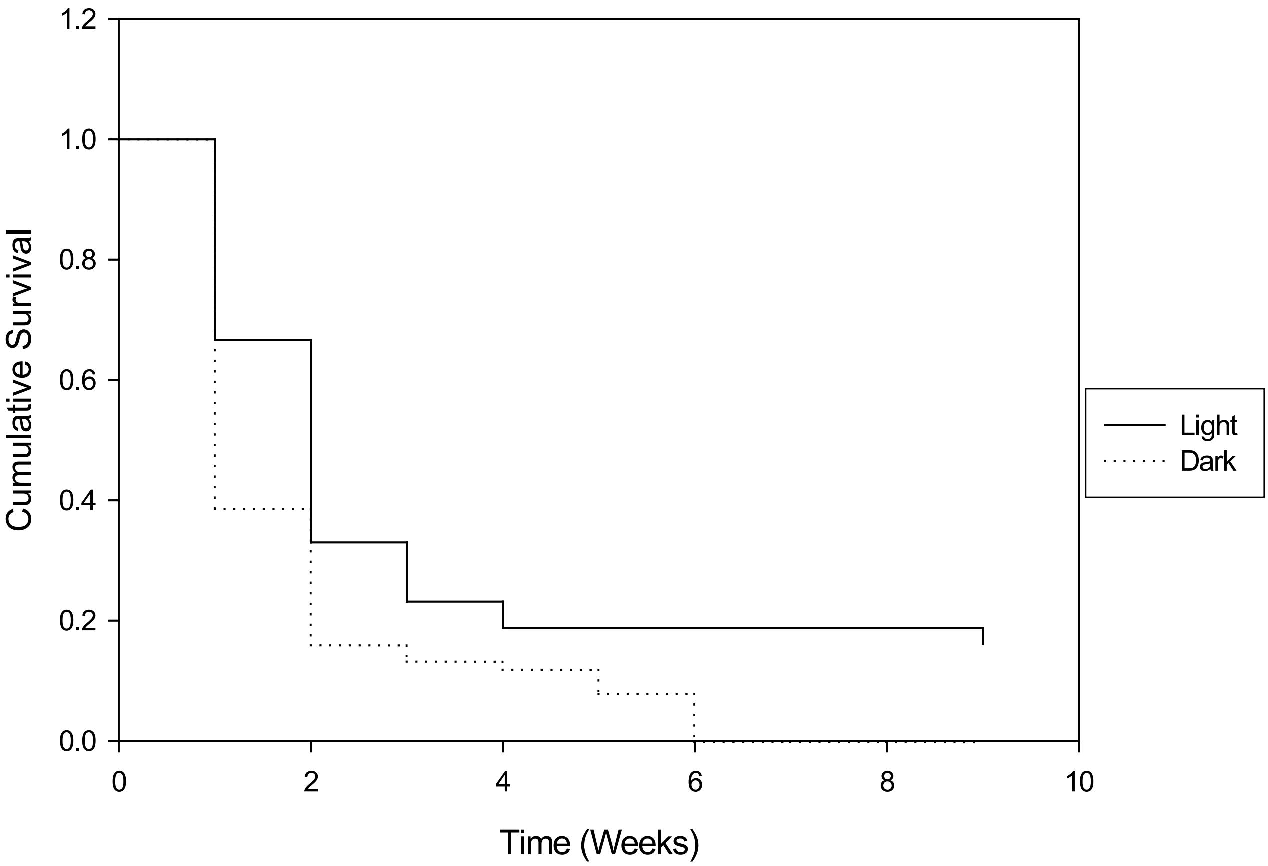


Figure 3.9 Survival of laboratory populations of *A. foliaceus* over time under light and dark conditions.

3.3.2 The effect of host difference on the survival of *Argulus* spp. populations

Figure 3.10 shows that there was no statistically significant difference in the survival of populations of *A. foliaceus* held on roach fry and carp. As in the previous experiment initial infection levels were high on both species but the infection level dropped rapidly to 30% of the level over the first week of the experiment. On both hosts the *A. foliaceus* populations survived for seven weeks.

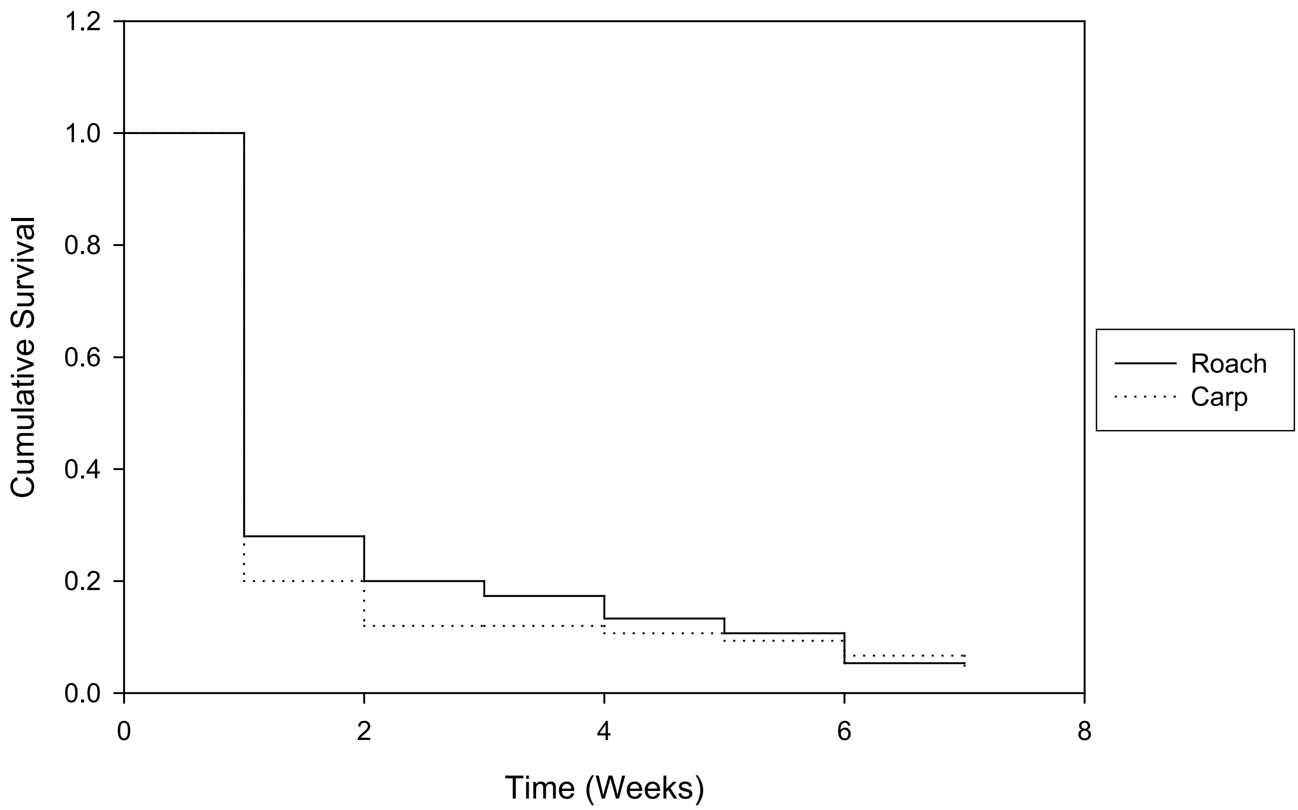


Figure 3.10 Survival of laboratory populations of *A. foliaceus* over time when held on different host species (carp &roach fry).

3.3.3 The effect of temperature on the survival of *Argulus* spp. populations

There was no significant difference in the survival of *A. foliaceus* populations at 10ºC and 20ºC (see Figure 3.11). However, survival was significantly longer ($p<0.05$) at these temperatures than at 25ºC. This result requires further investigation, as it is possible that the increase of 10ºC on day 1 of the trial to acclimatise the metanauplii to the 25ºC tank may have been too stressful (compared with a 5ºC change in the other tanks). The very rapid extinction of the 25ºC population (less than 2 weeks) may suggest this is the case.

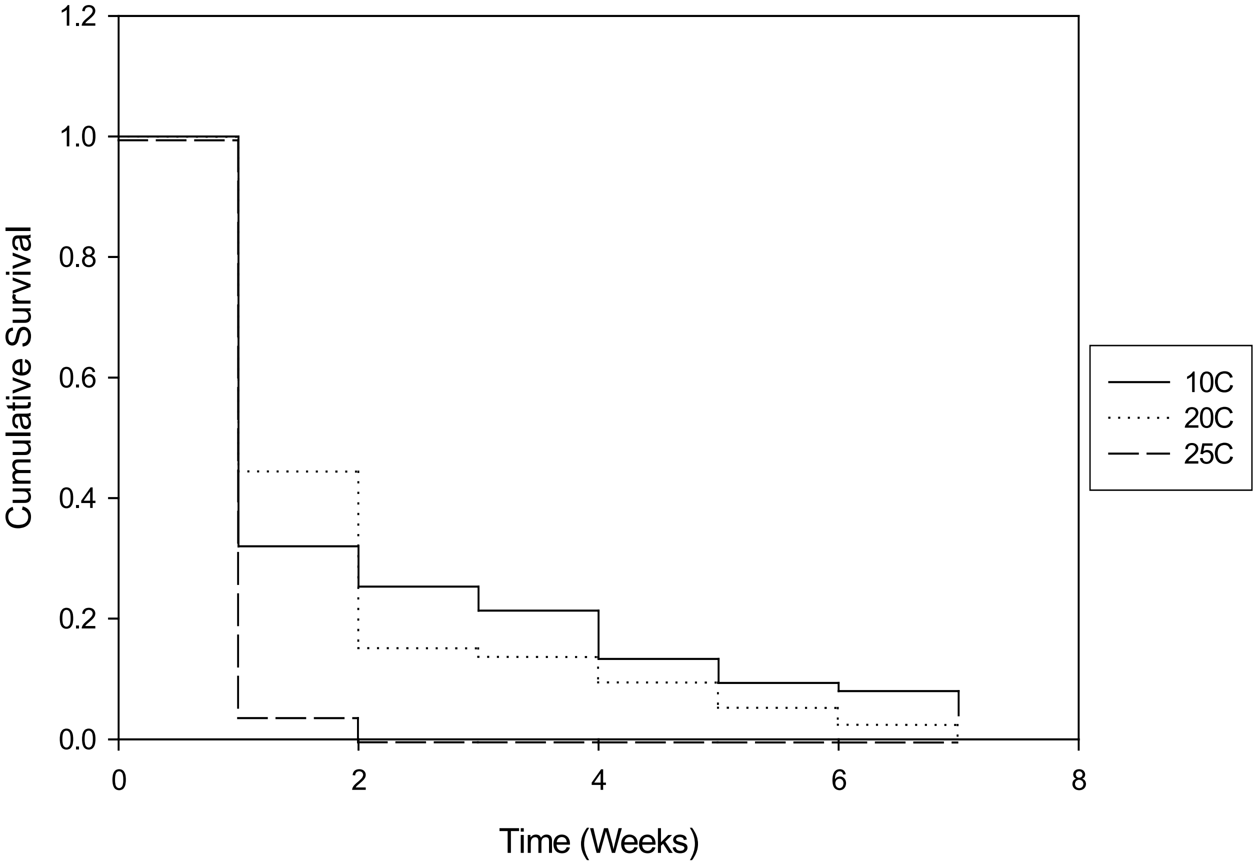


Figure 3.11 Survival of laboratory populations of *A. foliaceus* over time when held on different temperatures.

Although in real time the parasite populations held at 10ºC and 20ºC survived for the same period, those parasites held at 20ºC had developed to a greater size than those held at 10ºC. In order to take this into account the data was reanalysed in terms of degree-days (Figure 3.12). Results of this analysis showed that the populations held at 10ºC and 20ºC still survived significantly longer than the populations at 25ºC, but that survival was significantly higher ($P<0.05$) in the group held at 20ºC compared to the 10ºC group.

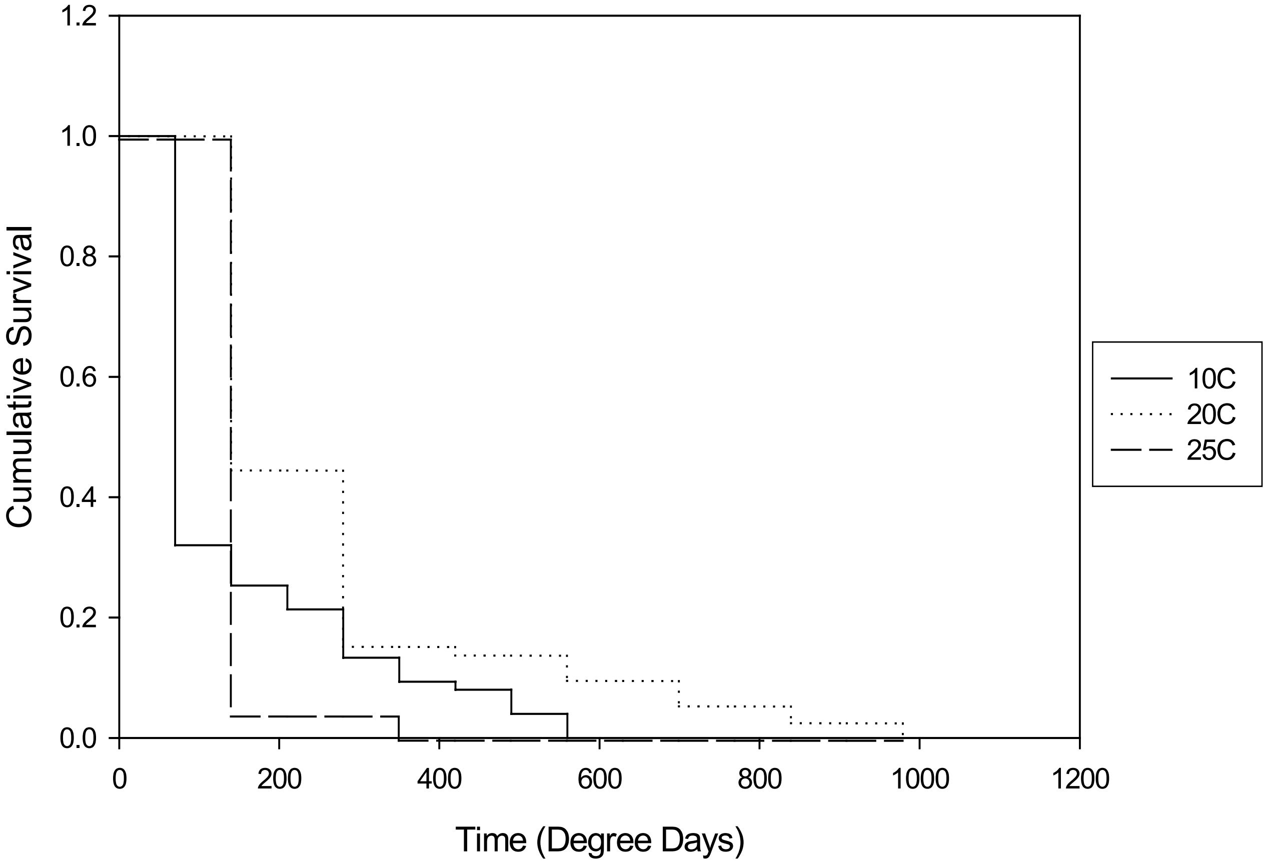


Figure 3.12 Survival of laboratory populations of *A. foliaceus* in terms of degree-days when held at different temperatures.

4 DISCUSSION

This study was designed to investigate the impact of *Argulus* spp. in UK stillwater trout fisheries, develop a greater understanding of the parasites biology, and evolve strategies to manage it. The study has successfully determined the nature of the problem and quantified the proportion of the industry affected. In addition, the study has given an up to date picture of the distribution of *Argulus* spp. in mainland Britain. Risk factors associated with problem infections have been identified, and correlations between these and the parasite population ecology established. From this information a number of management recommendations have been developed. This is the first time that the population ecology of *Argulus* spp. on mainland Britain has been studied, and has been examined in a variety of lakes with different management regimes.

4.1 The Perceived Problem and Prevalence of Infection

A cross-sectional study was conducted to establish the problems associated with *Argulus* spp. and the associated risk factors were identified. 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 site 2 in the longitudinal study shows that the rate of stock turnover (that can be taken as an indication of the number of fish caught) dropped to its lowest point as the abundance of *Argulus* spp. peaked. However, at this time of year, water temperature also peaked and turbidity reached its highest level due to an algal bloom. Two months later the stock turnover had returned to its normal level yet i.e. fish were being caught in large numbers, abundance of *Argulus* spp. was still high. The temperature however had dropped and the water clarity had increased. It may be, therefore, that if a heavy infection occurs but the lake remains cool and clear, a problem may not occur. 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% of them developed a problem in year 2000.

Based on the randomness of the 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) that response bias would be acting. It is possible that the prevalence is over-estimated, as those 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 less willing to participate owing to concern that their details may be released.

4.2 Distribution of *Argulus* spp.

Although the sample size is 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 south of the central highlands and Campbell (1971) reviews water bodies in Scotland where *Argulus* spp. has been found. More recent work by Northcott *et al.* (1997) described the occurrence of an epizootic of *Argulus* spp. in Perthshire. This is the most northerly record in the literature of *Argulus* spp. in the UK, however in 2003 a confirmed case in Aberdeenshire was reported to the authors. In some areas of the UK distribution map, the records of *Argulus* spp. look sparse, however there are no readily available records of the number of lakes in those areas so it is difficult to know how common the parasite actually is. Differences were noted through the survey between northern and southern UK: these were a lack of coarse fish and larger stillwaters in Scotland and northern regions. These factors were not shown to be statistically significant, as there were too few cases in the northern regions to make meaningful comparisons.

The distribution data suggests that *Argulus* spp. has been widely distributed throughout England and Wales since at least 1996, and has not spread northward in recent years. Insufficient data is available on any possible spread of *Argulus* spp. in Scotland, as there are few studies of *Argulus* spp. in this area.

When comparing the prevalence of problem infections in central and southern England and Wales, and after adjusting for the effect of confounding from other variables, risk factor analysis showed central regions to be at a significantly greater risk of suffering problem infections. This is perhaps surprising as a milder climate in the south might be expected to exacerbate the problem. Logistic regression models adjusted for variability caused by differences in the rate of stock turnover, lake depth and the presence of algal blooms between regions, show that these are not the only factors responsible for the differences in the prevalence of problem infections. It was not possible to determine other factors causing regional variations. One possible hypothesis is that there is a greater abundance of spring fed chalk waters in the South, which may result in cooler summer water temperatures. This is an important factor in determining the developmental rate of *Argulus* spp. and whether an infection will lead to a problem. Other factors that were not investigated, such as lake altitude, may also be important.

In the sites that were sampled and where *Argulus* spp. was present, only *A. foliaceus* was detected, with the exception of one site in which *A.coregoni* was found. This would suggest *A. foliaceus* to be the predominant species in UK stillwater trout fisheries. Environment Agency data suggests that *A. japonicus* follows a similar pattern of distribution to *A. foliaceus*, and supports the records of Rushton-Mellor (1992). Although not detected in the study, it is possible that *A. japonicus* is more abundant than presently thought due to 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.

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 other fish species in these trout lakes would be observed to the same level as trout. The longitudinal study found that fish species other than trout were infected with *Argulus* spp. demonstrating that they could therefore act as reservoir hosts. However, the prevalence and abundance of infection on these other fish species was always much lower than on the trout sampled, showing that they really were less susceptible to infection than trout.

Laboratory studies showed there was no difference in the survival of the parasite on carp and roach, and suggested that both species were equally susceptible to infection. It was not possible from the cross-sectional survey results to establish whether certain coarse fish species were more susceptible than others. Observations from the longitudinal study found pike to be the second most heavily infected species after trout and that perch were more heavily infected than roach. This corresponds to the findings of Pasternak, Mikheev & Valtonen (2000). The cross-sectional study produced no evidence to suggest that brown trout were more or less susceptible than rainbows, or if susceptibility was related to fish size. Survey data did suggest that over-wintered fish were more susceptible, but it is possible that these fish had been exposed to the parasite for longer and were therefore more heavily infected.

4.4 Methods of Control Currently Used

Treatment and management of *Argulus* spp. was often considered to be difficult for one or more of the following three 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. 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 increase in problem *Argulus* spp. infections.

In lakes in which it was possible, draining and liming was perceived as being effective. Fishery owners felt 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. It was not clear whether infections occurring after liming were a result of new introductions of *Argulus* spp. or a result of the treatment not being totally efficacious.

Few sites 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 suggested that high weed cover might increase the risk of a problem *Argulus* spp. 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. As a result the analysis was inconclusive and weed cover was not included in the final model, it would however be worth of further study.

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 survey, none of the fishery owners were using them. There were also concerns that boards would impede angler's casts.

None of the measures described by the fishery owners were perceived 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.

4.5 Risk Factors

Univariable analysis of the cross-sectional data set identified 20 potential candidate variables. These have not been detailed, as this technique for reduced the data set, pulled out the most important variables and allows for the identification of confounding when the logistic regression models are built. The 20 candidate variables identified by these means were not necessarily associated with a problem infection.

The logistic regression models identified three factors: algal bloom, stock turnover and depth, which can be used to predict whether a site has increased (or reduced) likelihood of suffering a problem infection. The higher the number of these factors present in a fishery the greater the risk of it suffering a problem *Argulus* spp. infection i.e. the chance of a problem infection is greater in a lake with two risk factors compared with a lake with just one. It must be noted that although these risk factors are associated with the outcome (presence of "problem" *Argulus* spp.), they do not necessarily have any direct causal effect upon it. Therefore, without further information 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. Some evidence as to how these risk factors are acting was provided through the laboratory trials and longitudinal study. This information is discussed for each risk factor in the following paragraphs.

4.5.1. Algal blooms

The cross-sectional survey showed that algal blooms were associated with an increased risk of suffering problem *Argulus* spp. 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 the 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 not felt to be truly representative.

It may be that algal blooms do not affect the *Argulus* spp. population but stress the fish in a way that would cause an infection by *Argulus* spp. to manifest itself as a problem. There is some evidence for this from the population survey. At site 2, in October, stock turnover returned to normal levels suggesting the fish were being caught; yet the abundance of *Argulus* spp. was high. At the same time there was a drop in the lakes water temperature and an increase in water clarity. This would suggest that it is a combination of these factors causing the reduced feeding by fish and not just the parasite burden. It is also possible that infection by *Argulus* spp. causes fish to rub themselves on the lakebed thus churning up the sediment and clouding the water. This was observed by a fishery owner and Shimura (1983), but would only explain a correlation between infection and turbidity and not algal blooms.

There is, however, also evidence to suggest water clarity directly influences the population dynamics of *Argulus* spp.. Observations in the literature suggest *A. foliaceus* to be most abundant in warm, shallow, eutrophic lakes (Gurney 1948). The clearest lakes in the population study were observed to experience the lowest burden of *Argulus* spp.. In site 2 there were rapid increases in the abundance of *Argulus* spp. in June 2002 and May 2003, corresponding to rapid decreases in lake turbidity caused by algal blooms. Laboratory trials to elucidate more information on effect of turbidity through holding *Argulus* spp. populations on fish under light and dark conditions did not provide any conclusive answers. The overall effect showed there to be no statistically significant difference, but in two out of three pairs of tanks the *Argulus* spp. population survived longer under dark conditions. This difference was not however statistically significant, and the trials were conducted on carp not trout. It could be that differences in behaviour, e.g. feeding and swimming, of carp would have changed the effect that the light/dark conditions may have had. The trial also showed there to be no difference between the initial infection success under the two conditions, although it should be noted that the trials were conducted in small tanks making it relatively easy for the parasite to find its host.

If algal blooms are having a direct effect on the population dynamics of *Argulus* spp. several hypotheses can be advanced to explain this. Trout are predominantly visual feeders and it could be that a reduction in the water clarity reduces the level of predation upon the parasite. Predation on *Argulus* spp. by trout was observed in the laboratory, and noted in the field by examination of the stomach contents of trout. Predation by other fish species has also been recorded in the literature (Wilson 1902, Thomas 1961). Changes in water clarity may also change the swimming behaviour of trout or stress the fish, suppressing its immune system, making it easier for the parasite to infect and survive.

Algal blooms have some potential for management, but water clarity less. Barley straw or some commercially available products may successfully reduce algal levels, and lake dredging may also do this and increase water clarity. 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. Water from fish farms, septic tanks or stagnant ditches or ponds should be avoided.

Coarse fish, especially cyprinids, are associated with turbid water due to their feeding behaviour. In addition to increasing turbidity, coarse fish may also act as reservoir hosts for the parasite. As a result their removal could be of benefit, although there is currently no information available to demonstrate the significance of coarse fish in maintaining *Argulus* spp. populations.

If algal blooms are reduced and water clarity increased, care should also be taken to manage weed levels as analysis of the survey data provided some suggestion that high weed levels may also be associated with problem *Argulus* spp. infections. This requires further investigation but if there is an association it could be making fish more sedentary, remaining in the weed and making infection easier. It could also reduce predation on the parasite by providing refuge for free-swimming individuals.

4.5.2. Average depth of lake

Logistic regression models suggest that for every 30cm increase in average water depth there is a slightly reduced risk of suffering a problem *Argulus* spp. infection. This is obviously a factor that in most cases is difficult to manage, but may be relevant when purchasing, designing or constructing a fishery.

It is difficult to understand how this factor is acting. Increased depth may lead to cooler areas in lakes, although the temperature data from the cross-sectional study suggests that this is not necessarily the case. It could also be that an increase in depth reduces stocking density. Although stocking density is well established an important factor in determining the rate transmission of a pathogen between hosts, it was not in this study significant factor possibly as a result of relatively small sample size and the large number of factors being screened. It may be that increased depth makes it less likely a fish will enter an area of high infectivity (possibly fish and parasite utilise different parts of the water column in deep water).

4.5.3. Stock turnover

Of the risk factors identified, stock turnover has the greatest potential for management. The data suggests that if the entire stock of trout in the lake can be replaced in less than 72 days (median cut-point value from data set) there is a statistically significantly lower risk of suffering a problem *Argulus* spp. infection. Longitudinal survey data from site 2 shows clearly that parasite burden increases in conjunction with temperature and a reduction in turbidity and stock turnover. As already suggested it is possible that the reduced turbidity could increase the infection success of *Argulus* spp., but if the stock turnover is also reduced at this time it would allow the parasite abundance to increase since the removal of parasites would also be reduced. If fish can be removed faster than the parasite can reproduce then the abundance of *Argulus* spp. would be reduced. This can be seen most clearly in the sites 1 and 2 after September, where the parasite population dies out rapidly after recruitment stops. In site 5 where there is no significant winter stock turnover the parasite survives the winter. These parasites then lay eggs a month earlier than in lakes where the winter population of *Argulus* spp. has died out and the establishment of the parasite population is due solely to the hatching of over-wintered eggs.

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 the lake for a long time. In addition to this many clubs have a closed season from October to the end of March, allowing the hatched parasite to over-winter, as the abundance is not reduced through fishing. The survey showed that these sites stock a large batch of fish in March, ready for the start of the season. Data from the population study shows that this unfortunately means that the stock density is at its highest when the first cohort of *Argulus* spp. emerges in April, which originates from over-wintered eggs. This is likely to aid infection success at the start of the year, leading to higher infection levels throughout the year.

It may 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 come to the fishery. In syndicate waters, and many others, it is not feasible to increase stock turnover throughout the year. Even if it were, in the summer months when a combination of factors act together to reduce feeding by fish, only the healthiest fish with the lowest burden of *Argulus* spp. will be caught, thus it would have little effect. It may however be possible to do this at certain key time points. If fishing is allowed to continue over winter then it is likely to at least reduce the parasite population, and minimise the number of eggs laid in April. If it is then possible to hold a closed season from the end of April until the end of May when fish numbers are very low (due to winter fish out), this would act as a fallow period preventing the seasons first cohort of *Argulus* spp. finding a host. Obviously the effectiveness of this strategy will depend on the number of reservoir hosts on which the parasite can settle. Alternatively if a closed season is not possible and many fish are stocked at this time, speeding up stock turnover from the end of April to the end of May should remove the parasite while fish are still healthy and before *Argulus* spp. has begun to lay eggs. Stock turnover could possibly be increased over this time by holding competitions, tuition or corporate days. There may be other means by which the most heavily infected fish can be targeted, making the strategy even more effective. These may include seine netting, which is likely to select the slowest, sickest fish, or by removing fish from cool water inlets, as observations during the study have shown that heavily infected fish tend to congregate in these sites.

4.6 Population Ecology in Individual Sites

4.6.1. Site 1

This site was very intensively managed with a very fast turnover of fish. The lake was known to be infected with *Argulus* spp., but the parasite had only ever been observed at low levels in the lakes two-year history. Abundance of *Argulus* spp. was very low during May, June and July 2002. This suggests that there were only extremely low levels of eggs or hatched *Argulus* spp. over wintering, 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. In August there was a sudden increase in the abundance of *Argulus* spp., composed of a cohort already adult. During this month the lake suffered an algal bloom, reflected by an increase in turbidity. This may explain the high infection success but does not explain the origin of the parasite. There are several possible explanations for this. Parasites could have hatched since the previous visit, grown and reached adulthood by the August visit, however this would mean 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 sites stock pond were found to be free of *Argulus* spp. The most likely explanation is that the parasite came from a reservoir source. The study lake was directly connected to an upstream lake, which was known to suffer a heavy *Argulus* spp. infection. Adult parasites leaving their host to lay eggs may have been washed into the study lake, alternatively, the study lakes contained a large number of sticklebacks infected with low numbers of juvenile *Argulus* spp. 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 after their death.

After the August site visit all fish were removed by netting and the lake stocked with clean fish in an attempt to eradicate the parasite. This did not remove eggs already laid in the lake and in September the abundance of *Argulus* spp. peaked as a large cohort of metanaupli hatched from eggs laid between the July and August samples. Recruitment was possibly aided by immigration of metanaupli from the upstream lake. After September there was no recruitment for the rest of the year, and the parasite population dropped to virtually zero. This was probably due to the very fast stock turnover removing fish and parasites. There was a 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 *Argulus* spp. population had virtually died out. Recruitment of *Argulus* spp. began in April from over-wintered eggs, but egg laying by this cohort did not start until May.

4.6.2. Site 2

This site was relatively intensively managed, and has suffered with *Argulus* spp. infections for over a decade. This is known as a 'big fish' water, only stocking fish over 3kg, compared to 1 kg in a standard water. A wide range of other fish species is present that has the potential to act as reservoir hosts. This site suffered the highest abundance of *Argulus* spp. of all the sites sampled, even though it had a relatively fast stock turnover. Data from the site demonstrated that as the abundance of *Argulus* spp. increased, in combination with other factors, stock turnover rates dropped as anglers could not catch fish.

Abundance of *Argulus* spp. was low in May 2002, increasing slightly in June, and then increasing rapidly in July, corresponding with an algal bloom. The possible impact of blooms has already been discussed (see page 42). However, if blooms were to increase infection success by some means, then this, in combination with large fish being able to carry high numbers of *Argulus* spp., and a reduction in stock turnover, could explain the high abundance observed. The *Argulus* spp. population peaked in August but dropped rapidly by September. After the August visit the site owner carried out an intervention (For the purpose of this reports an intervention is defined as 'a measure undertaken by a fishery owner in order to reduce the burden of argulids, the legality of such interventions was not documented). The intervention appeared to be successful as numbers of *Argulus* spp. dropped and no egg laying occurred. However, the intervention did not kill eggs already present in the lake and 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 to another algal bloom and increased turbidity. This cohort would be expected to emerge from eggs laid by the August cohort in September. However this cohort was apparently killed out before they 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 Finland, but other authors have found relatively conserved hatching periods in *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 *Argulus* spp. carried on reservoir hosts in the lake or on only some trout, as it was implemented in only one part of the lake. Although no 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. For example, pike inhabit reed and weed beds, and any *Argulus* spp. leaving these fish to lay eggs are unlikely to use the boards in open water.

4.6.3. Site 3

This was the deepest and second largest lake in the survey. It received trickle stocking and maintained a moderate stock turnover of around 84 days throughout the study. The lake water remained very clear throughout the survey and had the lowest abundance of *Argulus* spp.

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, as it is difficult to know where real differences lie, and sampling error occurs. Abundance of *Argulus* spp. appeared to remain constant and low from May 2002 to August 2002. Abundance then appeared to drop to about 50% of this level from September to December, before dropping to almost zero in January, where it remained for the rest of the study. Due to low sample sizes the length-frequency data is difficult to interpret. A distinct cohort was present in May 2002, and only low levels of recruitment occurred in June, suggesting few hatched *Argulus* spp. over-wintered and were able to lay eggs in April. Higher levels of recruitment were observed in July, probably resulting from eggs laid by the cohort observed in May 2002. There did not appear to be recruitment in August, but if there was it was at a very low level. This would be as a consequence of a small July cohort. No recruitment occurred in September suggesting the progeny of the July cohort had not successfully established itself. Lack of recruitment in September explains the low parasite abundance observed over the winter. Only low numbers of *Argulus* spp. emerged on April 2003 due to a small August cohort laying small numbers of eggs that over-wintered. There was no egg laying in April or May 2003, reflecting the low number of over-wintering hatched parasites and low April recruitment.

4.6.4. Site 4

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

Only low levels of *Argulus* spp. were observed in May 2002, and spanned a wide range of size classes. Substantial recruitment had occurred by June when, two clear cohorts were observed and the parasite abundance had increased, suggesting that substantial numbers of *Argulus* spp. over-wintered and laid eggs in April. A very small sample size in July makes in unlikely that the apparent drop in abundance observed was significant. Its seems likely that the abundance of *Argulus* spp. held relatively constant from June to September. Recruitment occurs in each of these months and suggests that five cohorts hatched in the year. Recruitment stopped after September and did not occur again until

April. Low numbers of hatched *Argulus* spp. over-wintered and this is reflected in the egg laying activity observed in April.

4.6.5. Site 5

This is a small privately owned site, lightly fished and receiving little management. It has had a long-term problem with *Argulus* spp. In the study year the lake was stocked just once, one month prior to the start of the survey. These fish then remained in the lake throughout the study with only a very small number being removed. This is a useful study population as it effectively demonstrates how a population of *Argulus* spp. behaves on trout when there is no management. Water clarity remained constant for the entire survey, with the exception of April 03. As a result we can assume turbidity had little impact on changes in the parasites abundance. Effectively the only stock turnover was from the investigator taking *Argulus* spp. from infected fish during the survey and restocking them free from *Argulus* spp.

Recruitment from eggs laid in April by hatched stages of *Argulus* spp. that have over-wintered occurs between May and June, causing parasite numbers to increase. Although recruitment occurs, abundance holds constant into July. This is probably due to adults from the June cohort dying or laying eggs, counteracting the effect of recruitment from the third cohort. There was a significant increase in abundance in August with the emergence of a fourth cohort. The abundance continues to rise slowly until it reaches its peak in October. There is a substantial drop in the abundance of *Argulus* spp. in November. This corresponds to the water temperature dropping to below 10°C and suggests that, as no egg laying was observed and there was no real stock turnover, that the low temperature may be responsible for the apparent high mortality. The finding corresponds with Kimura's (1970) observation that *A. japonicus* that had hatched in the autumn were very likely to die over the winter. After this initial drop, the abundance remained constant, suggesting that those parasites that survive the initial mortality survive the rest of the winter.

In April there was another significant drop in the abundance of *Argulus* spp., associated with the start of egg laying. The first metanaupli of the season were also found in April, but numbers were very low compared to the other study lakes, except 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).

4.7 Population Ecology – General Observations

It is clear from the data that there is considerable variation in the population ecology of *Argulus* spp. between sites, although a broadly similar seasonal pattern was observed. Abundance of *Argulus* spp. was low in the winter months, increasing in spring and peaking just after the hottest months, before dropping as the temperatures fell in autumn. The abundance of the parasite throughout the year varies between waters and appears to depend on the ecology and management of the lake. This may explain the variability of the periods when *Argulus* spp. were perceived to be present by fishery owners.

In a site where no significant intervention was employed there appeared to be five cohorts in a year. Four of these hatched, developed to adulthood and laid eggs between April and September, the fifth hatched in September but developed slowly over winter, laying eggs in the subsequent season. Eggs laid by the fourth cohort in September, over-wintered and hatched in the spring. Details of the general pattern of how these cohorts were produced and develop are described in Table 4.1. Length frequency data suggests that hatching at the start of the season (April) is relatively conserved in terms of the period of hatching. As the season progresses, the hatching periods appear wider and cohorts start to overlap, probably due to variability in the period when eggs were laid. Distinct cohorts can be seen through the length-frequency data. The distinct hatching periods observed in the study are similar to the situation observed by Pasternak *et al.* (2000) in Finnish trout farms, but differ to the situation they observed in lakes where hatching was highly variable.

Table 4.1. The timing of development and egg laying of cohorts of *Argulus* spp. in UK stillwater trout fisheries

Cohort	Produced From Eggs Laid In	Lays Eggs In	Eggs Hatch In
April	August	June	July
June	September	July	August
July	April	August	September
August	June	September	April
September	July	April	June
April	August	June	July

Temperature is an important factor in determining the hatching, development and egg laying of *Argulus* spp. 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 and Rahman 1995). The higher the temperature the faster the parasite develops from metanauplius through to adult. The length frequency data from all sites, except site 1, shows that the first cohort of the season, hatching from over-wintered eggs takes approximately six weeks to reach adulthood from the end of April until June. With the exception of the September cohort, subsequent cohorts take only one month to reach adulthood. The September cohort develops slowly reaching adulthood around November when the water temperature has dropped to just under 10°C. No further growth or egg laying was observed until the growth rate of the cohort increased with a rise in water temperature in the spring. The September cohort does not appear to lay its eggs until April when the water temperature again reaches approximately 10°C. Egg laying in all sites appeared to stop once the August cohort had laid 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 stops at temperatures below 10°C (Stammer 1959, 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 4.5mm in length, similar to Rushton-Mellor & Boxshall's (1994) estimate of 4. 67mm. The laboratory studies suggest that, although temperature does not directly affect the overall survival of *Argulus* spp., it does affect the proportion of the parasites that develop through to an advanced developmental stage. At higher temperatures a higher proportion of the parasite will develop to an advanced state. Laboratory and field studies suggest the optimal temperature for survival and development is around 20°C. Over-wintering populations of *Argulus* spp. appear to survive for around 8 months. Mortality in the over-wintering cohort appears to be associated with egg laying in the following spring. This is most clearly observed from the data from site 5, where the abundance of *Argulus* spp. averages around 17 *Argulus* spp. per fish over winter until April when it drops to 5, corresponding to the start of egg laying for the year.

Temperature profiles between the five sites studied in the longitudinal study were very similar, the abundance of *Argulus* spp. was, however, highly variable. This suggests that although temperature is important in controlling the pattern of development, it does not necessarily determine the overall success of the parasite population. Parasite populations often cycle in their abundance over time. Some of this cycling will be determined by the parasites abundance in the previous year, while other variability can be explained by environmental and management conditions. Unfortunately it was not possible in this study to follow populations of *Argulus* spp. for more than one year. This makes it difficult to understand the differences fully, but correlations observed between risk factors identified in the study, and the abundance of *Argulus* spp., appear to explain a substantial proportion of this variability between sites.

5. SUMMARY OF RECOMMENDATIONS FOR THE MANAGEMENT OF *ARGULUS* SPP. IN UK STILLWATER TROUT FISHERIES

1) If possible, trickle stocking is better than batch stocking as this will reduce the length of time a fish spends in a lake, thus increasing stock turnover rates.

2) Avoid stocking April/May and speed up stock turnover during this period as this is when the first cohort of *Argulus* spp. hatches. By keeping stock low in these months it will reduce the likelihood of a parasite finding a host. Increasing stock turnover during this period will remove parasites before they are able to lay eggs.

3) Killing hatched stages of the parasite over winter will reduce the number of eggs in the subsequent year. This suggests that no closed season over the winter period will be beneficial as fish capture over this period will reduce the *Argulus* spp. population (success will be dependent on the number of reservoir hosts present).

4) If possible/practical draining and drying/liming the fishery over-winter will kill many of the over wintering eggs.

5) If the trout population is fished out over winter, a closed season from the middle of April until the end of May will act as a fallow period just as the first cohort of *Argulus* spp. is hatching (success dependent on the number of reservoir hosts).

6) Targeting interventions early in the year before the parasite lays eggs, and towards the end of the summer, will help prevent over-wintering of eggs.

7) Increased water clarity may reduce the infection success and survival of *Argulus* spp.. It may be feasible to increase water clarity through the use of barley straw, a clean water source and removal of cyprinids.

8) Removal of alternate hosts to reduce the reservoir of infection.

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APPENDIX 1

Argulus spp. Questionnaire

Section 1 – The fishery DATE:

1) Location of fishery – NGR Postcode County
2) What do you perceive to be the main problems caused by *Argulus spp.*?
3) What is the land surrounding the fishery used for? A) Woodland B) Arable C) Pasture D) Other – Details – Ask about treatments on neighbouring land.
4) What is the fisheries primary water source: A) Spring B) River/Stream C) Static D) Other – Details
5) What is the fisheries secondary water source: A) None B) Spring C) River/Stream D) Static E) Other – Details
6) Is the systems geology primarily: A) Chalk B) Gravel C) Clay D) Other – Details
7) What was the average number of anglers per month throughout the year in 2000? (include months in which the fishery was closed)
8) In which month were numbers of anglers highest in the year 2000?
9) In which month were numbers of anglers lowest in the year 2000?
10) Who supplied you with fish in the year 2000?

Supplier	**Species**	**Number/proportion**

11) How many trout fishing lakes are there at the fishery?
12) What are their surface areas?

The following questions refer to the specific lake selected and no other lakes in the fishery:

Section 2 - Presence of *Argulus spp.* in the year 2000

13) Was this lake in use for trout fishing in the year 2000? Y N
(if no select another lake).
14) Have you seen *Argulus spp.* in this lake this year? - Y N
15) Were *Argulus spp.* present in the year 2000? - Y N
if no go to question 16
16) Were the levels of *Argulus spp.* present last year sufficient to cause the problems associated with *Argulus spp.*, that you described in the previous section (Question 2)?

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

17) Were any other fish health problems in this lake in 2000? Y N If yes which
18) Were there any other problems in the lake e.g. pollution, water quality, low DO ? Y N If yes give details
19) When numbers of *Argulus spp.* reached their highest in the year 2000 did you note any of the following: Yes No

Were *Argulus spp.* 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.
(On the grounds of the above questions – Epizootic Yes No Verify by netting a lake as a gold standard, and workout the reliability of these questions).

Section 3 - The lake details from the year 2000

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 spp.* present in the following years?

Year	**No**	**Partially**	**Fully**	**Argulus spp. 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 salmonids (list) Y N
Coarse fish (list) Y N
Minor species e.g. Gudgeon, minnows, sticklebacks, bleak etc. (list) Y N
49) If *Argulus spp.* was present did certain fish species seem more susceptible to infection?
Y N DN 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 spp.* than others?
Y N DN 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 spp.* as older fish?
Y N DN 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 Q72
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 spp.* 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 spp.* 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 spp.*? 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 rate was 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 spp.* (give details)?

Section 6 – History of *Argulus spp.*

90) Have *Argulus spp.* ever been present in this lake? Y N If no please go straight to section 7
91) In which months did *Argulus spp.* appear to be present (list)?
92) In which month were the numbers of *Argulus spp.* lowest?
93) In which month were the numbers of *Argulus spp.* highest?
94) Have *Argulus spp.* always affected this lake? Y N If yes please go straight to section 96
95) In which year did *Argulus spp.* first occur?
96) Have *Argulus spp.* occurred every year since they first appeared? Y N
    If yes please go straight to question 96
97) In which years did *Argulus spp.* not occur?
98) In years that *Argulus spp.* 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 spp.* infections? Please give details.
100) Are there any other observations about *Argulus spp.* 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