Investigations into *Ergasilus sieboldi* (Nordmann 1832) (Copepoda: Poecilostomatoida), in a large reservoir rainbow trout fishery in the UK.

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DECLARATION

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

Signed ....

For my parents Mark and Elizabeth Tildesley

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Abstract

Ergasilus sieboldi has been reported from a number of trout fisheries in England and Wales. The population dynamics of this parasitic copepod in Rutland Water, a large reservoir in Central England was studied from 2003 to 2005. A combination of angler and net caught fish were examined to record numbers of adult females and egg production throughout each year. The parasite overwintered in large numbers on trout and commenced egg production in April which then continued until October/November. The prevalence of infection and the abundance of the parasite were very high in overwintered rainbow trout but these parameters then decreased in March as large numbers of uninfected fish were stocked into the reservoir. The parasite population then increased until October. Infection levels in 2004 and 2005 were significantly lower than in 2003. Infections of cage-held rainbow trout showed that *E.sieboldi* could become ovigerous within two weeks of attachment to trout in July and August. New infections occurred from June until November. Several species of coarse fish examined were also shown to be infected by the parasite. Cage trials showed that triploid rainbow trout were infected by significantly higher numbers of the parasite than diploid rainbow, brown trout or "blue" rainbow trout.

Observations of infected fish in experimental tanks showed that overwintering parasites were stimulated to commence oviposition by increasing water temperatures. Photoperiod had no noticeable effect on the parasite. Egg viability and rate of development was studied using tank held infected fish and *in vitro* incubation techniques. Viability of eggs in sacs detached from the adult parasite was greater than those remaining attached. The rate of egg development was modelled and was shown to be predicted by temperature. Development of eggs was estimated to commence at

3.6ºC. Eggs developed more rapidly at higher temperatures and at peak production, inter-clutch interval was between 0 and 0.5 days. Egg production models estimated that an overwintered parasite could produce up to 19 clutches of eggs between April and October under normal temperature regimes measured at the reservoir. Ovarian development during the winter was confirmed using classifications of ovary size and shape based on parameters measured using image analysis techniques. The life span of *E.sieboldi* was estimated at 10-12 months.

Nauplii culturing techniques were compared, and nauplii to stage V were successfully developed. Nauplii hatched from the eggs of adult parasites occurring in the spring were larger and conditioned to develop at lower temperatures than those hatched later in the year. Nauplii were fed on 4 different types of algae held in monocultures but development occurred only in algal polycultures. A comparison was made of nauplii feeding preferences and development with algae recorded in Rutland Water in 2003 and 2005 but no correlations were found.

Fish stock assessment was carried out using models of angler catch, effort and stocking figures from the fishery. Parasite numbers on the overwintered fish were estimated at 12 million parasites in April 2003, 8.3 million in April 2004 and 1.2 million in April 2005. Stock assessments suggested a reduction in number of overwintering trout and effects of stocking policy to be at least partially responsible for the decline in the parasite population. The results of this study formed a management strategy for the operation of the trout fishery.

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Chapter 1: General Introduction

Studies of the parasitic Copepoda are as diverse as the animals themselves. A wealth of literature is available on the morphology, systematics and pathology of this sub-class, but surprisingly few studies have addressed the need to understand the dynamics of a population and potential control methods for some important species. The exceptions to this are the parasitic copepods that cause the greatest economical losses to intensive aquaculture, the caligids. Extensive research on species such as *Lepeophtheirus salmonis* and *Caligus elongatus* which are problem species to salmonid aquaculture in Europe, Canada and Chile (the largest producers of salmon and trout), has led to a better understanding of the population dynamics and how management techniques, fish husbandry and chemotherapy can assist in reducing the risk presented by these parasites.

The focus of this study was to identify aspects of the biology and epidemiology of the freshwater fish parasite *Ergasilus sieboldi*, that will further the understanding required to manage a population of this species in Rutland Water, a commercial trout fishery.

1.1 Ergasilidae

The Ergasilidae is a family within the order Poecilostomatoida and is comprised of 24 different genera and more than 140 species (El-Rashidy & Boxshall 1999), although many of the taxonomic parameters that define a species are regularly debated, and new species are consequently being described or relegated to synonyms. The general morphology of the parasite is cyclopoid and not dissimilar from entirely free living copepods (Kabata 1979) (see Plate 1). The family is considered to be relatively new to parasitism in evolutionary terms (Huys & Boxshall 1991;Kabata 1979). The features

that allow them to exploit a parasitic life style are few and less developed in comparison with other parasitic Copepoda. Features include modified antenna for attachment to the host by encircling or penetrating host tissue (Kabata 1979) (see Plate 2) and feeding appendages which permit the removal of host tissue or blood (Einszporn 1965) (see Plate 3), and in some species, extensive serrations on the ventral surface of the somites (see Plate 4) and margins of the swimming legs (see Plate 5) that may assist in anchoring the parasite to the host in strong water currents (Kuang & Liu 1992). In contrast, members of the family Pennellidae which utilise more than one host during the life cycle have developed a range of adaptations to parasitism and the ability for extended growth without moulting in a parasitic phase. In the genus *Lernaeocera* Blainville, 1822, the adult female undergoes metamorphosis in the final host, producing a hold-fast, an extended genital complex and a sigmoid trunk that allows the parasite to feed from the bulbus arteriosus or ventricle of the host (Bricknell, Bron, & Bowden 2006;Kabata 1979;Kearn 2004).

Although known to be pathogenic to the host under certain conditions, the presence of some ergasilid species in low numbers is generally thought to be tolerated by most host species unless additional factors contribute to the pathogenicity (Kabata 1970). As a consequence, in depth studies tend to take place following reports of severe infections which are expressed as the disease ergasilosis (Schaperclaus, Kulow, & Schreckenbach 1992).

The distribution of ergasilids is world-wide encompassing a diverse range of habitats and hosts (Fryer 1970). In the Ergasilidae, it is only the adult female that becomes parasitic in the life cycle, the other stages being entirely free living. Predominantly,

hosts are teleost fish, but there are also a small number of species that infect bivalve molluscs. They are found as parasites of host gills, fins, nasal cavities or the pericardia of bivalves (Alston 1994). *Ergasilus chautauquaensis* has never been observed as parasitic and planktonic ovigerous females have been reported from inland lakes of eastern United States (Bricker *et al*. 1978).

Ergasilids can be found in marine and brackish environments but the majority of known species inhabit freshwater (Abdelhalim 1990). Studies of ergasilids have been carried out in many parts of the world as befits the distribution of the parasites, the pathogenic effects of infection and host species importance. Given that ergasilids are relatively common in many types of water bodies, many studies are limited to the records of occurrence rather than comprehensive epidemiological investigation.

1.2 *Ergasilus sieboldi*

Ergasilus sieboldi was first described by von Nordmann in 1832 infecting various freshwater fish in Europe and is the type species of the genus. Like most parasitic copepods, the description of the species is based on the external morphology of the adult female and this has been subject to at least ten re-descriptions since von Nordmann, most recently a study using SEM by Abdelhalim *et al.* (1991). The life cycle comprises the eggs held in paired membranous sacs attached to the adult female, six free swimming naupliar stages, five free swimming copepodid stages, the free swimming adult male and the adult female. Prior to this 1991 study, various studies had cited between one and five naupliar stages for this species (Fryer 1978;Gnadeberg 1948;Zmerzlaya 1972), but Abdelhalim *et al.* (1991) confirmed that *E. sieboldi* conforms to the conventional six naupliar stages found in free living copepods (Huys &

Boxshall 1991). Again this suggests that in evolutionary terms, ergasilids have recently adapted to the parasitic form of life.

Difficulties in culturing the free living stages of *E.sieboldi*, and indeed all ergasilids, in controlled conditions has limited studies of the free living stages (Abdelhalim, Lewis, & Boxshall 1991;Alston, Boxshall, & Lewis 1996;Urawa, Muroga, & Kasahara 1980;Zmerzlaya 1972). Only Zmerzlaya (1972) and Alston (1996) have had any success in culturing *E.sieboldi* and, in both cases, the parasite was cultured from infected fish held in large aquaria with a range of *flora* and *fauna* that did not constitute controlled conditions. The actual feeding mechanisms and type of food is thus poorly understood for nauplii stages and unknown for copepodid stages. Early studies suggested that the nauplii may be lecithotrophic (Gnadeberg 1948), but at least some stages of the nauplii apparently feed on phytoplankton as a 'green amorphous mass' was observed within the gut (Alston 1994).

Limited information is available on the reproductive strategy of *E.sieboldi* and the male of the species has received little attention. It is thought that the male dies soon after copulation and is thus rarely encountered in fish studies (Fryer 1978). Once attached to the host, the adult female parasite continues to grow and the cephalothorax increases in size due to the production of eggs within (Wilson 1911). The cuticle membranes which form boundaries between the first pedigerous somite and the anterior somite are much enlarged due to this process, giving the impression of a fusion of somites (Abdelhalim 1990). Following this morphological change, the effective swimming capabilities of the parasite are reduced, and it is unlikely that the parasite would be able to detach and re-attach to a different host.

The wide distribution of *E.sieboldi* throughout Eurasia, and its low host specificity has led to reports of the occurrence of this species in a variety of environmental conditions encountered in lakes, canals and slow moving rivers from Finland (Ruotsalainen 1984) to Turkey (Sarieyyupoglu & Saglam 1992). *E.sieboldi* infection has been recorded from more than sixty species of fish (Alston 1994;Gnadeberg 1948) indicating the ability of this species to succeed in a wide geographical range and to infect a diverse range of host fish. However, the majority of these studies are concerned with infection of commercially important species such as pike (*Esox lucius*), the cyprinids tench (*Tinca tinca*), carp (*Cyprinus carpio*), roach (*Rutilus rutilus*), bream (*Abramis brama*) and the coregonids *Coregonus peled* and *Coregonus albula* (Abdelhalim, Lewis, & Boxshall 1991;Alston 1994;Balling & Pfeiffer 1997;Czeczuga 1980;Grabda-Kazubska, Baturo-Warszawska, & Pojmanska 1987;Kashkovsky & Kashkovskayasolomatova 1985;Kuperman & Shulman 1972;Naich, Bilqees, & Khan 2000;Ruotsalainen 1984;Tuuha, Valtonen, & Taskinen 1992), which are highly susceptible to infection.

E. sieboldi was first recorded in Britain on brown trout (*Salmo trutta*) in Yorkshire in 1967 (Fryer 1969). It was thought that the parasite had been introduced to Britain with the movement of fish from the European mainland. Given that the adult parasites are quite large, at up to 2mm in length, and easily discernible on the gill surface, it is likely that if they were native to Britain they would have been discovered before 1967. Additionally, there are a further six known species of ergasilid present in Britain: *Ergasilus briani* Markewitsch, 1933; *Ergasilus gibbus* von Nordmann, 1832; *Ergasilus lizae* (Krøyer, 1863); *Neoergasilus japonicus*, (Harada, 1930); *Thersitina gasterostei*, (Pagenstecher, 1861) and *Paraergasilus longidigitus* Yin, 1954 (Fryer & Andrews

1983;Kirk 2000;Mugridge, Stallybrass, & Holman 1982;Williams 2007). Of these, *E. gibbus* and *T gasterostei* are thought to be more host specific (Fryer 1982), infecting eels (*Anguilla* spp.), three spined sticklebacks (*Gasterosteus aculeatus* L.) and nine spined sticklebacks (*Pungitius pungitius* L.). *E. lizae* has a wide host range but is generally considered a marine or brackish water ergasilid (Cloutman & Becker 1977). *E. briani*, *N. japonicus, P. longidigitus* and *E. sieboldi* have a wide host range of freshwater teleost fish including commercially important species. In the UK, *E. briani*, and *E. sieboldi* are classed as category 2 parasites by the Environment Agency (Williams 2007). This classification places restrictions over the movement of fish that carry these parasites as they pose a significant disease threat when introduced to waters where they do not already exist, or alternatively, have an unknown disease potential and distribution. *N. japonicus* was recently (September 2008) removed from this list as it is considered less pathogenic due to its preferred attachment site of fins rather than gills.

Infection with ergasilids does not necessarily lead to clinical disease. In many cases, low levels of infection cause relatively minor effects to the host (Kabata 1970). In certain circumstances, quite severe pathological effects may be observed. Tolerable infection levels for any given host, and at what point infection becomes disease, must be defined in each individual study according to prevailing conditions of host and environment. To regard infection levels as high or low, implies parameters that can only be relevant to the study in question. This is particularly true when the infection is of a commercially valuable species of fish and a degree of management is required to limit the effects of the infection/disease if eradication is not possible (Piasecki *et al.* 2004).

The main causes of host pathology are the result of the attachment mechanism and the feeding of the parasite (Kabata 1970). Specific pathology is host and parasite dependant. *Ergasilus colomeus,* for example, inserts the entire antenna into the gill tissue of the host (Thatcher & Boeger 1983) whereas some species embrace a gill filament and most pathological damage is ascribed to the feeding mechanism (Oldewage & Van As 1987).

E. sieboldi is usually located on the proximal part of the gill surface attached to the outer surface of each hemibranch. *E. briani* however, is usually located between hemibranchs, often on the distal part of the filament (Alston 1994;Urawa, Muroga, & Kasahara 1991). *N. japonicus* is most frequently located on the fins or attached to the scaleless area close to the base of fins (Abdelhalim, 1990; Alston, 1994). Pathological changes caused by infection of ergasilids include epithelial hyperplasia, atrophy of secondary and primary lamellae, mucus cell proliferation, rodlet cell proliferation, infiltration of macrophages, lymphocytes and eosinophils (Paperna & Zwerner 1981; Abdelhalim 1990; Dezfuli *et al*., 2003). Other reported measurable effects to the host include a change in host blood parameters, inhibition in transformation of carotenoids, a reduction in the oxygen uptake efficiency of host gills, loss of appetite, emaciation and death (Einzporn 1965, Einzporn-Orecka 1970, 1973a, 1973b; Roubal 1986; Ojha & Hughes 2001).

1.3 Fisheries in the UK

Angling is the largest participation sport in the UK (National Rivers Authority, 1995). It was estimated that revenue of £1.5 billion and 29000 jobs were directly dependant on angling in England and Wales, and that this is a growth industry increasing annually.

Fisheries in the UK are diverse in form and function. They include freshwater and brackish rivers and still waters, and sea angling from shore or boat. As they are highly variable in physical nature and management, there is a lack of practical control measures for parasite infections (Taylor, Sommerville, & Wootten 2006). They can either be fished for native/wild stocks or supplemented with farm grown species.

The majority of rainbow trout fisheries in the UK are still water lakes, pools, lochs or reservoirs. Many of the smaller water bodies have been created with the express purpose of operating as a commercial fishery. The larger water bodies tend to view angling as an additional resource to be managed (Gaterell *et al.* 1995). The financial viability of a trout fishery depends upon sufficient numbers of anglers visiting who pay a permit fee to fish and remove a limited number of the stock. The margin of profitability is often tight and many fisheries rely on additional sales to anglers visiting them such as boat hire, fishing tackle shops, accommodation and restaurants (D. Moore pers. comm.). All of these commercial activities require considerable numbers of visitors to the site each year. Consequently, if anglers are unhappy with the quality or operation of the fishery, an economic loss is felt in many ways. Given the rural location of many fisheries, local communities rely on the passing trade that angling provides, and an economic loss in the fishery sector, therefore has a “knock on” effect in different industries.

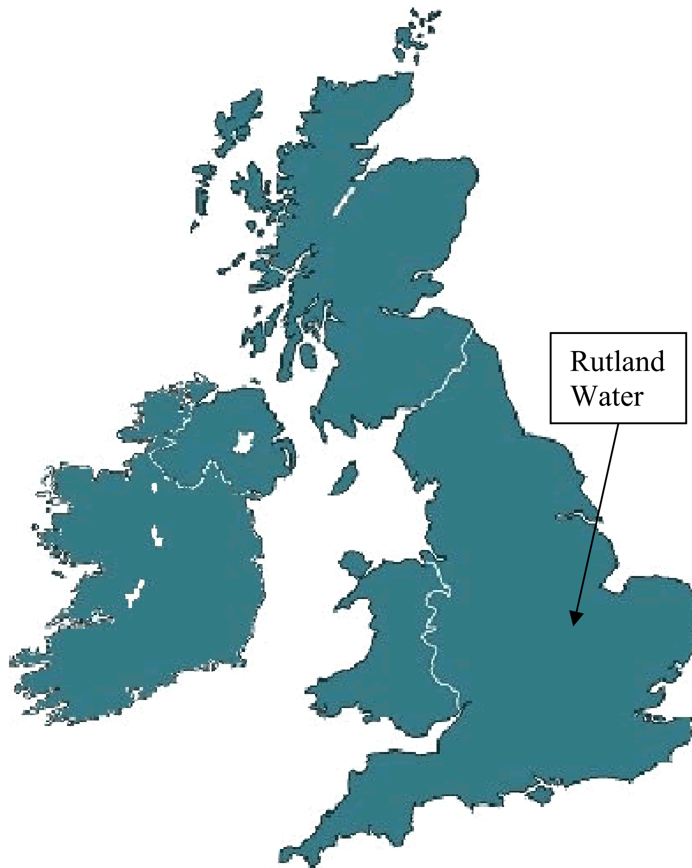
1.4 Rutland Water

Rutland Water is a water storage reservoir in the English East Midlands located at 52º 38´´ 52´N, 00º 39´´ 54´W (see Figure 1.1).

It supplies water to 1,500,000 people in the region via a large water treatment works at Wing, 7.6km south of the reservoir (Daldorph *et al*. 2001). Water is pumped into the reservoir from the rivers Nene and Welland and it is also served by direct inflow from the River Gwash. The reservoir was created between 1970 and 1976 by construction of an earth fill dam across the valley of the River Gwash to form a reservoir of 124 x 10^6 m^3 capacity and 12.4km^2 surface area (Gaterell, Morse, & Lester 1995), making Rutland Water the largest reservoir in the UK by surface area, and one of the largest artificial reservoirs in western Europe. The reservoir consists of a deep main basin to the east where the dam is located, and two arms (north and south) that extend westward from the basin, separated by the Hambleton Peninsular (see Plate 6). The western ends of these arms are shallow water zones important for the migratory and resident bird population. Angling is prohibited in these wildlife sanctuaries.

The maximum depth of the reservoir is 39 meters in the central basin. Shallow areas exist at the western extremes of the north and south arms. The mean depth of the entire reservoir at mid operating level is 10.7m (Bennion *et al*. 2005). The reservoir is considered to be eutrophic based on total P levels of water pumped in from local river systems, however depending on the timing of measurements taken, this classification has varied due to occasional ferric dosing at the water inlet (Bennion, Hilton, Hughes, Clark, Hornby, Fozzard, Phillips, & Reynolds 2005;Daldorph, Spraggs, Lees, Wheater, & Chapra 2001)

Figure 1.1 Map of British Isles showing location of Rutland Water.



Map reproduced from **www.castandcatch.com/ukmap.htm**

In addition to supplying a source of potable water, Anglian Water, the owners of Rutland Water, also exploit the reservoir as a recreational resource and nature reserve. In 1995, an estimated 400,000 visits were made to the reservoir by members of the public to enjoy the facilities of angling, horse riding, sailing and cycling (Gaterell, Morse, & Lester 1995). In 1983, Rutland was designated as a Site of Special Scientific Interest (SSSI) due to the important wetland habitats for birds and has since been granted the status of European Special Protection Area and also as a Ramsar site (Anglian Water web publication, http://www.anglianwater.co.uk, accessed May 2008).

Rutland Water is home to at least nineteen species of fish (Moore 1982). Of these, only rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) are stocked into the reservoir for anglers to catch. The coarse angling methods required to catch the other species are not permitted on the reservoir as it as operated for fly fishing only. Other species have been introduced naturally or through operations such as pumping water from the river systems that are used to fill the reservoir.

In 1975, the first stocking of rainbow trout into the reservoir began. This created the largest fishery in Europe to be dependent on farm reared trout. Currently, rainbow trout form the majority of the stock with brown trout as an additional resource. Rainbow trout are cheaper to produce and to stock but are not as long lived in UK waters as brown trout (Moore 1982).

Rutland Water is open to anglers from April to December. A closed season when no angling takes place is from 1st January to 31st March to protect the important conservation areas of the reservoir. The stocking policy in recent years has been to stock up to 100,000 rainbow trout per annum in a pattern from March to September that provides anglers with the best chance of catching fish. The mean size of trout stocked into the reservoir is 750g. The stocking policy structure has been to stock an initial 15,000 to 20,000 rainbow trout into the reservoir in mid to late March before the fishery opens to anglers, and then to stock additional fish each week until September, the weekly amounts depending on how many had been caught by anglers. In order to monitor numbers of caught fish, each angler is required to submit a “catch return”, a card detailing number and size of fish caught. Inevitably, not every angler submits this return and figures of caught fish are therefore considered to be an underestimate. Also

the mortality of stocked fish due to predation or other losses remain largely unknown, adding to the underestimate. For this reason fishery managers multiply the known catch figures by a factor to compensate for these under estimates. At Rutland Water, typically a factor of 2 multiplied by catch return figures is used to give a figure of what should be stocked to replace fish removed from the system.

One problem a fishery manager has is the need to ensure the optimal numbers of fish are available to be caught by paying customers at all times throughout the season. Anglers at Rutland Water are restricted to catching and removing a maximum of six fish in any one day. In economic terms, if the average number of fish being taken by each angler is more than four per day, the fishery will run at a loss due to the costs of stocking fish and running the fishery (D. Moore *pers. comm.*). If the average number regularly falls below three per day, anglers are unhappy because the fish are too difficult to catch (which is perceived by anglers as too few fish in the reservoir).

1.5 Aims of the study

In 2002, when *E.sieboldi* was first recorded in Rutland Water, the mean weekly catch rate was often below three and in some weeks below two fish per angler. In addition many anglers complained that fish were emaciated, in poor condition and with excess mucus production on the gills making an undesirable product. Soon after this, the angling press reported the fish in Rutland Water as having “disease” problems and the number of visiting anglers dropped markedly. Infected fish were sent to the Environment Agency and to Anglian Water laboratories where the presence of *E.sieboldi* was confirmed.

In 2003, Anglian Water contacted the Parasitology Research Group at the University of Stirling for assistance in researching the recent changes at the Rutland Water. David Moore, Recreation Manager at Anglian Water, responsible for the operating of the fishery, had consulted the available literature on *E.sieboldi* infections and had found a paucity of practical management solutions for a large trout fishery. Chemotherapeutants were impractical solutions for Rutland Water due to the large volume of water, and the function of the reservoir as a drinking water supply. Epidemiological studies on ergasilid infections in large water bodies that had been carried out, such as those by Zmerzlaya (1972) and Paperna & Zwerner (1976) were of infected fish in a natural environment rather than a managed fishery with a rapidly changing host population.

A study was designed in 2003 to initially sample fish on a monthly basis and to record the levels of infection and reproductive activity of *E.sieboldi* on the fish of Rutland Water. This study was extended by the sponsors Anglian Water into a PhD studentship over three years to monitor the population dynamics of the parasite during the study, to identify potential methods of control of the parasite that would allow management in a large reservoir containing potable water supplies (*i.e.* without the need for chemotherapeutants), and to add to the depth of knowledge required to model an *E.sieboldi* infection in a trout reservoir.

From 2003 to 2005, monthly samples of angler caught fish were obtained from the fishery and examined for the presence and reproductive stage of the parasite. The findings from field observations were combined with cage and tank experiments and laboratory investigations to fulfil the aims of this study. The following chapters are the results of this study.

Chapter 2: General materials and methods

The materials and methods contained in this chapter refer to techniques or processes that are relevant to several sections of the study. In addition, each chapter of the study contains a materials and methods section that details specific techniques that are relevant to that particular area of the study.

2.1 Terminology

Parasitological terminology referred to in this study follow the definitions of Margolis *et al.* (1982). Fisheries terminology is specifically defined in the chapter text where required.

2.2 Fish collection

Angler caught fish were collected by the wardens of Rutland Water in 2003 and by the author in 2004 and 2005. Anglers caught fish using fly fishing techniques from boat or from the shore of the reservoir. No attempt was made to randomise the fish collected. Fish were sequentially taken from anglers willing to donate their catch. Fish may have been caught in any area of the reservoir.

The seine net used was a 100 × 6 m net supplied by the Environment Agency. Mesh size was 1.5 × 1.5 cm (stretched). The net was deployed by boat and hauled to shore in a standard horseshoe configuration.

2.3 Holding conditions and husbandry

2.3.1 Aquarium tanks

Circular glass reinforced plastic tanks, 1 m diameter, 0.6 m^3 volume, were used for the study. Tanks were supplied with tap water filtered through activated carbon. Flow rates of 5l per minute were supplied to the upper layers of each tank via a spray bar. Flow rates were checked daily and adjusted as necessary. Experimental tanks were supplied from header tanks that were heated as required using standard 150w aquarium heaters in series. Temperature in each experimental tank was measured hourly with a submerged Tiny Tag sensor (Gemini UK Ltd.). Temperature records were downloaded from the sensors to a laptop computer on a regular basis. Aeration was supplied to each tank through air stones controlled by a central compressor. Lighting was by timer controlled fluorescent lights on the ceiling of the building or fluorescent lights contained in the tank lid. Water and air supplies were monitored by a 24 hour alarm system. Standard commercial 4.5mm maintenance trout pellets (Biomar UK Ltd) were supplied *ad libitum* to each tank daily. The stocking density of aquarium tanks did not exceed 5kg fish per m^3 at any time.

2.3.2 Experimental Cages

In July 2004, four experimental cages in a single block were constructed and moored approximately 100 m from the eastern tip of the peninsular separating the north and south arms of Rutland Water (see Figure 2.1). The cages were used for experiments in 2004 and 2005, and later served as a temporary holding pen for angler caught fish in 2006. The water depth in the area of the cages varied between 7 m and 9 m according to operational conditions of the reservoir.

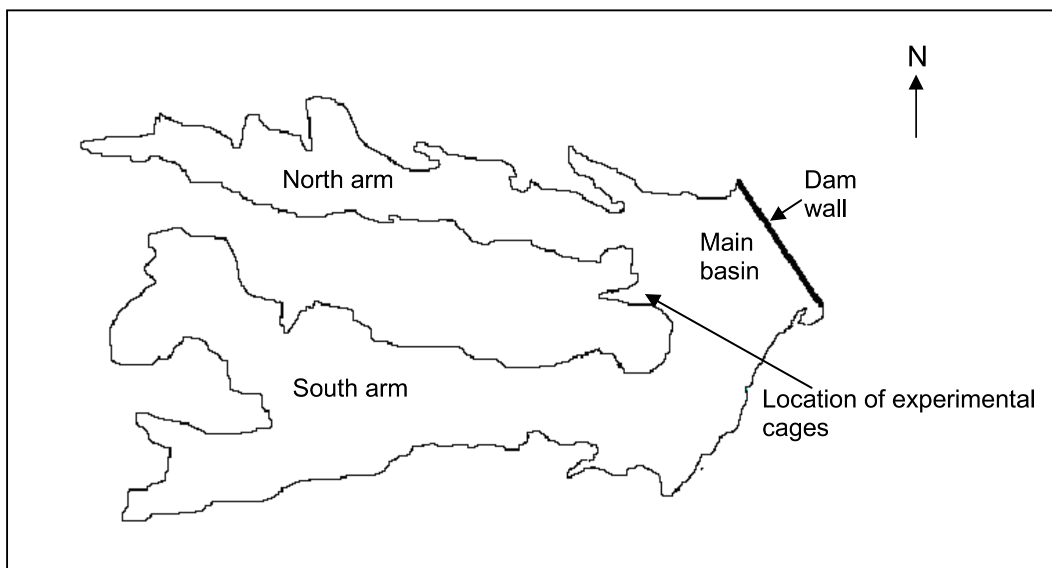
The cage system was designed by the author in consultation with Kames Ltd, Argyll, Scotland. The cage was assembled on the shore of Rutland Water from prepared materials and towed to the mooring location by boats.

The platform consisted of four identical net cages, each surrounded by a floating walkway collar and moored to a central floating pontoon (see Plate 7). The platform was constructed of pressure treated wood with stainless steel fittings. Net cages were supplied by Collins nets Ltd, Dorchester, Dorset, and measured 4m × 4m × 4m. Each was suspended from guard rails 1.5m above water level. Volume below the water level was therefore 4m × 4m × 2.5m (40m^3) for each cage. Net cages were constructed from 1 x 1cm nylon mesh (stretched) and had a secondary 10 x 10cm predator net secured laterally around the perimeter. Predator nets to the underside of the cages were not used. Plate 7 shows a photograph of the cages *in situ*. A Tiny Tag (Gemini UK Ltd) temperature logger was suspended 1.5m below the water surface under the central pontoon of the cage. This device was set to record temperature at 3 hourly intervals. Temperature records were downloaded from the sensors to a laptop computer on a regular basis. Fish held in cages were not fed an artificial diet. At weekly intervals, one half of cage netting of each cage was raised and brushed to remove fouling. The cage netting was then submerged and the same process was then applied to the second half of the cage. During all cage held experiments, stocking density of fish was below 5kg fish per m^3.

2.4 Transport of fish to Stirling

Rainbow trout were caught by Rutland Water wardens angling from boats in November 2006. These were transported to an empty experimental 4m × 4m × 4m experimental cage immediately after being caught.

Figure 2.1. Map of Rutland Water indicating the location of the experimental cages.



In December 2006, the cage was lifted and the fish transferred to shore in a 200l plastic tank. Fish were transferred to a 400 litre plastic transport tank on a trailer and transported immediately to the aquarium facilities of the Institute of Aquaculture, University of Stirling, Stirling, Scotland. Additional oxygen was supplied to the transportation tank as required to keep levels above 6mg/l.

2.5 Imaging

2.5.1 Light micrographs

All light micrographs were captured using a Carl Zeiss MRc Axiocam attached to a trinocular head of an Olympus BX51 microscope with or without phase contrast, or to a trinocular head of an Olympus SZ 40 dissecting microscope. Images were captured using MR Grab version 1.0.0.4 (Carl Zeiss Vision GmbH) in tagged image file format (tiff).

2.5.2 SEM processing and equipment

Adult female *E.sieboldi* were gently removed from the gills of freshly caught rainbow trout using mounted needles. They were gently cleaned of excess tissue and mucus with a fine paint brush and placed in a solution of Tween 30 (3 drops in 100ml of distilled water) and gently agitated for 20 minutes in a Kerry Pul 60 sonicator. Specimens were then removed, rinsed in distilled water and fixed in 2.5% glutaraldehyde (buffer cacodylate) at 4°C for 3 days, then transferred to cacodylate for at least 4 hours. Secondary fixation was performed in 1% osmium tetroxide for 2 hours. Specimens were then transferred through graded ethanol series 30%, 60%, 90%, 100% (1 hour in each). Specimens were then critical point dried, attached to aluminium stubs with carbon tabs and sputter coated with gold. Images were viewed and recorded using a Jeol 6460 LV SEM at 5-10kV.

Chapter 3: Population dynamics and epidemiology

3.1 Introduction

For salmonids infected with ergasilids, and particularly the non-native rainbow trout (*Oncorhynchus mykiss*), little information exists on infection dynamics in the UK beyond the record of infection occurring. Although the first record of *E.sieboldi* in the UK was from a brown trout (*Salmo trutta*) in a reservoir in Yorkshire (Fryer 1969), there has been only one previous study in the UK to examine seasonality of infection and associated parameters on trout species. Abdelhalim (1990), conducted an experiment using a cage stocked with 400 rainbow trout at Sandhurst Lakes, Camberley, Surrey. Samples of fish were removed every 7-10 days between March and November and examined for the presence of *E.sieboldi*. In addition, angler caught fish from the lake were also examined. In his PhD thesis of 1990, he reports the levels of infection and the reproductive state of the parasite during this season. His conclusions on host susceptibility were that tench (*Tinca tinca*), bream (*Abramis brama*) and pike (*Esox lucius*) were the most susceptible to infection in terms of prevalence and intensity but when rainbow trout were introduced, they rapidly became the species with the highest infection levels.

In order to understand the dynamics of infection in an artificially stocked water body, it is necessary to understand the dynamics of the potential host population. Rainbow trout are not native to the UK and do not normally breed in stillwaters. For this reason, rainbow trout are stocked into fisheries after being reared on commercial fish farms.

Fish that are recently stocked into a trout fishery are accustomed to being fed commercial feed pellets at regular intervals. The fish are held at high stocking densities

in the farm and competition between fish is important in stimulating a feed response (Alanara & Brannas 1996). When the fish are transferred to the reservoir, they receive no artificial feeding and, after a short initial settling in period, are relatively easy for anglers to catch. If an individual fish is not captured for some time, it adapts to the very low stocking density of the reservoir and begins to consume a natural diet. They also grow in size, and take on better condition in terms of fin growth, colouration and musculature (Hunt & O'Hara 1973). Condition factor declines in the absence of an artificial diet as stored fat levels are utilised. Fish that have been in the reservoir for an extended period of time are readily distinguished from recently stocked trout and are perceived as hard fighting fish, highly prized by anglers.

Studies of other parasites have suggested that a degree of resistance to infection may be acquired by previously infected fish, or fish which are part of a population where genetic traits for resistance have been selected (García de Leániz *et al.* 2007). This mechanism has not been demonstrated for all fish ectoparasites. Studies on the infection of rainbow trout with *Argulus coregoni* found that prior infection with the parasite did not result in any reduction in their susceptibility (Bandilla *et al.* 2005). The trout that are stocked into Rutland Water are naïve as they are hatchery reared and regularly checked at the fish farms for infection with parasites such as *E.sieboldi*. As they have not been previously exposed to the parasite, there is no possibility of resistance or local adaption. The coarse fish population, however, is natural and individual fish may be subjected to multiple infective periods of *E.sieboldi*.

A stocked trout fishery presents unique challenges and solutions in studying the infection dynamics of any parasitic infection. Changes in the stocking density of the

reservoir directly affect host availability to the parasite. An influx of naïve fish can lead to a large upsurge in the parasite population. Conversely, reducing the fish stock at certain times could present a management option to a serious parasite problem.

Typically, for parasitological surveys, unless the fish is migratory, it is assumed that the host fish is available for parasitism throughout its life apart from any period when infective stages of a parasite are not present. For this reason, age classification of sample fish is often used to determine the period of time in which they have been available to the parasite. In a stocked fishery this is not the case. The host fish only becomes available to parasitism after it is stocked into an infected water body and size and age of stocked fish can vary. In this study, where appropriate, an assessment of sampled fish has been made that estimates the amount of time the fish has been in the reservoir and thus the amount of time that it has been available to parasite infection. The classification of a sampled fish as overwintered, early stock or stock fish is explained in section 3.2.

In a study to examine abiotic effects on infection dynamics of *Ergasilus celestis* occurrence on American eel (*Anguilla rostrata*) in eight freshwater localities in Nova Scotia, Canada, Barker & Cone (2000) found that parasite component population, mean abundance and prevalence correlated positively with water pH and negatively with stream velocity. This correlates well with the known distribution of *E.sieboldi* in the UK. The parasite has been recorded in still or slow moving freshwaters in the south and east of England with a few additional sites in Wales (Alston 1994;Hawkins 2001). These water bodies tend to be of a higher alkalinity than UK water bodies further north. For example, *E.sieboldi* has never been recorded in Scotland, where water bodies tend

to be more acidic and there are few slow moving rivers. However, given the wide host range in different environments that *E.sieboldi* has shown, it is possible that the distribution within the UK will increase.

Following the identification of *E.sieboldi* on the fish of Rutland Water in 2002, a study was designed and carried out from March to December 2003. The purpose of this study was to describe the seasonal infection dynamics of the adult female parasite on rainbow and brown trout throughout the year, specifically to quantify numbers of parasites infecting the stocked trout and the rate at which this occurred.

It was decided to continue the monthly sampling of angler caught trout in order to assess any changes in parasite levels between 2003 and the following seasons. In 2004 and 2005, further monthly trout samples were obtained by angling and dissected and examined by the author at the Anglian Water central laboratories in Huntingdon. In addition, cage trials were conducted to further the analysis of seasonal infection data by examining the transmission rate of infective stage of *E.sieboldi* at 2 weekly intervals.

A further objective of this study was to compare the relative susceptibility to *E.sieboldi* infection of other species of fish from Rutland Water, and to compare the relative susceptibility of other commercially available trout species or types that could be used in the stocking policy of the fishery. These aims were addressed by sampling for coarse fish with various methods and the use of cage trials to compare trout species/types.

Brown trout are native to Britain and are stocked commercially for angling purposes in still waters and rivers where they do not naturally occur, or to supplement existing

stocks. Brown trout are slower growing than rainbow trout and are more expensive for fisheries to purchase. Some anglers regard the species as harder to catch than rainbow trout and consequently, rainbow trout are more popular in many fisheries. Brown trout have been stocked into Rutland Water in small numbers every year since the 1970s. It is thought that they have not been able to spawn successfully in the reservoir but mature fish have been found attempting to swim up small feeder streams in the reservoir in an attempt to spawn. No juvenile brown trout that would indicate successful reproduction of larger stocked fish have ever been caught by anglers. It is possible that the eutrophic nature of the water body and the substrate of the feeder streams are not suitable for spawning or development of eggs.

The blue trout is a genetic variant of the rainbow trout with a cobalt blue dorsal surface, governed by an autosomal recessive gene (Blanc *et al.* 2006). It is commercially available for restocking into fisheries in the UK from a limited number of fish farms. In recent years many fisheries have experimented by adding trout varieties such as blue trout or golden trout to the stocking policy in order to provide some variety for anglers. As it is still of limited availability, costs of stocking this strain of rainbow trout are high.

All female diploid rainbow trout are the main production species of UK trout farms. Production of these fish is mainly for the table market and in 2003, 6413 tonnes of diploid female rainbow trout were produced in the UK for this purpose compared with 3156 tonnes for restocking in fisheries (mostly triploid rainbow trout) (Anon 2004; Trout news, Cefas). Sterile triploid trout have tended to be the preferred option for stocking into fisheries due to increased somatic growth rates in the absence of gonadal development (Lincoln & Scott 1984). The potential for loss of the genetic wild

population caused by interbreeding of introduced diploid fish with wild stocks (Allendorf & Leary 2005), has also favoured the use of triploid fish in a variety of fisheries.

3.2 Materials and Methods

3.2.1 Infection levels 2003

In each month between March and December 2003, samples of trout from Rutland Water were caught and donated by anglers or caught using a seine net (see Chapter 2 for details on sampling procedure). The fish were measured for standard length (tip of snout to end of caudal peduncle) and weight, then heads were removed with gills intact, placed in individual sealable bags in reservoir water and sent on ice to Stirling University by next day courier for further examination. Most fish samples were caught by anglers using rod and line, however the March sample and the coarse fish sample from June were seine netted from the Normanton Bay area of the reservoir south shore using a 100m net with a 6m drop side. Further seine netting proved ineffective due to fish moving further from shore, large amounts of rocks/debris that inhibited the net and the high demands on staff time. As the March sample comprised only two fish, it was not possible to perform a statistical analysis of the bias caused by the sampling methods. Additional coarse fish were examined during the study that were capture using a 40m multi-mesh gill net with mesh sizes of $1cm^2$ to $15cm^2$.

In the earlier months of the year, a differentiation was made between overwintered fish that had been stocked in the previous year and recently stocked fish. From May to September, a third category was added – early stock fish, indicating fish that had been stocked earlier in that sample year. From October to December, no assessment of

stocked time was made, as by October it had become too difficult to differentiate between the groups of fish. The assessments were made by the experienced fishery warden team of Rutland Water and were prior to any parasitological examination. Factors used to make this categorization are purely qualitative and include fin condition, skin colouration and fish shape and, for obvious reasons this judgment is treated with caution. However, biological data did correlate with these determinations as shown in later in this chapter and Chapter 4.

The categories are described as follows:

1) Overwintered rainbow trout: A fish that was stocked in the previous year - Complete fins with no or few splits, especially caudal fin that should be large and have a defined linear edge when opened fully; body shape lean with little obvious fatty tissue; colouration predominantly silver without red colouration of lateral line.

2) Early stock: A fish that was stocked in the early part of the sample year - Complete fins with or without splits; caudal fin showing increased growth from stock fish; body leaner than stock fish but still with fat reserves present; colouration darker than overwintered fish with red lateral line present.

3) Stock: A recently stocked fish - Fins incomplete with splits; caudal fin incomplete often with rounded posterior edge; body shape indicating large fat reserves; dark colouration with red lateral line present.

Examples of an overwintered fish and a stock fish are shown in Plate 8.

At the Institute of Aquaculture, gills were removed from the fish and examined under an Olympus SZ40 dissecting microscope at magnification of ×10. Each gill was then examined, on the inner and outer hemi branch and between primary filaments. As they

were recorded, parasites were noted for egg sac development stage and then removed using fine needles. The parasites were then fixed in 80% ethanol. The water in which the fish heads were transported was also examined for any parasites but none were found to have detached.

3.2.2 Infection levels 2004-2005

From March 2004 to November 2004, and March 2005 to December 2005 fish were collected monthly by donation from anglers. The same method of dissection and parasite evaluation was used as described above.

3.2.3 Cage trials 2004-2005

Four experimental cages were built and moored in July 2004 as described in Chapter 2, General materials and methods. Details of experimental procedures are described below.

3.2.3.1 Cage trial 1. *E.sieboldi* recruitment cage trial

This experiment was set up to provide data on the rate of parasite transmission during the season. Parasites were also examined for signs of reproductive development (see Chapter 4).

Cage 4 was stocked with 30 triploid rainbow trout (mean weight 750g) in mid July 2004. After 2 weeks these were sampled by raising the cage netting and removing all fish with a hand net. Fish were removed, killed, dissected and examined. The cage was restocked with 30 new (naïve) triploid rainbow trout from the same batch originating at Gwash Trout farm as soon as possible after harvest. This process was

continued in approximately 2 week intervals until 2 consecutive batches had been seen to be free from infection, thus indicating the end of the parasite infective period for 2004.

This experiment was repeated in May to December 2005, again with sampling and restocking in 2 week intervals, stocking 15-20 triploid rainbow trout per batch from the same fish farm that was used in 2004.

3.2.3.2 Cage trial 2. Susceptibility of different trout types to infection with *E.sieboldi* in mixed population experimental cages.

This experiment was to study the susceptibility of different species and ploidy levels of commercially available trout in experimental cages where each of the types would be equally subjected to the infective stage of the parasite. By sampling the fish in 2 batches at different stages of the winter (December 2004 and March 2005), the ability of *E.sieboldi* to overwinter was also determined.

Cages 1, 2 and 3 were each stocked in mid July 2004 with 4 groups of fish. Each cage contained:

50 triploid rainbow trout

50 diploid rainbow trout

50 blue rainbow trout (triploid)

50 brown trout (mixed sex diploid)

At the end of stocking, each cage contained 200 fish of a mean weight of approximately 750g per fish.

At the end of December 2004, 50% of the fish of each type were sampled from each of these cages. The nets were lifted and every other one of each species/type were killed with a priest for later examination as described previously. The remaining 50% of fish in each cage were returned after minimal handling to be sampled in March 2005 prior to the onset of the new infective period. Numbers of fish sampled in different groups are not equal due to mortalities during the trial. Fish fed on a natural diet and no artificial feed was given at any stage.

3.2.3.3 Cage trial 3. Susceptibility of triploid *vs.* diploid rainbow trout to infection with *E.sieboldi* in separate and mixed population experimental cages.

This experiment was to further the findings of Cage trial 2, by stocking cages with diploid and triploid rainbow trout in separate and mixed populations.

In April 2005 the cages 1, 2 and 3 described above were removed from the water, dried and cleaned in preparation for Experiment 3. In the first week of July 2005 each cage was stocked with rainbow trout in the following groups.

Cage 1. 100 triploid rainbow trout

Cage 2. 100 diploid rainbow trout

Cage 3. 50 triploid rainbow trout plus 50 diploid rainbow trout

In mid December 2005 the cages were lifted and all fish killed, dissected and examined as previously described.

The reason for using single population cages and a mixed population cage was to see if parasite infection levels would be different if both types of host were equally available to the parasite *i.e.* if triploids became more heavily infected than diploids, and as a consequence it was decided to stock only diploids, would the infection levels of diploids still be lower or would the parasite levels increase as choice of host would then be more limited?

3.3 Results

3.3.1 Infection levels 2003

From March to December 2003, gills from 209 rainbow trout were examined for *E.sieboldi*. Table 3.1 shows the sample dates and summary statistics for these fish.

Table 3.1 Summary of monthly *E.sieboldi* infection data for all rainbow trout sampled in 2003.

Rainbow Trout All Fish			*E.sieboldi* numbers				
	n	Prevalence (%)	Min.	Max.	Mean Intensity	S.D. of Mean Intensity	Mean Abundance
13/03/03	2	100	256	1659	958	992	958
13/04/03	30	33	0	5258	2770	1538	923
14/05/03	27	44	0	5046	2445	1532	1087
18/06/03	11	100	46	2834	361	822	361
13/07/03	28	100	102	922	375	216	375
16/08/03	32	91	0	2752	566	756	513
21/09/03	29	100	216	4278	1223	831	1223
24/10/03	26	100	1400	6196	2688	1036	2688
23/11/03	20	100	1008	4862	2411	834	2411
27/12/03	4	100	1762	3250	2530	657	2530

The high variation in infection levels compared to the relative mean was a result of more than one stock being present in the sample (e.g. overwintered and recently stocked fish). When data is presented as a mean for all fish sampled, there was clearly bias

towards the most infected fish which in the earlier part of the year was overwintered fish. Tables 3.2 to 3.5 show the data separated into categories when a judgement of fish status (*i.e.* overwintered, early stock, stock fish or not assessed) was applied to the data.

Table 3.2 Summary of monthly *E.sieboldi* infection data for overwintered rainbow trout.

Rainbow Trout Overwintered			*E.sieboldi* numbers				
	n	Prevalence (%)	Min.	Max.	Mean Intensity	S.D. of Mean Intensity	Mean Abundance
13/03/03	2	100	256	1659	958	992	958
13/04/03	10	100	730	5258	2770	1392	2770
14/05/03	12	100	798	5046	2445	1387	2445
18/06/03	1	100	2834	2834	2834	0	2834
13/07/03	1	100	646	646	646	0	646
16/08/03	1	100	2074	2074	2074	0	2074
21/09/03	2	100	814	974	894	113	894

Table 3.3 Summary of monthly *E.sieboldi* infection data for early stock rainbow trout.

Rainbow Trout Early stock fish			*E.sieboldi* numbers				
	n	Prevalence (%)	Min.	Max.	Mean Intensity	S.D. of Mean Intensity	Mean Abundance
13/03/03	0	-	-	-	-	-	-
13/04/03	0	-	-	-	-	-	-
14/05/03	2	0	0	0	0	0	0
18/06/03	2	100	140	210	175	50	175
13/07/03	6	100	302	922	648	207	648
16/08/03	0	-	-	-	-	-	-
21/09/03	13	100	216	4278	1488	1025	1488

Table 3.4 Summary of monthly *E.sieboldi* infection data for <u>stock</u> rainbow trout.

Rainbow Trout Stock fish			*E.sieboldi* numbers				
	n	Prevalence (%)	Min.	Max.	Mean Intensity	S.D. of Mean Intensity	Mean Abundance
13/03/03	0	-	-	-	-	-	-
13/04/03	20	0	0	0	0	0	0
14/05/03	13	0	0	0	0	0	0
18/06/03	8	100	46	170	114	38	114
13/07/03	21	100	102	716	284	132	284
16/08/03	31	90	0	2752	512	712	462
21/09/03	14	100	284	2372	1023	620	1023

Table 3.5 Summary of monthly *E.sieboldi* infection data for rainbow trout that were <u>not classified</u> as overwintered, early stock or stock fish.

Rainbow Trout Not assessed			*E.sieboldi* numbers				
	n	Prevalence (%)	Min.	Max.	Mean Intensity	S.D. of Mean Intensity	Mean Abundance
24/10/03	26	100	1400	6196	2688	1036	2688
23/11/03	20	100	1008	4862	2411	834	2411
27/12/03	4	100	1762	3250	2530	657	2530

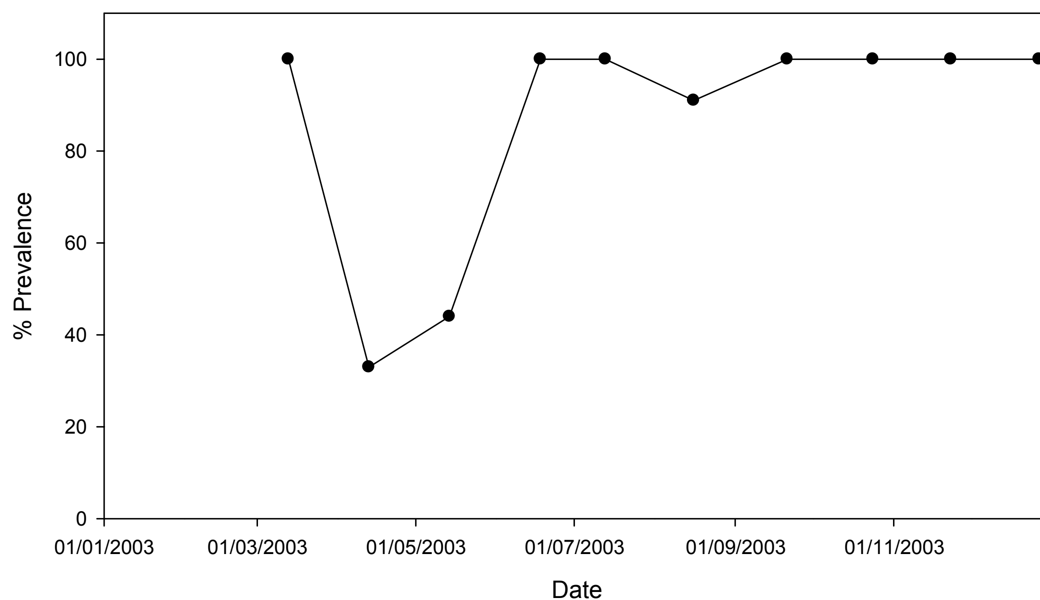
Where possible, samples of 25-30 trout were examined. Unfortunately, for some months this was lower as few angler caught fish were available. The first sample of fish in March was collected by seine net as it was prior to the angling season opening and also prior to commencement of the 2003 stocking programme and thus would contain only overwintered fish. The difficulties in obtaining a suitable sample size with this method, suggested that a more balanced sampling programme would be to use angler caught fish. Despite the inherent bias in this sampling method, it was decided that it would be suitable for a study of this type and would satisfy the aims of the study. Further attempts at seine netting resulted in too few fish to be able to compare the level of bias inherent in a study based on angler caught fish. It is suggested that fish with the

highest levels of infection are likely to have a suppressed appetite and therefore be less likely to be caught by anglers. The sampling methods used in this study are thus likely to be an underestimate of the true infection levels of fish in Rutland Water

3.3.1.1 Prevalence of infection 2003

The percentage prevalence of infection for 2003 is illustrated in Figure 3.1. The prevalence of infection on the overwintered fish in March, April and May 2003 was 100% but prevalence of all the fish sampled fell sharply in April and May as the newly stocked fish joined the population (see Appendix 4 for stocking details). Parasite prevalence for all fish increased to 100% in June as the first new generation of 2003 *E.sieboldi* (designated F1 generation) reached an infective stage and were present on all fish sampled indicating rapid and widespread infections taking place in the reservoir since the previous sample in May.

Figure 3.1 *E.sieboldi* prevalence of infection for all rainbow trout sampled in 2003.



In the sample taken on 16th August 2003, the prevalence had fallen slightly to 91% overall. The August sample comprised 31 recently stocked fish and 1 overwintered fish. The drop in prevalence was due to three uninfected recently stocked fish in the sample. At this stage of the study, a slight drop in prevalence was ascribed to a larger sample size than had previously been taken and it was assumed that the three uninfected fish in the recently stocked group may have been caught very quickly after entering the reservoir. Later findings would question this assumption.

3.3.1.2 Intensity and abundance of infection 2003

The levels of infection for the all the rainbow trout sampled are graphically illustrated in Figures 3.2 and 3.3.

Figure 3.2 Mean abundance of *E.sieboldi* infection for all rainbow trout sampled in 2003 with 95% confidence intervals.

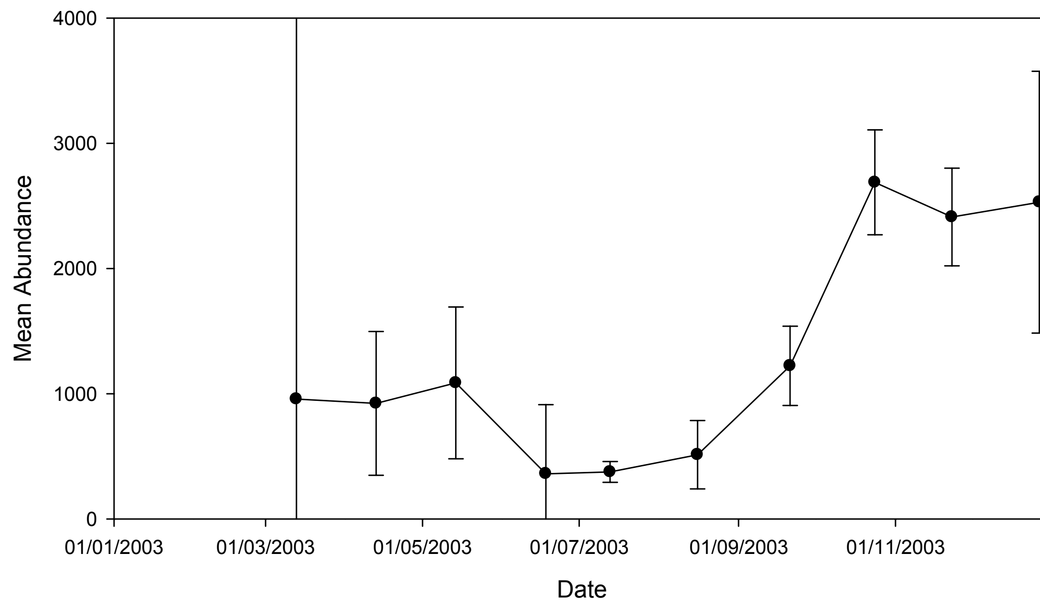
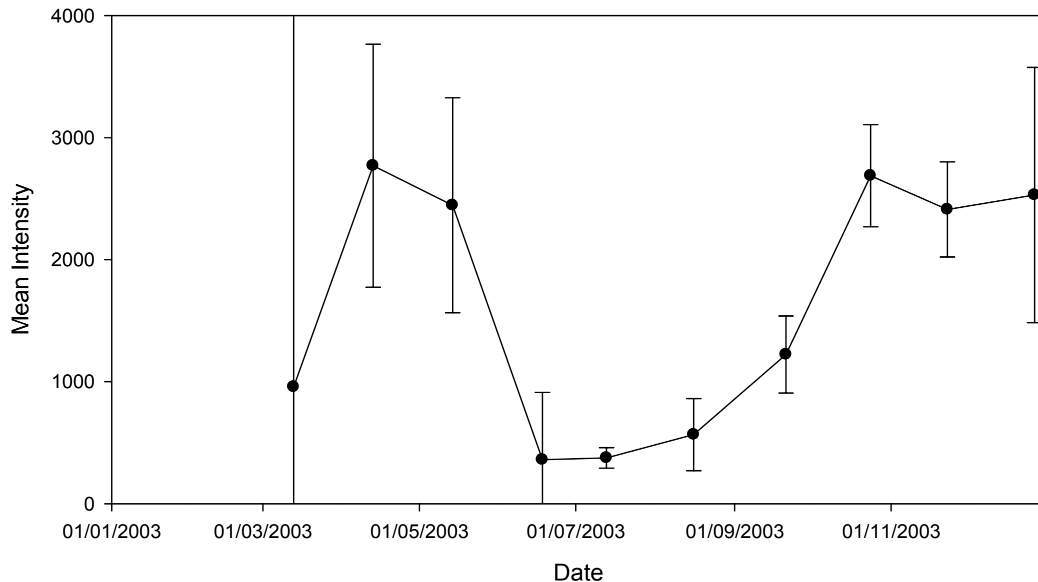


Figure 3.3 Mean intensity of *E.sieboldi* infection for all rainbow trout sampled in 2003 with 95% confidence intervals.



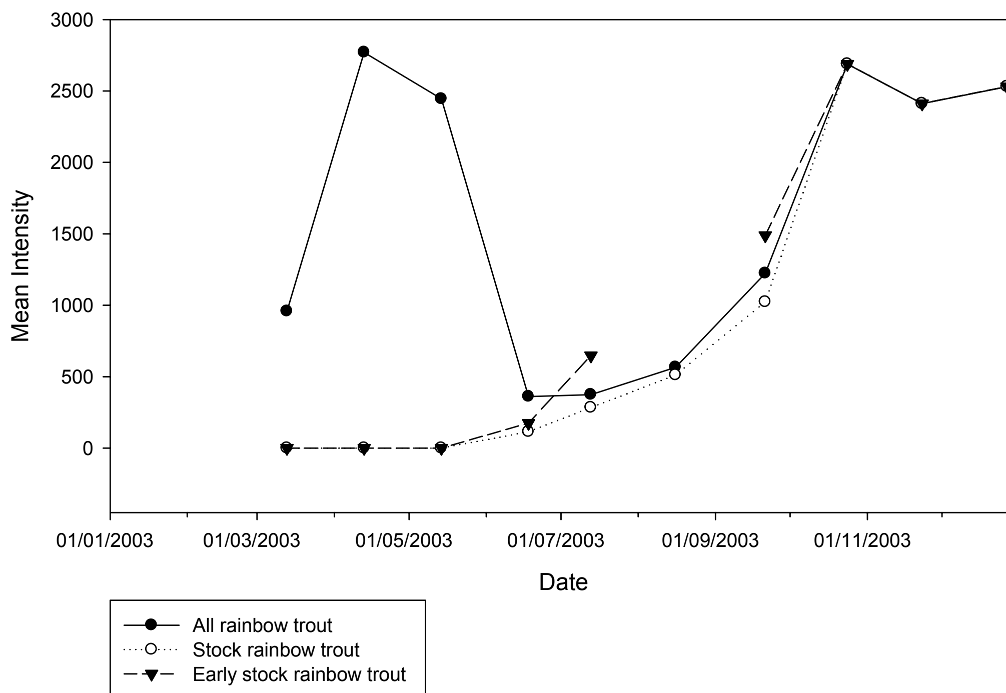
The 95% confidence intervals for the March sample are very large. This was due to the small sample size of just two fish. However, it was noted that fish stocked in 2003 did not become infected until the June sample. For this reason, mean intensity was a better indication than mean abundance of infection levels in the months prior to June as it was based only on the infected overwintered fish from the previous year. Pooling the overwintered infected fish from March, April and May gave a mean intensity of infection of 2457 parasites per fish. The range of infection in these fish was large at 256 to 5258 parasites per fish with a 95% confidence that the true mean intensity of the overwintered population was between 1866 and 3048.

In the sample taken on the 18th June 2003, recently stocked fish had become infected for the first time. Infection levels in these stock fish were initially low with a mean intensity of 114 parasites per fish. One month later the intensity had risen to 284 per fish. Six of the fish in this sample were described as early stock fish, and carried a

parasite mean intensity of 648 per fish. Figure 3.4 graphically illustrates the mean intensity of infection of the stock fish and early stock fish when compared with the sample of all rainbow trout.

The greatest increase in mean intensity occurred between the August and October samples. The last stocking of new trout ended at the beginning of September. By October it was not possible to specify which group a particular trout belonged to as by this stage of the season the condition of all the fish was the similar. After October the mean intensity did not increase further, indicating the end of the infective period for 2003. The mean intensity of infection of fish at the end of the infective period was similar to the levels of infection seen on overwintered fish at the start of the year.

Figure 3.4 *E.sieboldi* mean intensity of infection for different rainbow trout classifications in 2003.



3.3.2 Infection levels 2004

Monthly sampling of angler caught trout continued in 2004 in order to assess any changes in parasite levels between the 2003 and 2004 seasons. Monthly samples of angler caught rainbow trout were collected between March and November 2004. A total of 154 rainbow trout were examined for *E.sieboldi*. Table 3.6 shows the sample dates and summary statistics for these fish.

The March sample comprised of 6 overwintered fish that were caught prior to new stocks of fish being added to the reservoir for the 2004 season. The April sample comprised two infected overwintered fish and eleven uninfected stock fish. As no more overwintered fish were sampled during the 2004 season, and conditioning of stock rainbow trout was better in 2004 than 2003, no attempt to judge the length of time a particular fish had been in the reservoir was made beyond the identification of the overwintered fish at the start of the year.

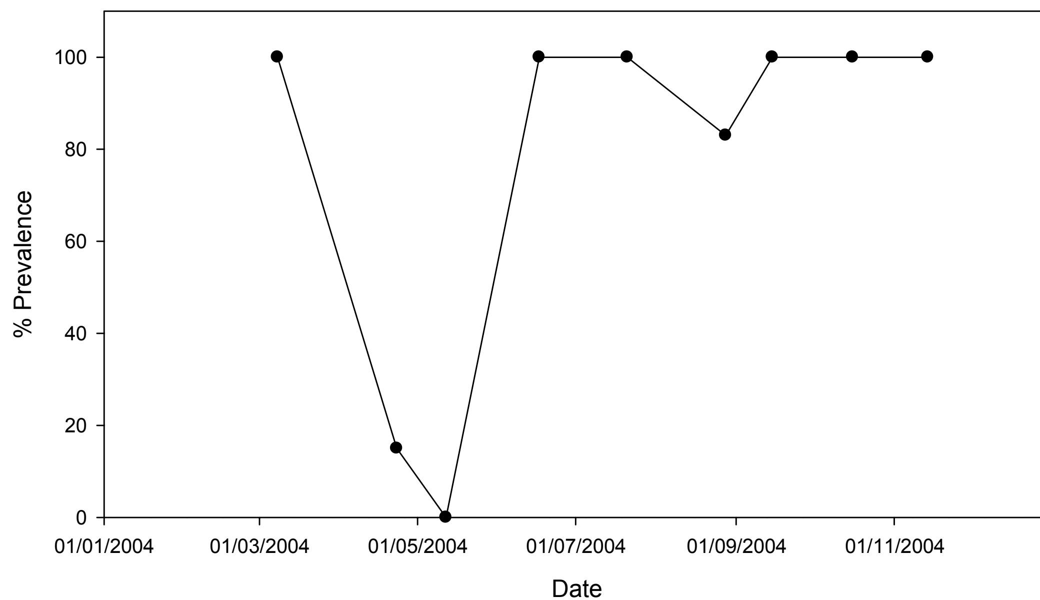
Table 3.6 Summary of monthly *E.sieboldi* infection level data for <u>all rainbow trout</u> sampled in 2004.

Rainbow Trout All Fish			*E.sieboldi* numbers				
	n	Prevalence (%)	Min.	Max.	Mean Intensity	S.D. of Mean Intensity	Mean Abundance
08/03/04	6	100	1280	2416	2406	785	2406
23/04/04	13	15	0	5975	3326	1652	512
12/05/04	20	0	0	0	0	0	0
17/06/04	18	100	1	12	6	3	6
21/07/04	22	100	12	377	89	62	89
28/08/04	24	83	0	472	160	87	133
15/09/04	25	100	92	676	233	95	233
16/10/04	12	100	8	760	479	173	479
14/11/04	14	100	230	891	565	197	565

3.3.2.1 Prevalence of infection 2004

The percentage prevalence of infection for 2004 is graphically illustrated in Figure 3.5. The pattern of infection in 2004 was remarkably similar to 2003. The prevalence of infection on the overwintered fish in March 2003 was 100% but prevalence of all the fish sampled fell once again in April and May as the newly stocked fish joined the population (see Appendix 5 for stocking details). Parasite prevalence was again 100% in mid June as the progeny of the overwintering parasites from 2003 infected the recently stocked fish of 2004 for the first time.

Figure 3.5 *E.sieboldi* prevalence of infection for <u>all rainbow trout</u> sampled in 2004.

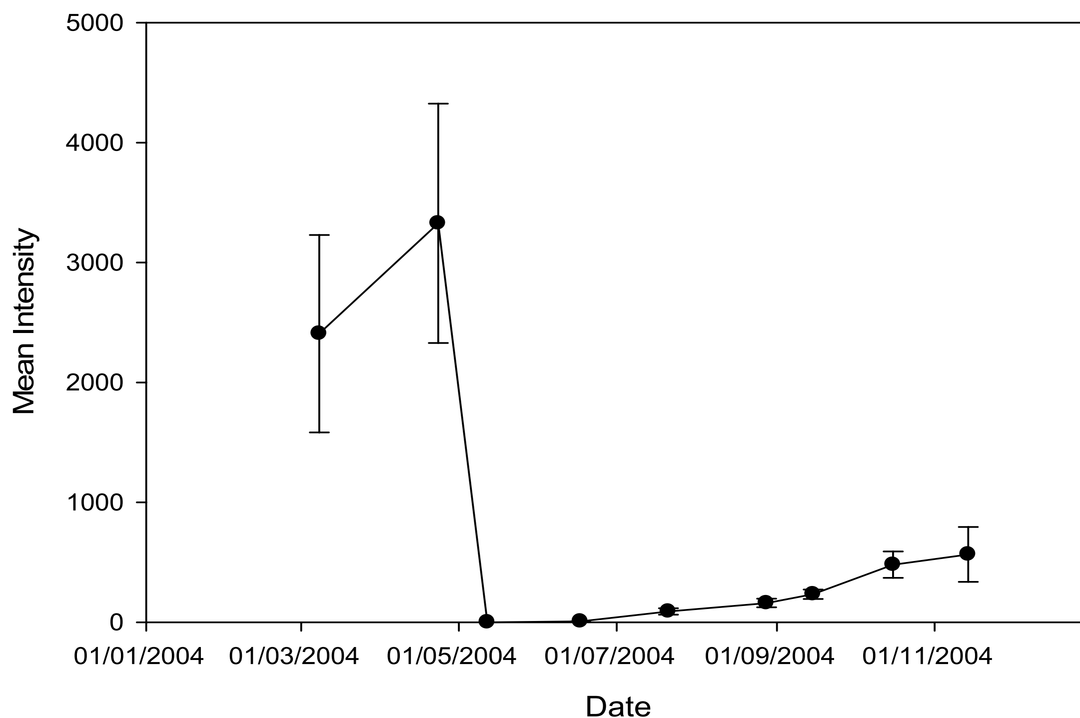


In the sample taken on the 28th August 2004, the prevalence had fallen slightly to 83% in the recently stocked fish. This mirrored the drop in prevalence seen in August 2003 and suggests that this was not a sampling induced effect. In subsequent samples the prevalence was again 100% indicating further infections taking place after August 28th.

3.3.2.2 Intensity and abundance of infection 2004

The mean levels of infection for all rainbow trout sampled are graphically illustrated in Figure 3.6.

Figure 3.6 Mean *E.sieboldi* intensities of infection for all rainbow trout sampled in 2004 with 95% confidence intervals.



The mean intensity of infection of overwintered trout at the start of 2004 was not significantly different to the trout sampled at the end of 2003 (t test P>0.05), implying that *E.sieboldi* had successfully overwintered on the rainbow trout hosts. The first new infections in 2004 were seen by 17th June at the same time as in 2003. Overall, infection levels were significantly lower in 2004 (mean intensity of infection of fish stocked and sampled in 2003 by month *vs.* mean intensity of infection of fish stocked and sampled in 2004 by month, independent t test value p= 0.04). The rapid increase in

infection levels seen between August and October 2003 did not occur in 2004. The sampled fish at the end of the year show that the mean intensity of infection was 565 parasites per fish. This figure was considerably lower than the overwintered fish at the start of the year with a mean intensity of 2406 parasites per fish.

3.3.3 Infection levels 2005

Monthly sampling of angler caught trout continued in 2005 in order to assess any changes in parasite levels between the previous seasons. Monthly samples of angler caught rainbow trout were collected between March and December 2005. A total of 126 rainbow trout were examined for *E.sieboldi*. Table 3.7 shows the sample dates and summary infection data for these fish.

Table 3.7 Summary of *E.sieboldi* monthly infection level data for all rainbow trout sampled in 2005.

Rainbow Trout All Fish			*E.sieboldi* numbers				
	n	Prevalence (%)	Min.	Max.	Mean Intensity	S.D. of Mean Intensity	Mean Abundance
10/03/05	4	100	192	488	322	129	322
12/04/05	12	0	0	0	0	0	0
01/05/05	20	10	0	629	604	186	68
27/06/05	16	13	0	22	8	6	1
18/07/05	16	50	0	162	128	72	60
02/08/05	17	59	0	226	144	57	85
01/09/05	15	100	22	567	234	92	234
04/10/05	14	100	84	622	366	111	366
08/11/05	12	100	200	1487	682	267	682
04/12/05	17	100	238	1228	872	210	872

In 2005, only six overwintered fish were available for examination, four fish in March and two fish in May. The mean intensity of infection for these six overwintered fish

was 416 parasites per fish. The figure was not significantly different to the mean intensity of 565 parasites per fish from the fish sampled in November 2004 indicating the parasites had again successfully overwintered (t test P>0.12).

3.3.3.1 Prevalence of infection 2005

The prevalence of infection showed a different pattern in 2005 than in previous years. In the data sets of all 3 sample years there was a 100% infection level at the start of the year when all the sampled overwintered fish were infected. This level fell to low levels as new fish were stocked in late March indicating that the infected overwintered fish were now a small percentage of the angler caught fish sampled. This may be due to these fish being harder to catch than recently stocked fish or that the size of the overwintered population of fish was much smaller than the new stock of fish The data for 2003 and 2004 showed that the prevalence level then increased rapidly in late June suggesting that the first new parasites of the year were finding hosts amongst the fish stocked in that year. From Figure 3.7, it can be seen that in 2005 this did not happen as quickly. It was not until 1st September that the prevalence of angler caught fish reached 100%.

If there had been a reduction in new infections causing a drop in prevalence in August/September of 2003 and 2004, this could not be seen in the angler caught fish data from 2005 as the prevalence was not as high in the preceding month.

3.3.3.2 Intensity and abundance of infection 2005

The mean levels of infection for the trout sampled in 2005 are graphically illustrated in Figure 3.8.

Figure 3.7 *E.sieboldi* prevalence of infection for <u>all rainbow trout</u> sampled in 2005.

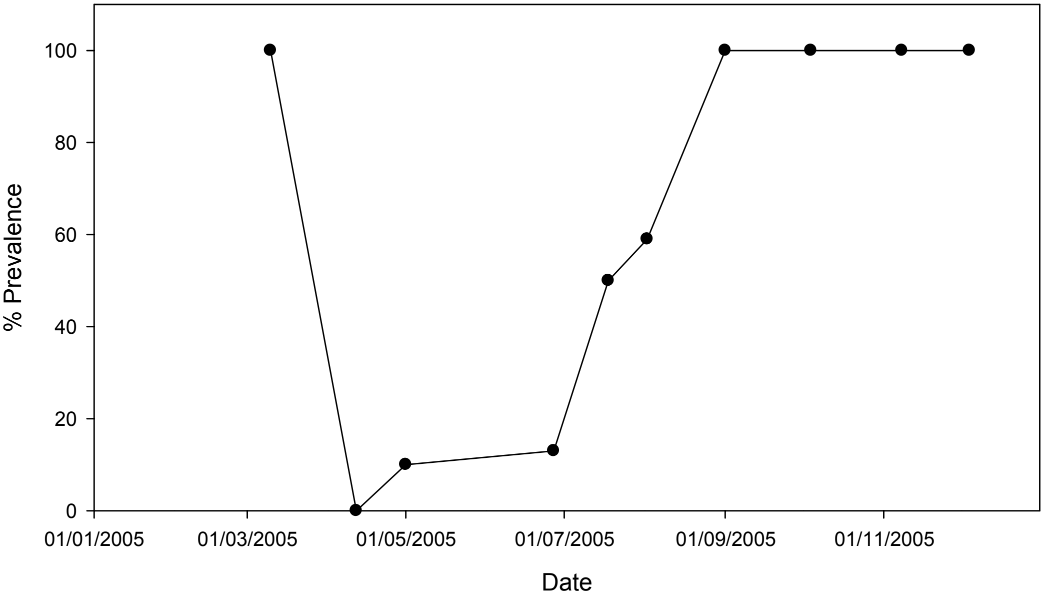
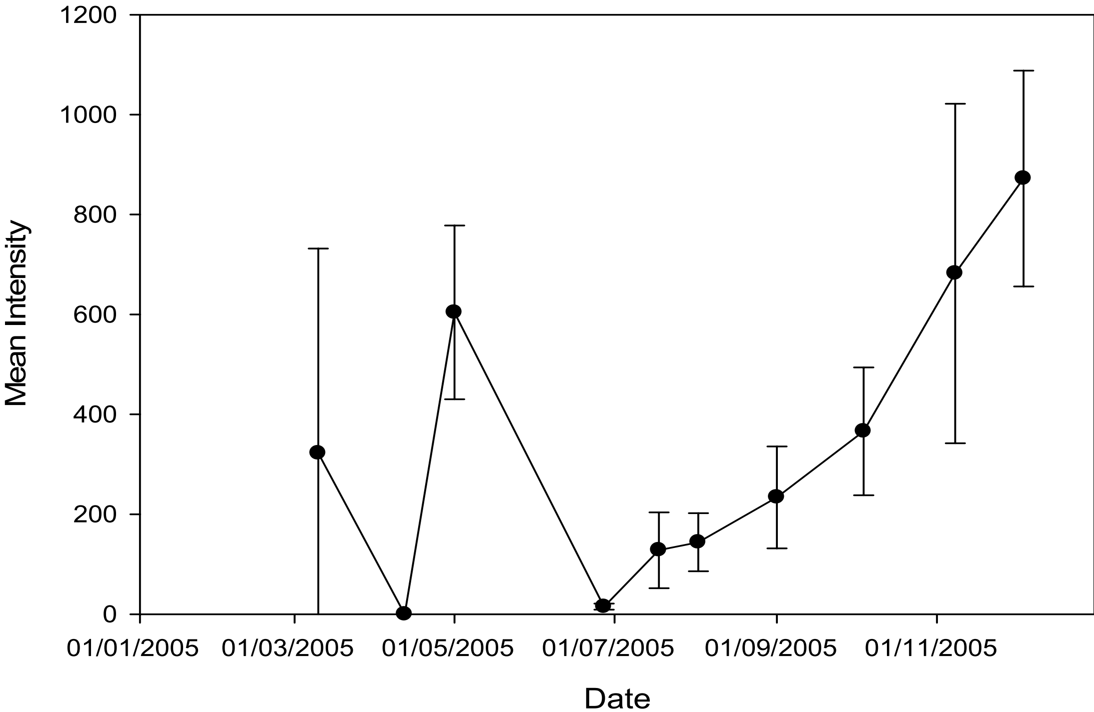


Figure 3.8 Mean intensity of *E.sieboldi* infection for <u>all rainbow trout</u> sampled in 2005 with 95% confidence intervals.



The mean intensity of infection, as seen in Figure 3.8 was similar in 2005 to 2004, with both 2004 and 2005 showing a marked reduction in infection levels compared with the data collected in 2003. The drop to a mean intensity of 0 parasites per fish in April 2004 was due to the sample containing only stock fish which at that time were not infected. The rapid increase in infection levels seen between August and October 2003 did occur in 2004 and 2005 although the effect was markedly less than was seen in 2003 when infection levels rose exponentially in the late summer months.

Figures 3.9 and 3.10 summarize the infection data for 2003 to 2005.

Figure 3.9 *E.sieboldi* prevalence of infection for all rainbow trout sampled in 2003 to 2005.

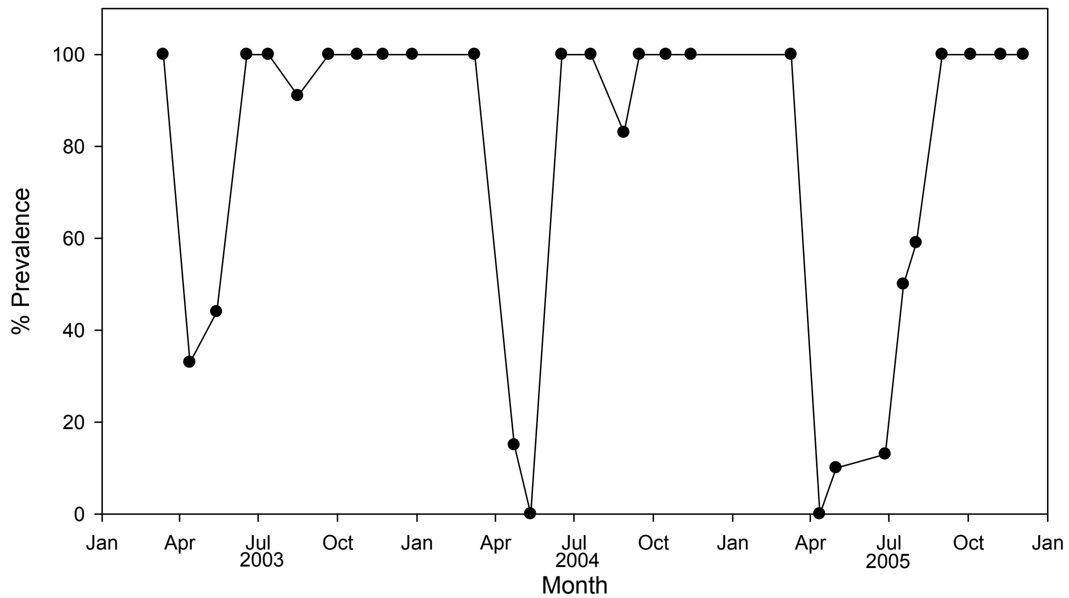
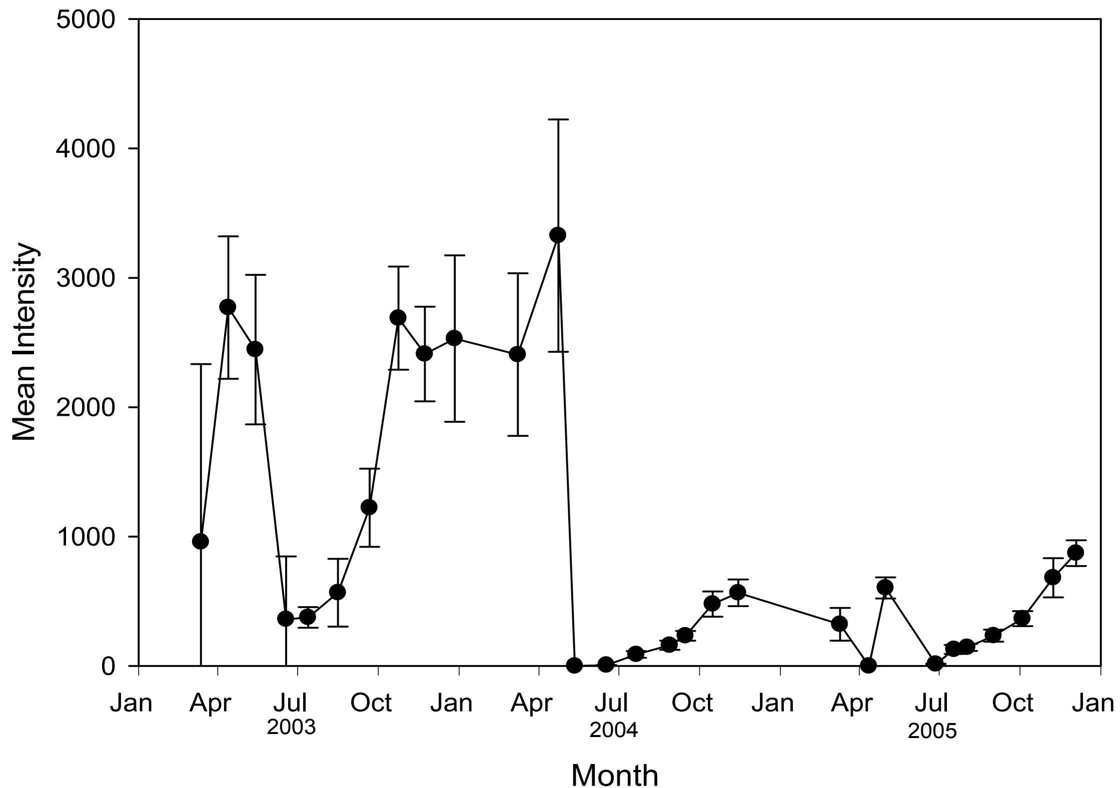


Figure 3.9 above shows the similarity in infection patterns in terms of prevalence between 2003 and 2005. Only in 2005 did it take slightly longer for all the sampled trout to become infected once the infective period had begun in June.

From the intensity of infection data illustrated in Figure 3.10, it becomes clear that 2003 was a year of greater success for the *E.sieboldi* population than subsequent years. The infective period of June to October remained fairly constant throughout the study.

Figure 3.10 Mean intensity of *E.sieboldi* infection for all rainbow trout sampled in from 2003 to 2005 with 95% confidence intervals.



3.3.4 Cage trial 1. *E.sieboldi* recruitment cage trial

Table 3.8 and Figure 3.11 show the parasite mean intensities and abundances of *E.sieboldi* found on the triploid rainbow trout of cage 4. This cage was the 'all in all out' sample cage that was harvested and restocked at approximately 2 weekly intervals.

Parasite infections took place from the first sample in July and ended after the first week of November when the temperature had fallen below 12 ºC. The sample of fish that was

stocked in the cage on the 19th August and harvested on 2nd September showed no infection. Interestingly, this drop in prevalence at this time of year was also seen in reservoir fish in previous data from 2003 and 2004 angler caught fish and supports the findings from the angler caught fish that there was a reduction in the number of new infections at this time and it was not an effect caused by sampling error. Prevalence of infection for the 2004 two weekly recruitment cage is graphically illustrated in Figure 3.12. Due to the late stocking of fish into cage 4 in 2004, the start of the infective period was not identified using the cage trial.

Table 3.8 Summary of monthly *E.sieboldi* infection data from 2004 two week recruitment cage (cage restocked every two weeks and then fished out).

Cage 4 Harvests (2 weekly recruitment)							
Stocking Date	Harvest date	n	Min	Max	Prevalence %	Mean intensity	Mean abundance
13/07/04	27/07/04	25	0	12	72	5	4
02/08/04	16/08/04	25	1	17	100	4	4
19/08/04	02/09/04	30	0	0	0	0	0
09/09/04	23/09/04	30	0	19	77	8	6
24/09/04	08/10/04	30	0	10	63	6	4
08/10/04	22/10/04	30	0	7	70	5	4
23/10/04	06/11/04	30	0	7	40	5	2
10/11/04	24/11/04	15	0	0	0	0	0
26/11/04	10/12/04	15	0	0	0	0	0

Although parasite infection levels in the cage held fish were relatively low compared with free ranging angler caught fish, the rate of infection as seen in Figure 3.12 did show a similar pattern to prevalence data in angler caught fish and also corresponded to measured water temperatures illustrated for 2004 in Figure 3.13. Infection rates were highest in early August when the water temperature was above 18 ºC. There was then a dearth of new infections in the last 2 weeks of August. Infections again took place during September, October and November but rate of new infections declined as the temperature fell.

Figure 3.11 *E.sieboldi* infection levels from 2004 two week recruitment cage (cage held fish sampled and restocked every two weeks).

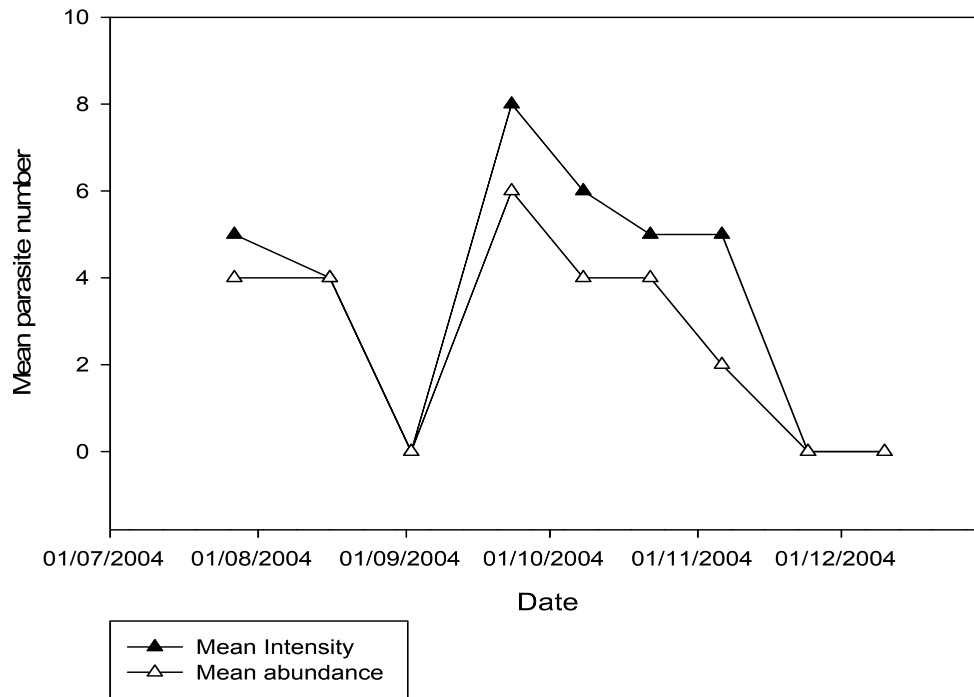


Figure 3.12 Prevalence of *E.sieboldi* infection from 2004 two week recruitment cage (cage held fish sampled and restocked every two weeks).

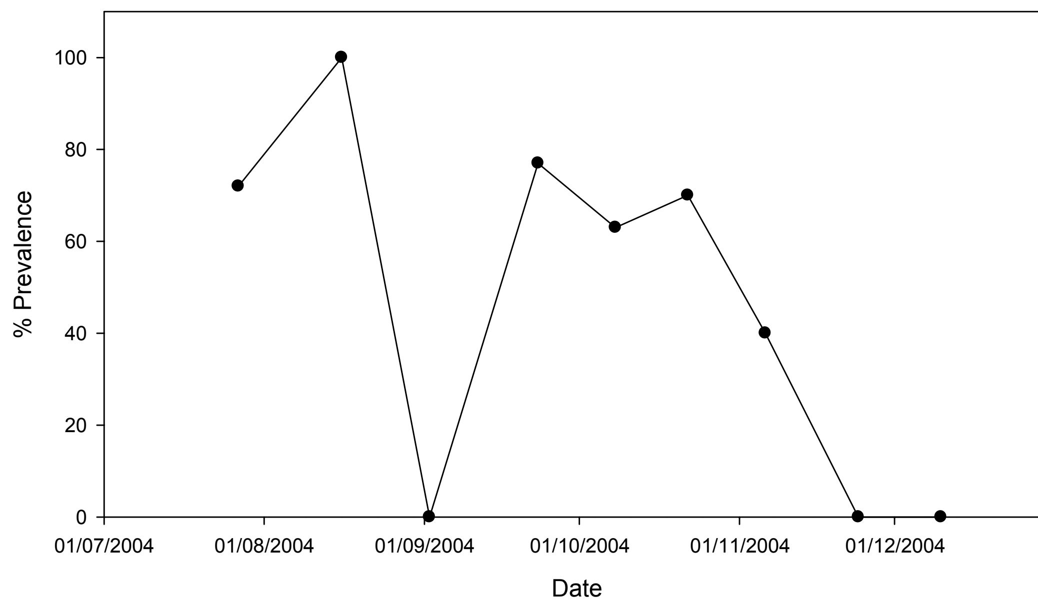
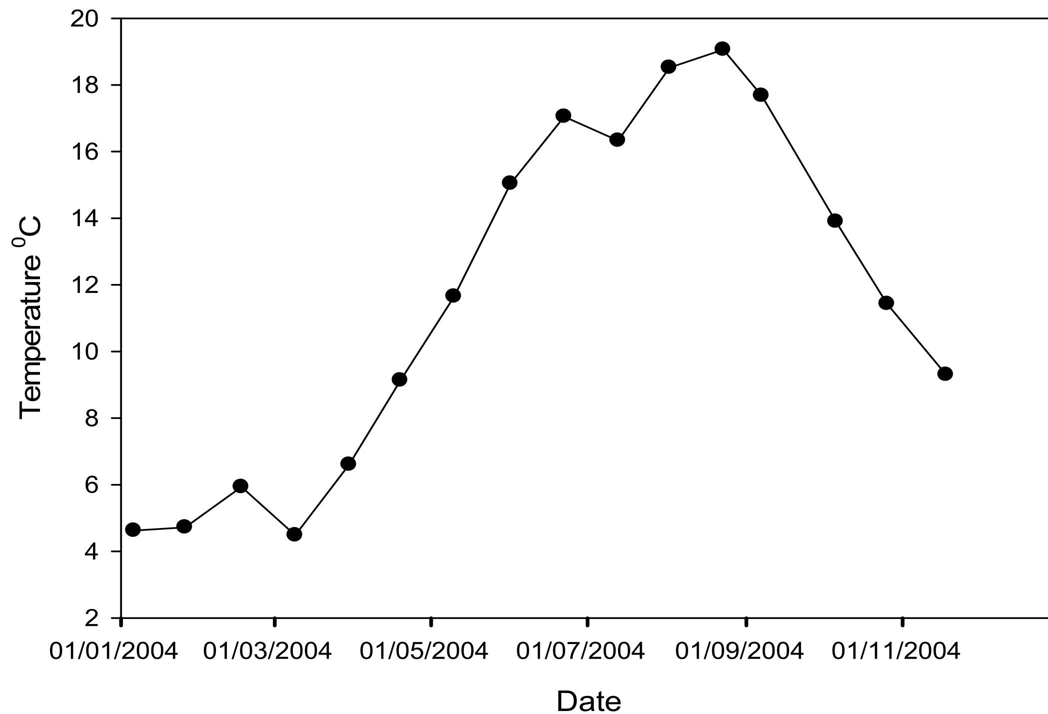


Figure 3.13 Temperature profile for 2004 measured every 2-3 weeks 2m below the surface at the Lim Tower sampling site.



The experiment was repeated in 2005. As in 2004, one cage was stocked with triploid rainbow trout and sampled by removing all the fish every 2 weeks but sample size was twenty per batch to assess the rate of recruitment of parasite infection.

The sampling regime began earlier in 2005 than in 2004. Starting the sampling programme earlier in 2005 enabled initial samples to be taken every week order to identify more precisely the period of the first new infections of the year. Table 3.9 shows that the first new infections were detected in the week prior to the 24th June. It should be noted that the 2004 data on occurrence of new infections is based upon angler caught samples and different sample dates to the methods employed in 2005 and therefore it not possible to compare the two. From then on, samples were taken at two weekly intervals with a further 20 fish stocked in their place after each sampling. The

summary infection data from 2005 cage samples is shown in Table 3.9. The prevalence of infection is graphically illustrated in Figure 3.15 and the levels of infection are shown in Figure 3.14.

Figure 3.9 Summary *E.sieboldi* infection data from 2005 2 week recruitment cage.

Cage 4 Harvests (2 weekly recruitment)							
Stocking Date	Harvest date	n	Min	Max	Prevalence %	Mean intensity	Mean abundance
26/06/05	02/06/05	20	0	0	0	0	0
03/06/05	10/06/05	20	0	0	0	0	0
10/06/05	17/06/05	20	0	0	0	0	0
17/06/05	24/06/05	20	0	6	40	3	1
30/06/05	14/07/05	20	0	8	75	5	3
15/07/05	29/07/05	20	2	12	100	6	6
31/07/05	14/08/05	20	0	10	65	6	3
15/08/05	29/08/05	20	1	8	100	4	4
31/08/05	14/09/05	20	3	10	100	7	7
14/09/05	28/09/05	20	0	9	70	6	5
28/09/05	11/10/05	20	0	8	65	4	3
17/10/05	31/10/05	20	0	5	45	3	2
31/10/05	14/11/05	20	0	0	0	0	0
15/11/05	29/11/05	20	0	0	0	0	0

Again infection levels were low in cage held fish in 2005 compared with infection levels of angler caught fish. Figure 3.14 shows the pattern of infection in 2005 was similar to 2004 (Figure 3.11). The drop in intensity and abundance in late August was seen again in 2005. The accumulated evidence from 2003-2005 further supports the theory that there is a reduction in new infections occurring in the reservoir at this time. A potential increase in numbers of uninfected fish stocked into the reservoir at this time may affect the results from the angler caught fish but would not affect cage trial results unless it was sufficiently large a stock to reduce the numbers of infective stages of the parasite throughout the reservoir including the area where the cages were held.

Figure 3.14 *E.sieboldi* infection levels from 2005 two week recruitment cage (cage restocked every two weeks and then fished out).

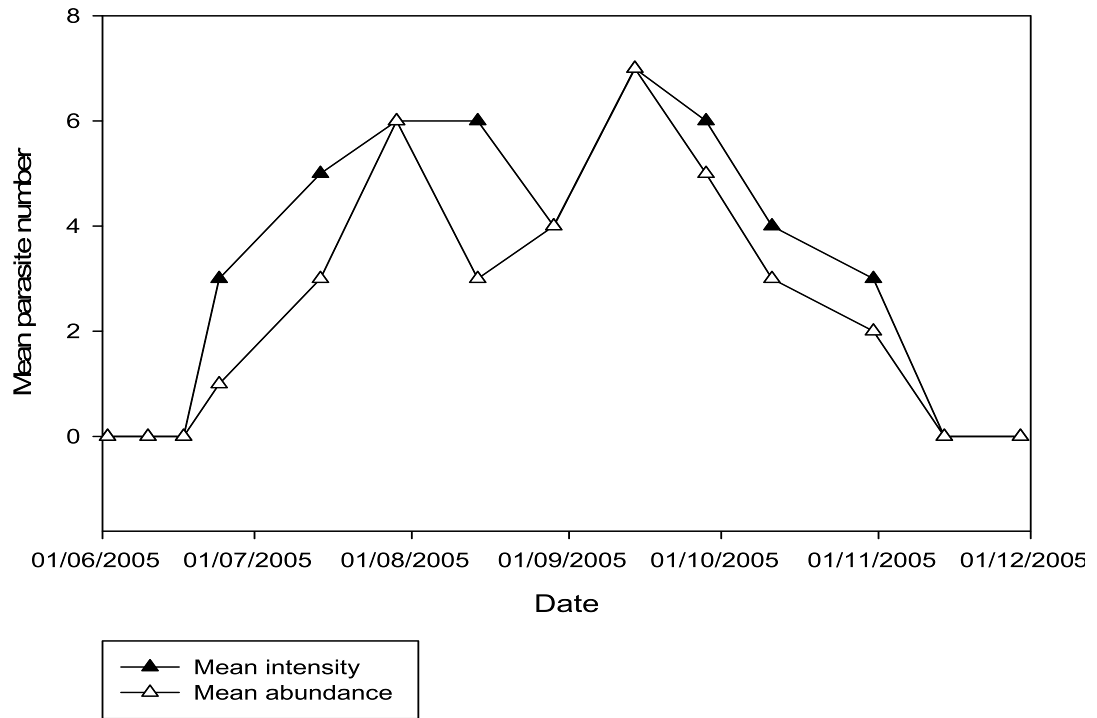
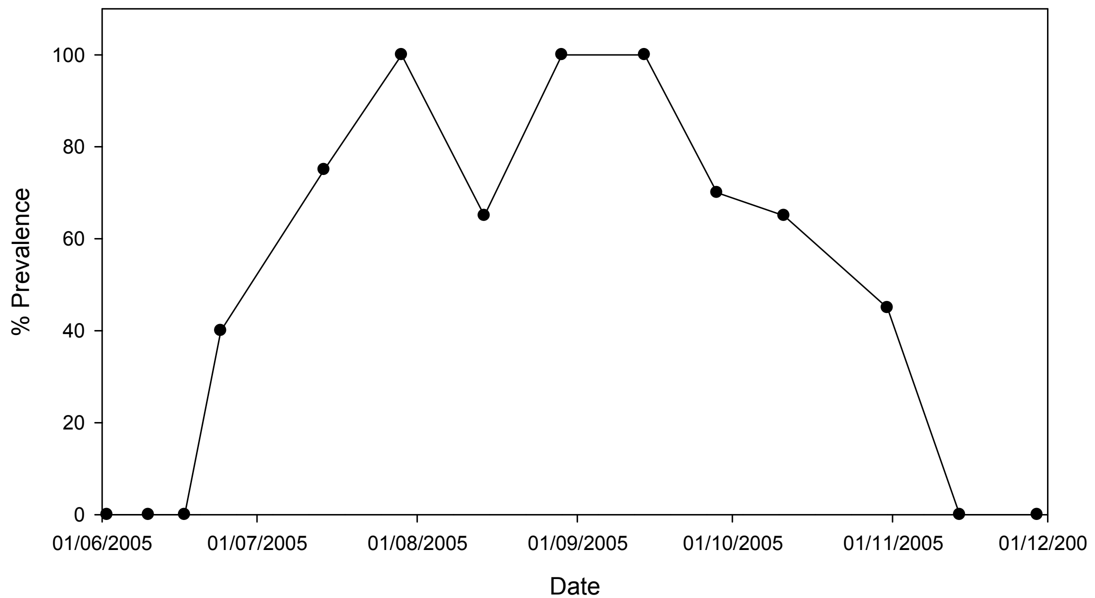
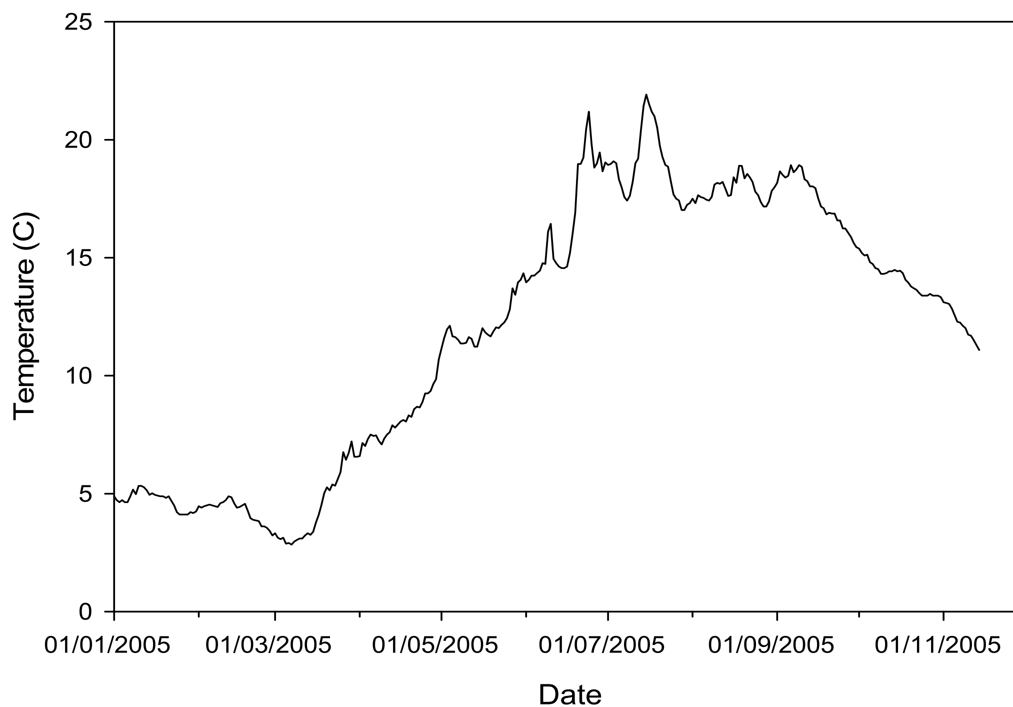


Figure 3.15 Prevalence of *E.sieboldi* infection from 2005 two week recruitment cage (cage restocked every two weeks and then fished out).



From the dates of the harvest it may be questioned whether fish were in the cage for different lengths of time. The reason for the dates not being exactly 2 weeks apart were that fish were not always able to be stocked on the exact day due to availability, weather conditions or other unavoidable circumstances. It should be noted, however, that each batch of fish remained in the cage for exactly 2 weeks. Occasionally, due to these difficulties, the cage may have remained empty for a few days until restocking could take place. The last observed infections were in the last 2 weeks of October 2005. The measured temperature profile in 2005 is illustrated in Figure 3.16 for comparison.

Figure 3.16 Temperature profile for 2005 measured every 3 hours 2m below the water surface at the experimental cage site.



3.3.5 Susceptibility of different hosts to infection with *Ergasilus sieboldi*.

3.3.5.1 Coarse fish

Coarse fish were obtained by seine net in the Normanton Bay area of Rutland Water on the 18th June 2003. A total of 29 roach (*Rutilus rutilus*), 9 perch (*Perca fluviatilis*), 1 ruffe (*Gymnocephalus cernuus*), 1 pike (*Esox lucius*) and 1 zander (*Sitzostedion lucioperca*) were sampled. These fish were examined using the same methods described for trout previously. Table 3.10 shows the counts of *E.sieboldi* found to be present on each species sampled.

Table 3.10 *E.sieboldi* infection and size data for coarse fish sampled in June 2003.

Species	n	Minimum length (mm)	Maximum length (mm)	Mean length (mm)	Prevalence %	Mean intensity	S.D. of Mean intensity	Mean abundance
Roach	29	110	260	179	62	7	10.9	4
Perch	9	105	200	139	22	1	0.4	0.2
Pike	1	350	350	350	100	2	n/a	2
Zander	1	205	205	205	0	0	n/a	0
Ruffe	1	105	105	105	0	0	n/a	0

Unfortunately, coarse fish sample sizes were too small for any detailed statistical analysis but it was noted that roach, perch and pike were infected by the parasite. Infection levels were substantially lower than rainbow trout but sample size and fish size must be taken into consideration. A single ruffe and a single zander were caught and were uninfected.

Additionally, sixteen perch of size range 120mm-295mm were obtained by gill net in May 2004 in the sailing club area of the reservoir at the mid-way point of the south arm. Prevalence of infection was 29%, mean intensity of infection was 1.5 parasites per fish

and mean abundance 0.4 parasites per fish. The gill net was used again in June 2005 in the Normanton area of the main basin and twelve further zander of size range 190mm-310mm were obtained. These were examined and were uninfected by *E.sieboldi*.

One European eel (*Anguilla anguilla*) of length 1010mm was caught by seine net in July 2005 and was infected with two *E.sieboldi*.

3.3.5.2 Angler caught brown trout

Occasionally during the study, brown trout have been caught by anglers and donated for examination. Table 3.11 details the findings of examination of these fish for the presence of *E.sieboldi*.

Table 3.11 *E.sieboldi* infection data for angler caught brown trout sampled from 2003-2005.

Date of capture	n	Prevalence %	Mean intensity	Mean abundance	*Mean intensity of rainbow trout
14 May 2003	2 overwintered	100	45	45	2445
18 June 2003	1 stock	100	68	68	114
21 Sept 2003	1 stock	100	48	48	1023
24 Oct 2003	1 not assessed	100	2192	2192	2688
21 July 2004	7 stock	100	48	48	89
28 Aug 2004	5 stock	100	68	68	160
15 Sept 2004	4 not assessed	100	111	111	233
10 March 2005	4 overwintered	100	155	155	322

*mean intensity of rainbow trout is the mean intensity of all comparable rainbow trout sampled at the same time as the brown trout, on a like by like basis *i.e.* newly stocked rainbow trout *vs.* newly stocked brown or overwintered rainbow *vs.* overwintered brown where the sample of brown trout is comprised of overwintered fish *etc*.

Throughout the study, the brown trout that were examined were less parasitized with *E.sieboldi* than the rainbow trout that were thought to have been resident in the reservoir for a similar period of time and thus subject to infection for a similar period of time. However, the numbers of brown trout sampled were low and do not permit a statistical

evaluation of susceptibility to infection. Also judging the length of time that a brown trout had been in the reservoir was more difficult than with the rainbow trout. When brown trout arrive from a fish farm to be stocked into Rutland, they are often in a better condition than a comparable rainbow trout in terms of fin condition, and colouration is more variable ranging from deep brown colours to almost silver. For these reasons, it was decided to conduct cage trials to subject brown trout to infection for the same period of time as rainbow trout and to judge susceptibility in a controlled manner.

3.3.5.3 Cage trial 2. Susceptibility of different host fish to infection with *E.sieboldi* in mixed populations

Tables 3.12 and 3.13 below detail the findings of the examinations of fish in Cage trial 2 at the two sample points in December 2004 and March 2005. Data is divided by cage and by group on each sample date.

The first analysis of these data sets was to determine if there was any difference between the parasite numbers on fish harvested in December 2004 and March 2005 and to thus determine how successfully the parasite had overwintered. Comparisons were made using unpaired t test on single groups by harvest date to test the null hypothesis that there would be no difference in mean parasite abundance in a group harvested in December 2004 or March 2005. The t test assumed that the groups compared against each other came from populations with equal standard deviations (tested using Bartlett's statistic) and Gaussian distributions (tested using Kolmogorov and Smirnov method). This was found to be true at the 95% confidence interval for all pairings and assumptions were therefore not violated. Table 3.14 below illustrates t and p values for each comparison made between harvest dates.

Table 3.12 Trout susceptibility cage trial sampled in December 2004. *E.sieboldi* numbers and summary statistics from each of the 3 cages of mixed trout types.

December 2004	**Cage 1**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	9.0	3.0	3.9	3.6
number of fish	25	19	18	25
min. infection	1	0	0	0
max. infection	21	8	9	10
standard deviation	5.6	2.7	2.7	2.2
lower C.I. 95%	6.6	1.7	2.6	2.7
upper C.I. 95%	11.3	4.3	5.3	4.5
December 2004	**Cage 2**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	7.4	2.9	4.0	2.7
number of fish	18	16	21	21
min. infection	0	0	1	0
max. infection	15	7	9	6
standard deviation	3.8	1.7	2.0	1.6
lower C.I. 95%	5.5	2.0	3.1	2.0
upper C.I. 95%	9.3	3.8	5.0	3.4
December 2004	**Cage 3**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	6.8	2.2	4.2	3.8
number of fish	23	15	19	23
min. infection	0	0	1	0
max. infection	17	6	8	13
standard deviation	4.3	2.1	2.2	2.8
lower C.I. 95%	5	1.1	3.1	2.6
upper C.I. 95%	8.7	3.4	5.2	5

Table 3.13 Trout susceptibility cage trial sampled in March 2005. *E.sieboldi* numbers and summary statistics from each of 3 cages of mixed trout types.

March 2005	**Cage 1**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	8.9	3.2	4.1	4.3
number of fish	25	19	18	23
min. infection	1	0	0	0
max. infection	18	7	8	9
standard deviation	4.3	2.2	2.2	2.8
lower C.I. 95%	7.1	2.1	2.9	3.1
upper C.I. 95%	10.7	4.3	5.2	5.5
March 2005	**Cage 2**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	7.9	3.2	4.3	2.4
number of fish	18	16	20	20
min. infection	2	0	1	0
max. infection	14	8	8	6
standard deviation	3.3	2.1	2.0	1.7
lower C.I. 95%	6.3	2.1	3.4	1.6
upper C.I. 95%	9.6	4.3	5.2	3.2
March 2005	**Cage 3**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	6.5	2.9	4.3	4
number of fish	20	14	19	23
min. infection	1	0	1	1
max. infection	15	5	9	9
standard deviation	3.3	1.7	2.2	2.2
lower C.I. 95%	5.0	1.9	3.2	3.1
upper C.I. 95%	8.0	3.9	5.3	4.9

In all groups, the mean parasite abundance in December 2004 was not statistically different from the mean parasite abundance in March 2005. This indicates that the parasite was very successful in overwintering as no obvious losses had occurred. Previous data showed that no new infections took place at that time of year. The following analysis is based on the data for each group pooled from both sampling dates and is shown in Table 3.15.

Table 3.14 t test analysis of differences in mean parasite abundance for each group of fish in trout susceptibility cage trial between December 2004 and March 2005.

Cage	Group	Test parameter	t value	p value
Cage 1	Triploid rainbow	Dec 04 *vs.* Mar 05	t = 0.02825	p = 0.9776
Cage 1	Diploid rainbow	Dec 04 *vs.* Mar 05	t = 0.2642	p = 0.7931
Cage 1	Triploid blue	Dec 04 *vs.* Mar 05	t = 0.1335	p = 0.8946
Cage 1	Diploid brown	Dec 04 *vs.* Mar 05	t = 0.9796	p = 0.3324
Cage 2	Triploid rainbow	Dec 04 *vs.* Mar 05	t = 0.8540	p = 0.3991
Cage 2	Diploid rainbow	Dec 04 *vs.* Mar 05	t = 0.4571	p = 0.6509
Cage 2	Triploid blue	Dec 04 *vs.* Mar 05	t = 0.4050	p = 0.6877
Cage 2	Diploid brown	Dec 04 *vs.* Mar 05	t = 0.5291	p = 0.5997
Cage 3	Triploid rainbow	Dec 04 *vs.* Mar 05	t = 0.3950	p = 0.6949
Cage 3	Diploid rainbow	Dec 04 *vs.* Mar 05	t = 1.022	p = 0.3158
Cage 3	Triploid blue	Dec 04 *vs.* Mar 05	t = 0.1466	p = 0.8842
Cage 3	Diploid brown	Dec 04 *vs.* Mar 05	t = 0.2384	p = 0.8127

With the pooled data, some groups violated the assumptions of Gaussian distribution and equality of standard deviation. For this reason, pooled data analysis was carried out on data that had been transformed by Log (x+1). The transformed data sets then conformed to the assumptions of parametric analysis.

To analyse if the cage used had an effect on the parasite distributions between trout types, a general linear model to quantify the effects of cage, trout type and cage*trout type was carried out. Results are illustrated in Table 3.16.

Table 3.15 Trout susceptibility cage trial. Pooled samples of fish examined in December 2004 and March 2005. *E.sieboldi* numbers and summary statistics from each of the 3 cages of mixed trout types.

Pooled samples	**Cage 1**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	8.9	3.1	4	3.9
number of fish	50	38	36	48
min. infection	1	0	0	0
max. infection	21	8	9	10
standard deviation	5	2.4	2.5	2.5
lower C.I. 95%	7.5	2.3	3.2	3.2
upper C.I. 95%	10.3	3.9	4.8	4.7
Pooled samples	**Cage 2**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	7.7	3	4.2	2.5
number of fish	36	32	41	41
min. infection	0	0	1	0
max. infection	15	8	9	6
standard deviation	3.5	1.9	2	1.6
lower C.I. 95%	6.5	2.3	3.5	2
upper C.I. 95%	8.9	3.7	4.8	3
Pooled samples	**Cage 3**			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	7	2.6	4.2	3.9
number of fish	43	29	38	46
min. infection	0	0	1	0
max. infection	17	6	9	13
standard deviation	3.9	1.9	2.2	2.4
lower C.I. 95%	5.9	1.8	3.5	3.2
upper C.I. 95%	8.2	3.3	4.9	4.6

Table 3.16 Results of General Linear Model assessing effect of trout type, cage number and combination of trout type and cage against the recorded measures of parasite numbers from each fish.

Analysis of Variance for Log(x+1), using Adjusted Sum of Squares for Tests.

Source	Degrees of freedom	Adjusted sum of squares	F	P	Significant at 95 % confidence
Cage	2	0.17474	1.61	0.201	No
Trout type	3	9.44031	58.04	<0.001	Yes
Cage*type	6	0.68497	2.11	0.051	No

As the analysis of cage effect showed that no significant difference in parasite mean abundance between test groups was due to the cage used, further analysis was carried out on pooled data from all three cages and analysed by trout type only.

Table 3.17 Summary of *E.sieboldi* numbers from mixed cage susceptibility trial, pooled data for all cages by trout type.

Pooled cages	Cage 1-3			
Group	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
mean abundance	7.95	2.92	4.13	3.5
number of fish	129	99	115	135
min. infection	0	0	0	0
max. infection	21	8	9	13
standard deviation	4.29	2.12	2.19	2.31
lower C.I. 95%	7.21	2.5	3.73	3.11
upper C.I. 95%	8.69	3.34	4.54	3.89

When all the data was pooled according to trout type, some groups did not conform to the assumptions required for parametric analysis and transforming the data was unsuccessful at rectifying this. Therefore non-parametric analysis was performed on the data set when pooled by trout type.

Table 3.18 gives the p statistic for Mann-Whitney tests performed on each trout type paired against each other trout type.

Table 3.18 P values for Mann-Whitney tests of *E.sieboldi* abundance between trout type groups on pooled cage data.

	Triploid rainbow	Diploid rainbow	Triploid blue	Diploid brown
Triploid rainbow	n/a			
Diploid rainbow	p=<0.0001*	n/a		
Triploid blue	p=<0.0001*	p=0.0002*	n/a	
Diploid brown	p=<0.0001*	p=0.0862	p=0.0137*	n/a

* indicates statistical significance at 95% confidence level

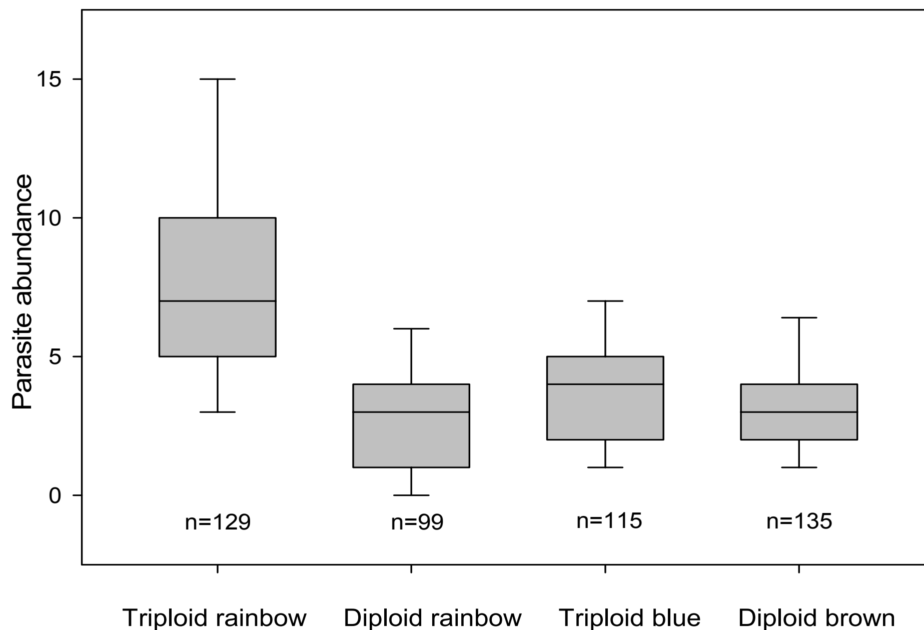
In the mixed cage trials from July 2004 to March 2005, triploid rainbow trout were the most susceptible to infection with *E.sieboldi*. The median abundance of infection for triploid rainbow trout was statistically higher than the other 3 types of trout tested.

Triploid blue trout were the second most susceptible to infection in this trial and median infection levels were significantly lower than triploid rainbows and higher than diploid rainbows or diploid brown trout.

Diploid brown trout were significantly less infected than triploid rainbow or triploid blue trout. There was no statistical difference in infection levels between diploid brown and diploid rainbow trout.

Diploid rainbow trout had the lowest infection levels of the groups tested but the median abundance of infection was not significantly different to diploid brown trout. The findings of this trial are graphically represented in Figure 3.17.

Figure 3.17 Box and whisker plot of parasite abundance on four types of trout held in mixed experimental cages from July 2004-March 2005.



The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. The error bars on each box represent the 10th and 90th percentiles for each group.

3.3.5.4 Cage trial 3. Susceptibility of different host fish to infection with *E.sieboldi* in mixed and separate populations

In 2004, it was demonstrated that there was a significant difference in the infection levels of different species and ploidy level. Triploid rainbow trout became more infected than diploids or brown / blue trout during the course of the trial. As it was necessary to repeat this experiment to confirm this finding and also to further explore these results, it was decided to concentrate on triploid and diploid rainbow trout for 2005 trials. These fish are the most commonly available and cost effective stocking options for trout fisheries in the UK.

Cage 1 was stocked with 100 triploid rainbow trout.

Cage 2 was stocked with 100 diploid rainbow trout.

Cage 3 was stocked with 50 diploid and 50 triploid rainbow trout.

The 2004 trials showed that there were no noticeable cage effects. Therefore it was assumed that the fish in each cage had an equal opportunity for infection by the parasite.

All the cages were stocked with fish in the first week of July 2005, and the fish were removed and killed for examination in December 2005 when no new infections were observed in the recruitment rate cage. During this trial 8% of the triploids and 11% of the diploids died.

Table 3.19 shows the descriptive statistics of the parasite infections in cages 1, 2 and 3. In order to compare these results statistically, independent sample t-tests have been used for the data. This tests the null hypothesis that there was no difference between groups. The independent samples t test assumes that there are equal variances between the groups to be analysed and that the groups are sampled from a Gaussian distribution. To check this, Bartlett's statistical test was performed and concluded that variances between groups were not significantly different and Kolmogorov and Smirnov values indicated that each group conformed sufficiently to a Gaussian distribution. Independent sample t tests are valid to compare the means of the different groups.

Table 3.19 *E.sieboldi* numbers from mixed and separate cage susceptibility trial in December 2005.

Dec 05				
	Cage 1	Cage 2	Cage3	
Group	Triploid rainbow	Diploid rainbow	Triploid rainbow	Diploid rainbow
mean abundance	22.46	12.98	21.65	11.4
number of fish	92	90	46	43
min. infection	0	0	0	0
max. infection	37	29	37	24
standard deviation	8.98	8.75	9.67	7.77
lower C.I. 95%	20.59	11.14	18.78	9.01
upper C.I. 95%	24.32	14.81	24.52	13.79

Figure 3.18 Box and whisker plot of parasite abundance on triploid and diploid rainbow trout held in separate and mixed experimental cages from July 2005-December 2005.

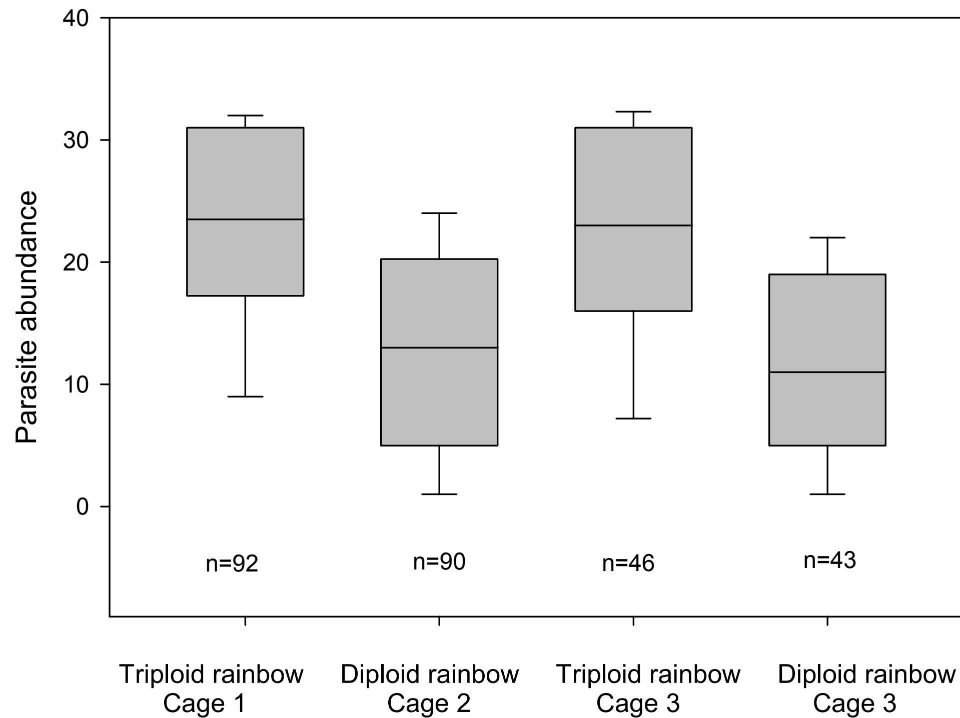


Table 3.20 P and t statistics for the results determined using a independent samples t tests of the *E.sieboldi* abundance between triploid and diploid rainbow trout held in separate and mixed experimental cages.

	Cage 1 Triploid rainbow	Cage 2 Diploid rainbow	Cage 3 Triploid rainbow	Cage 3 Diploid rainbow
Cage 1 Triploid rainbow	n/a	t = 7.214 p = <0.0001*	t = 0.4837 p = 0.6294	t = 6.952 p = <0.0001*
Cage 2 Diploid rainbow	t = 7.214 p = <0.0001*	n/a	t = 5.281 p = 0.0001*	t = 1.011 p = 0.3139
Cage 3 Triploid rainbow	t = 0.4837 p = 0.6294	t = 5.281 p =0.0001*	n/a	t = 5.497 p = <0.0001*
Cage 3 Diploid rainbow	t = 6.952 p = <0.0001*	t = 1.011 p =0.3139	t = 5.497 p = <0.0001*	n/a

* indicates statistical significance at 95% confidence level

The data gathered in 2005 supported earlier findings that triploid rainbow trout became more infected with *E.sieboldi* in Rutland Water than diploid rainbow trout. The statistical tests confirmed that the difference was significant at 95% confidence levels for both the mixed cage and for the separate cage populations. This suggests that triploid rainbow trout were more susceptible to infection with *E.sieboldi* than diploid rainbow trout, whether in mixed populations or when separated.

3.4 Discussion

3.4.1 Possible source of infection

E.sieboldi was first identified as parasitizing the fish of Rutland Water in 2002. At that time infection levels are believed to have been high although this was one year prior to any quantitative assessment being made of infection levels. Anglers reported emaciated fish with large amounts of mucus on the gills and abnormally large numbers of fish jumping from the water. The fishery managers reported that anglers were catching fewer fish, especially during the summer and that growth rates of the fish were lower than normal (based on fish sizes at the end of the year). This was attributed to the

effects of the parasite but as no measurements of effects were taken, it was a perceived change in the fishery.

It is very unlikely that *E.sieboldi* first infected the fish of Rutland Water in 2002. It is more likely that the parasite had been present in previous years but the potential effects of parasitism had gone unnoticed or infection levels were lower and there had not been a reduction in catch rates or fish condition that gave cause for close examination.. However, a survey of rainbow and brown trout by Moody & Gaten (1982) was carried out on the fish of Rutland Water in 1974-1979 following infections of *Diplostomum spathaceum* and *Tylodelphys clavata*. This survey did not record the presence of *E.sieboldi*. Although this survey examined the eyes for the presence of these parasites, the size of *E.sieboldi* on the gill surfaces would have made them likely to have been found by parasitologists. It is possible that *E.sieboldi* was discovered but not reported. However, Anglian Water staff that were employed at the time of this study and previous investigations, recall no mention of additional parasite species being found.

There are a number of possible routes for the parasite to have entered the reservoir. Fish are regularly stocked and infected fish could have entered the reservoir in this manner. However, all the fish stocked into the reservoir are from reputable trout farms in the UK. These farms are inspected on a regular basis by Cefas, the agency of the UK Government's Department for the Environment, Food and Rural Affairs (Defra), responsible for health surveys of salmonid farms in England and Wales. In addition, pre-stocking samples of trout from all the farms that supply Rutland were examined for infection by the author in 2004 and 2005 and none were found to be infected.

The reservoir is supplied with water from two river systems, the Nene and the Welland, and the status of the parasite in these systems is unknown. If the parasite is present in one or both of these systems, it is probable that infected fish and/or the free living stages of *E.sieboldi* have entered the reservoir in the pumped water supply from these rivers. The parasite could also have been transferred by anglers who had fished at other infected sites by transfer on angling equipment. Anglers commonly use ice boxes or "bass bags" to hold any dead fish after capture to keep them fresh. A bass bag is a permeable bag which holds the dead fish and is placed in the water to keep a small flow of water over the fish to reduce temperature. If a bag had held infected fish it is possible that the parasite could be transmitted to a new site in this manner. It is not a common practice at UK trout fisheries to require anglers to disinfect equipment such as bags and nets.

There is also the possibility of parasite transfer between water bodies caused by birds or other wild life that could transfer parasite eggs or infected fish through movement or feeding. There have been no studies to identify the potential risks of *E.sieboldi* transfer between sites but it was observed in this study that if kept in water at a suitably low temperature (<8 ºC), adult female *E.sieboldi* can remain alive for up to 1 week after removal from the host. In addition, egg sacs attached to the parasite were viable for a longer period, whether attached to live or dead adult female or removed from the parasite altogether (see Chapter 4).

3.4.2 Infection levels over time

Throughout the study, angler caught fish infected with small numbers of *E.sieboldi* (less than 100 per fish), rarely showed any obvious gross pathology and were in good

condition. At high infection levels (more than 1000 parasites per fish) it was likely that a greater bias in the method of fish capture existed as fish may have became less likely to be caught by anglers. Anglers will only catch fish which are able to feed and this, therefore, is biased towards the healthier specimens. Seine and gill netting at Rutland Water proved difficult due to the shore topography and man power requirements. Unfortunately too few fish were caught by this method to compare the extent of bias inherent in each capture method.

The initial examination of overwintered infected fish in 2003 showed that *E.sieboldi* was present in large numbers. Adult female *E.sieboldi* was present on some fish in every monthly sample during the year. Parasite numbers infecting rainbow trout in 2003 varied from 0 to 6196 per fish. Stocking of trout in 2002 had ceased in September of that year. As a result, it was relatively easy to distinguish overwintered and early stock fish in the early months of 2003 compared with the newly stocked fish but more difficult after September.

As *E.sieboldi* is only known to overwinter as an adult female attached to fish and not as an egg or any of its free living stages, it is clearly important to have an assessment of the numbers of parasites on overwintering fish at the start of the year as these form the source of all subsequent infections later in the season. The prevalence of infection of overwintered fish was 100% in 2003 indicating that all fish stocked in 2002 that had overwintered into 2003 had become infected, including the last fish to be stocked in September 2002. There was a wide range in the infection levels of these overwintered fish (256-5258 parasites per fish). Individual host behaviour and susceptibility may have been in part responsible for this variance in infection levels, but the largest factor

is likely to be the duration of time that the fish was subject to the infective stage of the parasite, *i.e.* some of these fish were stocked earlier in the year than others and are therefore more likely to be infected with higher numbers of the parasite. A survey of *Argulus* spp. in rainbow trout fisheries also acknowledged that duration of the retention time or 'stock turnover' in a managed fishery was correlated to the observed intensity of infection (Taylor, Sommerville, & Wootten 2006).

A total of 24 overwintered fish were examined in 2003 prior to the infective period beginning in June. The mean intensity of infection of these fish was 2457 parasites per fish. In November and December 2003, after the infective period had ended, a further 24 fish examined had a mean intensity of 2431. Thus the relative population of *E.sieboldi* per host had maintained itself successfully in 2003. The number of infected hosts at the start and end of the year cannot be known exactly and therefore the total size of the *E.sieboldi* population in Rutland Water also is not known.

The first new stockings of rainbow trout in 2003 took place in late March. This had the effect of reducing prevalence of infection by diluting the standing stock of infected fish with naïve uninfected fish. These fish remained uninfected in samples obtained until 18th June when all fish examined were then infected and prevalence had increased to 100%. At this point, 50750 rainbow trout had been stocked into the reservoir. Of these, 26210 had been reported as caught and removed. From this we can say that there were up to 24540 newly stocked rainbow trout in the reservoir on the 18th June 2003. This is clearly an overestimate as it does not take account of additional losses due to mortality, predation or unreported catches. Standing stock and estimation of parasite numbers will be considered further in Chapter 6. Ten newly stocked fish were available for

examination on the 18th June 2003. The fish were caught from different areas of the reservoir and each of these ten fish was infected with at least 46 *E.sieboldi*. Despite the sample size being small, it does indicate that the first transmission of infection in 2003 was widespread and the onset of infection was rapid as no infected stocked fish were present in the sample one month earlier.

From the June sample to the October sample in 2003, mean intensity of infection and mean abundance of infection increased rapidly. Only in the sample of fish collected on the 16th August were there uninfected fish present in the sample (3 out of 31 newly stocked fish). It is possible that these uninfected fish were caught very quickly after being stocked into the reservoir and before they had encountered the infective stage of the parasite. It is also possible that there was a reduction in the transmission rate of new infections at this time. New infections continued to occur until the sample taken on the 24th October. From this point on the mean intensity of infection for each sample remained fairly constant at approximately 2500 parasites per fish indicating that the infective period had ended for 2003.

In 2004, eight overwintered fish were examined prior to the onset of new infections in June. The mean intensity of infection of these fish was 2636 parasites per fish indicating that the parasites had successfully overwintered from the 2003 season as infection levels were very similar. No further overwintered fish were examined in the samples taken in 2004, possibly indicating that there were fewer overwintered fish in 2004 than in 2003 or that more had been caught by anglers in the first few months of the season.

Again the prevalence of infection and mean abundance of infection fell with the stocking of new fish from March onwards. These fish remained uninfected until 100% prevalence of infection was again seen in a sample taken in June. Of 18 newly stocked fish sampled on the 17th June, all were infected but at lower levels than had been seen at the same time point in 2003 (mean intensity of 6 parasites per fish in 2004 compared to 114 parasites per fish in comparable fish from 2003).

After the start of the infective period in June of 2004, prevalence remained at 100% for the rest of the year, apart from a sample of 24 fish taken on the 28th August. Of these, 4 fish were uninfected. The pattern of prevalence of infection in 2004 is almost identical to 2003.

Mean intensity of infection in 2004 increased from June until the final sample in November which was expected to be after the end of the infective period, and was confirmed as such by the recruitment cage experiment. The rate of increase in parasite mean intensity was lower in 2004 than in 2003. The final mean intensity of fish at the end of the year was 565 parasites per fish, substantially lower than the mean intensity at the start of the year.

The parasite successfully overwintered into 2005 with 6 fish examined in March and May samples with a mean parasite intensity of 416 parasites per fish. The first infected stock fish examined in 2005 were on the 29th June with a mean intensity of infection of 8 parasites per fish. Mean intensity of infection continued to increase in monthly samples up to 793 parasites per fish in November and December combined.

In contrast to 2003 and 2004, the prevalence of infection in 2005 increased more slowly. On the 27th June 2005, prevalence of infection was 13% compared to 100% in at this point in the previous two seasons. It was not until 1st of September 2005 that a sample of angler caught stock fish reached 100% prevalence of infection. The reduction in prevalence in late August to early September that was observed in 2003 and 2004 is not apparent in the data from angler caught fish in 2005. As the prevalence of infection did not reach 100% until later in the season, any such drop in recruitment rate would be undetectable.

To summarise, the infection levels seen between 2003 and 2005, the patterns of infection are very similar. Overwintering fish with large numbers of overwintering parasites are present at the start of the year. These give rise to the first new generation of parasites which attach to the newly stocked fish in June and infection levels continue to increase until late October or early November. The increase in mean infection levels was exponential in 2003 but the increase seen in 2004 and 2005 was a more linear increase until the end of the infective period resulting in lower mean intensities than had been seen in 2003. There are several potential explanations for a reduction in infection levels in 2004 compared to 2003. Little is known of the free living stages of the parasite and factors that affect its feeding, development, reproduction and ability to find a host. It may be that conditions were more optimal for one or more of these factors in years that saw higher levels of infection. At the start of both years, mean infection rates of trout were about the same. However we do not know how many of these trout were present in the reservoir and consequently the size of the population of *E.sieboldi.* Also there may have been fewer fish available for infection at the optimal times for the parasite. It is also possible that the total population size of *E.sieboldi* had remained the

same in all the study years and there are simply more infected fish with lower mean intensities. Chapters 4-6 will examine these possibilities in more detail as potential reasons for the change in *E.sieboldi* infection levels reported in this study.

3.4.3 Infection levels of different species

It is thought that Rutland Water is populated with many species of coarse fish but as no systematic surveys of populations have ever taken place, quantities are unknown. Therefore the role that these additional species have in hosting the population of *E.sieboldi* is also unknown. The levels of infection of the coarse fish that were sampled from the reservoir were very low compared to the levels of infection in trout, but unfortunately, relatively few fish were obtained and they did not represent all species and size ranges thought to be present.

Given the wide host range recorded in the literature for *E.sieboldi* (Abdelhalim 1990;Gnadeberg 1948;Williams 2007), it is likely that many of the nineteen fish species known to be present in Rutland Water are infected by *E.sieboldi*.

An incidental observation was that in the summer of 2004, there were high numbers of coarse fish fry in many areas of the reservoir but especially in the harbour areas and the area of the experimental cages. No quantitative measure was taken, but it was commentated by the staff and regular anglers of Rutland that this was the largest number of fry seen for many years. Netting of these fry indicated that they were mostly roach with some roach / bream hybrids. As roach are planktivorous during the summertime (Balling & Pfeiffer 1997), it is possible that they had a role in reducing the number of *E.sieboldi* free living stages present in the reservoir at that time which led to

reduced intensities of infection in 2004 / 2005 compared with 2003, when fewer coarse fish fry were seen.

The two species of coarse fish most associated with infections of larger numbers of *E.sieboldi*, common bream and tench (Abdelhalim 1990;Alston 1994) were not encountered in this study. Both species are, however, present in the reservoir in unknown numbers. Bream are occasionally caught by fly fishermen in Rutland Water, and tench have been seen in the shallow areas of the south arm. The coarse fish of the reservoir were removed in 1974 by a combination of electro-fishing, draining and netting, and finally treating feeder streams with 5% rotenone. This was carried out while the reservoir was at low levels in the early phase of construction and was intended to reduce the competition for food during the early growth stages of stocked trout (Moore 1982). Re-colonisation was, however, inevitable and successful.

It is unlikely that the mean intensity of infection of bream would be in the same order as that for trout. Large bream with the highest infection levels of *E.sieboldi* have been recorded with up to 150 parasites but are usually less infected (Abdelhalim 1990;Dezfuli et al. 2003;Naich, Bilqees, & Khan 2000;Pojmanska & Dzika 1987;Zharikova 1993). Tench have been recorded with high numbers of *E.sieboldi* in previous studies. Abdelhalim (1990) recorded more than 13000 from the gills of a single 2kg tench in the south of England. It is unlikely, however, that a large biomass of tench exists in Rutland Water as the shallow, heavily vegetated areas favoured by the species (web publication, http://www.fishbase.org, accessed March 2008) constitute only a small part of the reservoir at the western ends of the north and south arms.

Additionally, the annual draw down of water levels as the reservoir is in full operation, reduces the littoral fringes and vegetation present.

Some authors have considered that high infections of *E.sieboldi* on fish such as tench, bream and pike are due to the benthic and sluggish nature of these fish allowing them to come into contact with the infective stages (Gnadeberg 1948;Piasecki, Goodwin, Eiras, & Nowak 2004). Rainbow trout do not share these characteristics, yet have been shown to be a preferred host for the parasite in this study and that of Abdelhalim (1990). Numerous studies have also indicated that larger fish have a tendency towards higher infection levels with *E.sieboldi* than smaller fish of the same species (Alston 1994;Dorovskich, Ekimova, & Rocheva 1985;Molnar & Szekely 1997;Starovoytov 1990).

In this study, no conclusions could be drawn on the size of the fish in relation to infection levels as firstly, there were insufficient numbers of coarse fish obtained for analysis; and secondly, the duration of exposure to the infective stage in trout is determined by the time since it was stocked, and as this could only be estimated, such conclusions would be tenuous. However, the author was present at Rutland Water when 2 large rainbow trout and 2 large brown trout were caught by anglers (different dates in 2004 and 2005). These fish were not donated to the study but a cursory examination of the gills *in situ* suggested that *E.sieboldi* was present in very low numbers (<100 in total for all 4 fish). These fish varied in size between 4 and 6kg and had probably overwintered for at least two consecutive seasons. These larger fish may have been able to mount an immune response to the parasite, or perhaps behaviourally, they inhabited areas of the reservoir where transmission rates were low, although the most likely

reason for a reduction in infection levels is due to size of gill filament. *E.sieboldi* attaches to the filament using the antennae. On thinner filaments of small fish, the parasite may overlap two or three filaments in order to achieve purchase with both antennae (*pers. obs.*). In a very large fish, the maximum span of an adult female *E.sieboldi* may be insufficient to affect purchase on the gill. Other authors have suggested that increased ventilation rate in larger fish may account for increased intraspecific infection with ergasilids (Hudson, Bowen, & Stedman 1994). The findings of Alston (1994) indicated that *Ergasilus briani* preferred smaller bream than large specimens. *E.briani* is a much smaller parasite than *E.sieboldi*, which supports the theory that gill filament width is important in host selection within a given species, and there is an optimum size depending on antennal length and attachment mechanism, above and below which, lower infection levels are seen.

Given that *E.sieboldi* has been associated with large fish, of a similar size to the trout stocked into Rutland Water, and that the small coarse fish examined in this study were infected with comparatively low levels of the parasite, it seems likely that the stocked trout are hosts to a large proportion of the *E.sieboldi* population in this reservoir. Although this is cause for concern to a fishery manager, it does suggest that suitable management of the trout population, may be successful in reducing the population of the parasite overall. Further analysis of the role of coarse fish as hosts for *E.sieboldi* in Rutland Water should be pursued however, in order to understand the full extent of infections.

Angler caught brown trout were examined too infrequently during the study to form any statistically reliable data on their role as hosts for *E.sieboldi* in Rutland Water.

Anecdotal reports from anglers and fishery management did suggest that they perceived the problem infection to be mainly affecting the rainbow trout. Only one brown trout examined in the study was infected with high numbers of *E.sieboldi* (2192 from a fish caught 24th October 2003).

The observations made of infection levels on angler caught brown trout were further studied by using experimental cages in 2004. Diploid brown trout were shown to be less susceptible to infection than triploid or diploid rainbows at a statistically significant level. Three replicate cages held mixed populations of triploid rainbow, diploid rainbow, triploid blue rainbow and diploid brown trout. Many anglers consider brown trout to be behaviourally different to rainbow trout and harder to catch (Moore 1982). There are also perceptions that brown trout inhabit deeper water but it has been shown that both species feed on a similar invertebrate diet and become piscivorous in larger individuals (Frost 1974). Holding fish in confined mixed populations ensured that each type would be equally available to infection with *E.sieboldi*.

Brown trout have been shown to be less susceptible than rainbow trout to a number of pathogens, especially where rainbow trout have been introduced into an area where they are not indigenous. Studies have shown increased susceptibility in rainbow trout to the very different pathogens including: *Myxobolus cerebralis* (cause of whirling disease) (Hedrick *et al.* 1999); *Discocotyle sagittata* (Monogenea) (Rubio-Godoy & Tinsley 2004); *Diplostomum* sp. (Moody & Gaten 1982) and *Diphyllobothrium latum* (Torres *et al.* 2004). The mechanisms involved in susceptibility and immunity are still poorly understood in most cases. In evolutionary terms, the rainbow trout originating from North America and only being present in Britain since the 1880s (Rubio-Godoy &

Tinsley 2004), may not have adapted a response to the pathogens of Europe, including *E.sieboldi*, to which generations of brown trout have been exposed. The rainbow trout is therefore at a disadvantage. This was demonstrated in a study on *Myxobolus cerebralis,* where an experimental infection was carried out on rainbow trout of a German strain and rainbow trout of an American strain under controlled conditions (Hedrick *et al.* 2003). The naïve American strain was more susceptible to infection in terms of intensity, prevalence and pathological effects than the German strain which had been cultured through many generations in a region where the pathogen exists.

3.4.3.1 Infection levels of diploid and triploid rainbow trout.

The cage trial observations in 2004 indicated that diploid rainbow trout were less susceptible to infection than triploid rainbow trout. This finding was supported by the results of the cage trial in 2005 where diploid and triploid rainbow trout were stocked into cages in mixed and separate populations. The results suggested that if triploid rainbow trout were replaced with diploid rainbow trout, there would be a reduction in the success of *E.sieboldi*, rather than parasites simply increasing on diploid trout to triploid levels.

The finding that brown trout were less susceptible to infection is useful for fishery management. To substitute the stocking of the triploid rainbow trout with less susceptible fish at key points in the infective season of the parasite is potentially a good control measure for managing the effects of the parasite. Brown trout, however, are expensive to stock and less popular with some of the anglers of Rutland Water. Diploid rainbow trout may form an acceptable substitute as they are commercially available and cheaper to stock as they are the mainstay of trout farm production for the table market.

The process of inducing triploidy can be achieved in several different ways, the results in each case being an increase in the ploidy level by incorporation of an extra set of genetic material. The increased cell nucleus size results in many of the cells of different tissues of the fish also becoming enlarged. Consequently in many systems, there are fewer cells than in a diploid fish. Difference in leukocyte cell counts has been attributed to a reduction in immunological performance of the host to invasive pathogens in some studies (Budino et al. 2006;Jhingan et al. 2003). The mechanisms are still poorly understood and seem to vary according to the host / pathogen interface.

Triploid rainbows are generally preferred to diploids for fishery restocking for a number of reasons. Triploid rainbow trout are often perceived by fishery managers to grow faster due to the lack of gonadal growth, and utilisation of energy in somatic growth, although this may be nullified to some extent by lower steroid levels which have an anabolic effect. The results being that growth may be increased during some growth stanzas but overall little difference exists (Myers & Hershberger 1991).

The description of *E.sieboldi* infections of fish Rutland Water in this study have identified what may be a major change in the success of the parasite in terms of population size. Environmental and biological factors will be considered in order to understand the shift in population dynamics that this study has identified between 2003 and 2005. Chapter 4 will consider any changes in the reproduction of the parasite during the study period as a potential cause for the reduction in *E.sieboldi* numbers.

Chapter 4: Reproductive development

4.1 Introduction

The reproductive development of ergasilids is important to the understanding of population dynamics of an *E.sieboldi* infection in a trout fishery. Chapter 3 examined the occurrence of new infections and the infection levels of *E.sieboldi* on the fish of Rutland Water. The results showed that in 2003, higher levels of infection were observed in fish sampled than in 2004 or 2005. This chapter will examine the development of the adult female and the eggs of the parasite during the course of the study with a view to understanding the observations made in Chapter 3.

Adult *E.sieboldi* mate when free living. Copulation, as in all poecilostomatoids, involves the male grasping the female on the posterior dorsal surface using its maxillipeds (Huys & Boxshall 1991). Spermatophores are deposited over the copulatory pores of the female by the male. Filiform spermatozoa then enter the genital complex of the female. The female, once fertilised, then enters the parasitic stage of life by infecting a host fish, usually by attaching to the gills. The mechanisms by which a female finds a host and enters the opercular cavity the fish are unknown.

Females, having mated only once, are equipped to fertilise multiple clutches of eggs (Baud 2004). The period between attaching to a host and beginning oviposition is unknown. The number of egg clutches produced is also unknown but it has been suggested (Abdelhalim 1990) that 2-4 clutches of eggs are produced by the overwintered parasites before they die and fall away from the host.

The production of an egg clutch entails a number of stages. Oocytes are developed in the paired ovary of the adult female. During oviposition, eggs are extruded via paired oviducts. They are fertilised by spermatozoa held in spermatheca anterior to the genital complex (Wilson 1911). The fertilised eggs pass out of the genital pores into paired egg sacs attached to the anterior portion of the genital complex. Eggs are initially white and opaque. As they develop, eggs become more translucent as yolk is assimilated by the developing nauplii (Alston 1994). Eggs then take on a blue/black pigmentation due to the colour of cells lining the naupliar gut wall and dorsal shield. While this occurs a new clutch of oocytes develops in the ovary of the adult female. Shortly after reaching the advanced stage, nauplii hatch from eggs, usually as the egg sac ruptures in one or several places to expel the eggs. There is then a period of time before the next clutch is extruded. This is termed inter-clutch interval in this study.

The male ergasilid is not parasitic and remains free swimming during its life time (Gnadeberg 1948). The modified antennae and maxilliped of the male assist in grasping the dorsal lateral area of the female urosome during copulation. The antennae are less specialised in males reflecting the non-parasitic mode of life. As such, the male is rarely encountered in parasitic studies and had been recorded only in plankton surveys or when the parasite had been cultured artificially. It had been suggested from these limited records that the adult male is short lived, viable to mate for only two weeks before death (Abdelhalim 1990;Halisch 1939). There is also no evidence that the male is able to overwinter. For this reason, it is assumed that the population of adult female parasites overwintering on the fish are responsible for both male and female progeny to form the new generation/s of a reproductive season. An understanding of the reproductive development of *E.sieboldi* during and after overwintering and the

consequent egg production is, therefore, vital in determining population dynamics of the parasite in a trout fishery.

There have been several studies that have described the reproductive season of *E.sieboldi* in specific geographical areas, but few have tried to relate the occurrence of the parasite with biotic or abiotic factors that could influence the success of a parasite population. The exceptions to this are the effects of temperature and light. It has been shown that the reproductive state and infective period of ergasilids follow a seasonal pattern in temperate climates (Pojmanska 1984) with new infections occurring from spring to autumn. In lakes in Poland, artificially heated by thermal pollution, Pojmanska recorded that the infective and reproductive period is longer than non heated lakes in the same area. A similar temperate season of infection was recorded for ergasilid species in China (Nie Pin 1998;Zhang 2004), Russia (Kashkovsky & Kashkovskayasolomatova 1985;Kuperman & Shulman 1977), Hungary (Molnar & Szekely 1997) and United States (Bricker *et al.* 1978). However it has been noted that ergasilid species in tropical regions were capable of producing eggs and infecting fish throughout the year (Fryer 1969).

Kuperman & Shulman (1977) suggested that photoperiod was involved in the mechanism that regulated egg production in ergasilids. They concluded that the parasite were able to discern a reducing photoperiod as a cue to end the reproductive season in preparation for overwintering despite its micro habitat being located on the gill of a fish where little light would enter past the operculum.

This study also investigated the effects of temperature and light on the reproductive development of *E.sieboldi*. For a manager of a trout fishery, it is critical to be able to understand and if possible, predict the times at which the stocked fish are most vulnerable to infection. As this is reliant on the production and development of eggs following the overwintering period, this study examined the ovarian development of parasites during the winter and the subsequent period when eggs were being produced. The aims were to provide information on the start of egg production, the rate of egg development at different times of the year, and also the time when egg production ended.

The fecundity of *E.sieboldi* has previously been examined by Abdelhalim (1990). His study showed that *E.sieboldi* produces sequential clutches of eggs in paired egg sacs made up of up to seven discontinuous rows of eggs. His results suggested that the number of eggs produced in each clutch may depend on the age of the parasite but that the mean number of eggs in a clutch was 220 and due to there being different age classes of parasites present at all times during the year, the mean was a good estimate of the number of eggs produced at any point during the reproductive season. His estimate of 2-4 clutches of eggs in the life span of the parasite was based on field observations of the occurrence of ovigerous and non-ovigerous parasites during a reproductive season. To fully elucidate the ability of the parasite to produce clutches of eggs, this study examines angler caught fish from Rutland Water over three years, and combines the findings with data from infected fish held in experimental cages and aquarium tank systems. Development rates of eggs at different temperatures are investigated using *in vivo* and *in vitro* methods in order to add to the information required to build a model of infection for *E.sieboldi*.

The potential for increased temperatures to affect reproduction rates and thus be responsible for the increased infection levels observed in 2003 compared with 2004 and 2005 are considered.

4.2 Materials and methods

4.2.1 Field examination

During the course of the sampling programme described in Chapter 3, adult female *E.sieboldi* were removed from the gills of rainbow trout using a fine pair of examination needles. As they were removed, the development stage of the egg sacs on each parasite was recorded. This was recorded as either non-ovigerous, ovigerous white (Plate 9 presence of 1 or 2 white opaque egg sacs), or advanced (Plate 10 presence of 1 or 2 translucent egg sacs with at least 50 % of the eggs having a blue/black colouration of the developing nauplii visible).

4.2.2 Egg production following overwintering - *in vivo experiments*

In November 2006, twenty one rainbow trout (900-1500g) were caught by angling in Rutland Water and transferred to an empty 4m × 4m × 4m cage. In December 2006, they were transferred to an aquarium at the Institute of Aquaculture, University of Stirling in an aerated transport tank (see Chapter 2).

These fish were used to monitor the egg production of post overwintering *E sieboldi* under controlled conditions where no possibility of new infections existed.
Fish were housed in seven 1m stock tanks and maintained according to husbandry practices described in Chapter 2 General materials and methods.

In January or February 2007, depending on the group, fish were randomly allocated to each of the seven 1m experimental tanks under the conditions detailed in Table 4.1. The exact date at which individual fish were moved from stock tanks to experimental tanks is recorded in the results section. A Gemini Tiny Tag data logger was placed in each tank to record the water temperature in degrees Centigrade at 1 hour intervals. Mean daily temperature (ºC) was used in all further calculations.

Table 4.1 The environmental conditions within each tank under which the *E.sieboldi* infected rainbow trout were held.

Group	Tank	No. of fish	Temperature	Photoperiod
1	2	3	Ambient	14hour light 10 hour
2	6	3	Ambient	Constant light
3a	1	3	Ambient	Dark
3b	7	3	Ambient	Dark
4	3	3	10 ^{0}C	Dark
5	4	3	10 ^{0}C	Dark
6	5	1	15 ^{0}C	Dark
7	5	1	15 ^{0}C	Dark
8	5	1	15 ^{0}C	Dark

4.2.2.1 Commencement of oviposition following overwintering

Rainbow trout gills were examined every 2 to 3 days to confirm commencement of oviposition in the *E.sieboldi* population. Fish were individually netted and removed to a tank containing a dilute solution of 2-phenoxy ethanol. Under light anaesthesia, gills were examined every two to three days using a CLS 150 endoscope and light source inserted through the buccal cavity and above the gill surface. Using this technique, it was confirmed that when the fish arrived at Stirling, all parasites were in a non ovigerous state. Commencement of oviposition was recorded when egg sacs were

visible on at least 10% of observable parasites. This figure was chosen to represent a significant proportion of the population commencing oviposition.

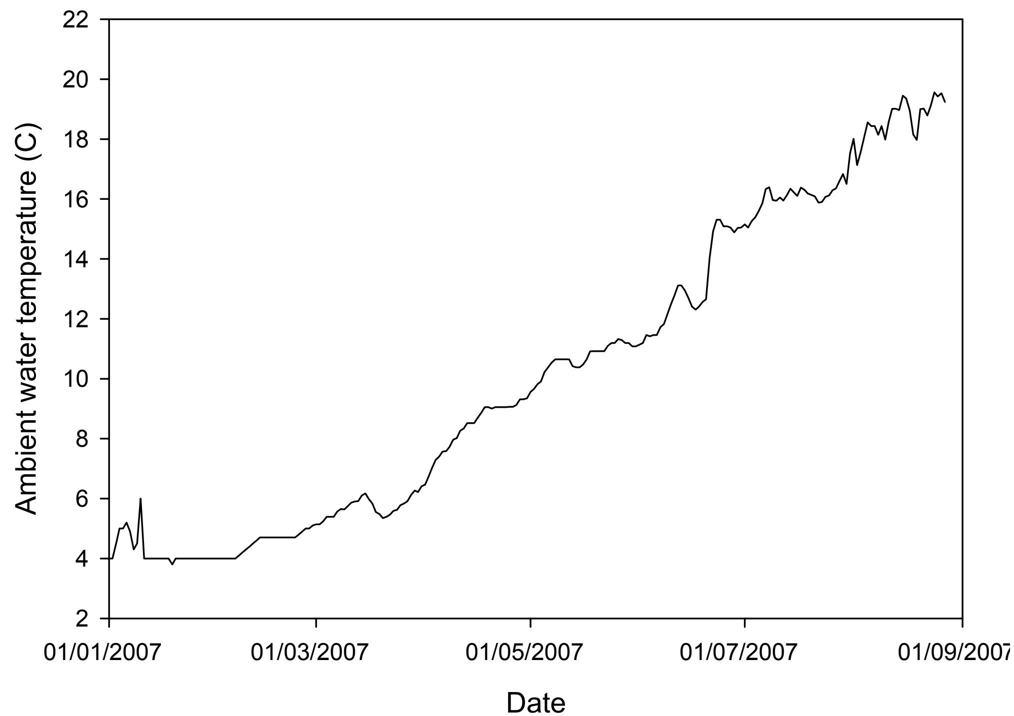
4.2.2.2 Post overwintering egg clutch production

Following confirmation of first post winter oviposition, each fish was examined two to five times per week. Each fish was removed from its respective tank and lightly anaesthetised using 2-phenoxyethanol. The fish was placed on a wet surface and each operculum was gently opened to reveal the gills. The outer hemibranch of each gill was photographed with a Nikon D40 6.1 with an 18-55mm lens. Photographs were taken by Mr Denny Conway, Institute of Aquaculture, while the author manipulated the fish under a transmitted light source. Multiple digital images were captured to provide a comprehensive map of the distribution of the parasite, and the stage of egg production of each ergasilid on the outer surface of each hemibranch was recorded using the same criteria used in the field observations described in 4.2.1. Multiple photographs were also necessary to ensure that focused images of each parasite were available on each sample day. Digital photographs in tiff format were transferred to a computer to view individual parasites in detail using Microsoft Picture Viewer.

At least 5 parasites per fish were identified by location on individual filaments and the development of their egg sacs was monitored and recorded for the duration of the experiment. The parasites recorded during the experiment were chosen as they were in the mid region of the outer surface of the gill and therefore visible in each digital image and also located far enough from other parasites to avoid confusion in identifying the individual. Examples of digital images of parasites recorded in this manner are shown in Plates 14 to 16.

The ambient water in non heated tanks on 1st January was at a temperature of 4ºC. The experiment commenced from 1st January as this was considered to be the mid–winter period in terms of egg production (*i.e*. 3 months since the end of egg production and 3 months before the beginning of new egg production (see later results section 4.3.1). Calculations using degree days of temperature received by individual fish are recorded from this time point using 0 ºC as a base threshold. Figure 4.1 shows the temperature of ambient water supplied to non heated tanks (Groups 1,2 3a and 3b) during the course of the experiment

Figure 4.1 Temperature of water (ºC) supplied to Groups 1, 2, 3a and 3b during course of tank experiment. Temperature was recorded by data loggers located in each tank and recorded temperature at 1 hour intervals. Plot is of mean daily temperature. "Ambient temperature" in the text refers to this profile during the study.



Following transfer, all fish appeared healthy but appeared to reject an artificial pellet diet that was given each day. Uneaten feed was removed from the tanks daily. Consequently, the condition factor of fish fell and with anticipated emaciation, fish were removed and killed before their welfare was likely to be compromised. The experiment was terminated on the 17th May 2007 as it was no longer possible to photograph fish on a regular basis due to available technical support. However, there were three fish remaining at this time and as they were in a healthy condition, they were maintained for until 27th August 2007, with only occasional examination to determine the longevity of *E.sieboldi*.

4.2.2.3 Longevity of the post overwintered *E.sieboldi*

The three fish maintained until August 2007 were from Group 3a (see Table 4.1, ambient temperature and dark conditions). A count was made of all the *E.sieboldi* present in the photographed area of the 8 outer surfaces of gill hemibranchs. Similarly, a count was made of parasites visible on each of the sample dates from 2nd April 2007 onwards for each of these 3 fish using the images previously captured. These fish were killed by overdose of anaesthetic followed by cervical dislocation. Gills were then removed and examined for remaining parasites.

4.2.3 Egg development and hatching – *in vitro* experiments

In Rutland Water, at the peak of the reproductive season, the water temperature often exceeds 18ºC. However, due to welfare concerns it was not possible to perform the tank trials at this temperature. Therefore, hatching of parasite eggs was carried out *in vitro,* to give an estimate of the viability of eggs under different conditions, and

development rates at different temperatures, and also to compare the findings of the *in vivo* tank experiments with *in vitro* data from this study and from existing literature.

In the following descriptions of experiments, parasites and eggs from different sources were used at different time points during the study. For ease of reference, Table 4.2 is a summary of the material used for the *in vitro* experiments.

Table 4.2 Summary information on 4 *in vitro* experiments to investigate viability of eggs and development rate of egg sacs

Exp. No.	Test type	Test subject	Quantity	Source	Date of experiment
1	Viability	Eggs	(A_1) 80 adults with 160 egg sacs attached, (B_1) 160 detached egg sacs and (C_1) eggs removed from 16 egg sacs	Newly attached F1 parasites from 22 rainbow trout (sample 1)	July 2006
2	Viability	Eggs	(A_2) 80 adults with 160 egg sacs attached, (B_2) 160 detached egg sacs and (C_2) eggs removed from 16 egg sacs	Overwintered parasites from 1 rainbow trout (sample 2	May 2007
3	Development Rate	Egg sacs	96 detached egg sacs for each group. Groups incubated at 8,10,12,15,18 and 20 ºC	Newly attached F1 parasites from 19 rainbow trout (sample 3)	July 2006
4	Development Rate	Egg sacs	96 detached egg sacs for each group. Groups incubated at 8,10,12,15,18 and 20 ºC	Overwintered parasites from 1 rainbow trout (sample 4)	May 2007

4.2.3.1 Experiment 1 and 2. Viability of eggs in egg sacs attached to adults, in egg sacs detached from adult and eggs removed from egg sacs

During the course of this study, it was noted that egg sacs could be easily detached from adult females. This was especially true in the more advanced stages of egg sac

development when the membranous sac appeared to become thinner and was easily broken with examination needles. Experiment 1 was to test any difference in viability of eggs hatching either within egg sacs attached to adult parasites, in egg sacs detached from adults using fine examination needles, or eggs removed from egg sacs entirely.

In July 2006, 168 newly attached ovigerous adult females were removed from the gills of 22 recently stocked angler caught rainbow trout (sample 1) immediately after the fish were killed. The parasites were initially pooled in chilled (<10 ºC) reservoir water. The parasites were then sequentially allocated to groups A_1 (80 parasites), B_1 (80 parasites and C_1 (8 parasites).

Group A_1 80 Adult female *E.sieboldi* with paired egg sacs attached placed in 10 replicates of 8 parasites (16 egg sacs) per Petri dish. Each 50mm Petri dish contained 15mls of 0.2 µm filtered tank water.

Group B_1 160 detached egg sacs removed from 80 parasites placed in 10 replicates of 16 egg sacs per Petri dish. Each 50mm Petri dish contained 15mls of 0.2 µm filtered tank water.

Group C_1 eggs removed from 16 white egg sacs placed in 15mls of 0.2 µm filtered tank water held in a single 50mm Petri dish.

Petri dishes for all groups were placed in a Binder environmental chamber maintained at 15ºC and under dark conditions.

Each day hatched nauplii and non-viable eggs (dark eggs with no indication of naupliar development within) were removed from each Petri dish and counted using a finely drawn out glass pipette. There was a 50% water change per day.

Experiment 2 was a replicate of Experiment 1 but was carried out in May 2007 using overwintered ovigerous female parasites from a single angler caught overwintered rainbow trout (sample 2). The three groups of parasites/eggs were designated A_2, B_2, and C_2. The numbers of parasites/egg sacs used and the incubation conditions were the same as for Experiment 1.

4.2.3.2 Experiment 3 and 4. Development rate of egg sacs

Experiments 3 and 4 examined the hatching rate of detached egg sacs at different temperatures. Parasites used in Experiment 3 consisted of 288 ovigerous ergasilids with paired white egg sacs which were removed from 19 rainbow trout (sample 3) immediately after the fish were killed in July 2006. The parasites were initially pooled in chilled (<10 ºC) reservoir water.

Forty eight pairs of egg sacs were removed from adult female *E.sieboldi* using examination needles. A single egg sac was placed in each well of a 96 micro-well plate, each well containing 350µl of 0.2 µm filtered tank water. Individual micro-well plates, each containing 96 egg sacs, were then placed in an incubator in the dark at 8 ºC. This was repeated at temperatures of 10 ºC, 12 ºC, 15 ºC, 18 ºC and 20 ºC. These temperatures were chosen as being representative of the water temperatures in Rutland Water when ovigerous *E.sieboldi* have been recorded. A lid was placed over each well plate to avoid loss of water by evaporation.

The plates were removed twice per day at 12 hour intervals and placed on an insulated block maintained at the same temperature to avoid a rise or drop in temperature. The development stage of each egg sac was quickly determined under an Olympus SZ40 dissecting microscope at ×4 magnification and then the plate was returned to the dark conditions of the incubator. Egg sacs were recorded as white, advanced or hatched.

The egg sacs used came from *E.sieboldi* on newly infected rainbow trout. The parasites had not overwintered as the fish were identified as recently stocked fish and there was a low mean intensity of *E.sieboldi* present. Experiment 4 was a repeat of Experiment 3 using overwintered parasites from a single overwintered fish (sample 4) in May 2007.

4.2.4 Analysis of laboratory, tank and field data

In 2003 and 2004 temperature readings were taken monthly with a thermometer. From July 2004 to November 2005, a suspended sensor was left to record temperature at 3 hour intervals (see Chapter 2). This generated much more informative data than monthly sampling. For this reason, most of the analyses of laboratory experiments are compared with field data obtained in 2005.

Analysis of development times and temperatures were performed using regression analysis techniques from Minitab statistical software version 13.32 or Sigma Plot for Windows version 10.

When detaching egg sacs from *E.sieboldi* it was not possible to know when the egg sac had been extruded by the parasite. If this information had been known it would have

been possible to use conventional development analysis techniques to model the rate of egg development against temperature. It would also be possible to determine the theoretical low temperature threshold for development, below which, no egg development occurs. In this case, as extrusion time was not known, the maximum time for egg development at each temperature was taken to be the development time at that temperature (Alston 1994). However if extrusion time had been known and all the egg sacs used were extruded at approximately the same time point, development time would be calculated as the point at which 50% of the eggs had hatched and would therefore account for any variations in development rate between individual test specimens (Campbell *et al.* 1974). For the purposes of this study, the theoretical low temperature threshold for development was calculated using data based on the longest time to hatch and when 50% of egg sacs had hatched. The true figure for the theoretical low temperature threshold for development is likely to lie between the 2 figures. Calculation of theoretical low temperature threshold for development was performed using regression techniques where the reciprocal of development time was subjected to linear regression against incubation temperature. The point of intercept with the x axis (where y=0) is thus indicated as the theoretical low temperature threshold for development (Campbell, Frazer, Gilbert, Gutierrez, & Mackauer 1974).

4.2.5 Ovarian development

4.2.5.1 Ovarian development during the inter-clutch interval

The size and shape of ovaries were recorded following the extrusion of an egg clutch. Egg sacs were detached from parasites and hatched under *in vitro* conditions. The adults were fixed and the ovary examined. The duration of time taken for the egg sacs

to hatch was used as an indicator of when each of the egg sacs were likely to have been extruded.

Three rainbow trout were caught by angling in November 2005. They were transported to Stirling University and held live in aquarium tanks (see Chapter 2 for husbandry details). In April 2006 when *E.sieboldi* oviposition had begun, the fish were killed and the parasites were removed using examination needles. Ninety-six overwintered adult females with paired egg sacs were removed from these fish and a single egg sac was detached from each and placed in 350µl of 0.2 µm filtered tank water in each well of a micro-well plate. Care was taken to avoid damage to the egg sac of the adult parasite.

The micro-well plate was held in a dark incubator at 15ºC and egg sacs examined twice per day. Development of each egg sac was recorded over time until each had hatched. The adult female with the corresponding single attached egg sac was fixed in 5% phosphate buffered formalin later used to assess the status of ovary development using image analysis techniques. The size and shape of the ovary was then compared with the time to hatch of the detached and incubated egg sac.

The fixed parasites with a single egg sac attached were placed individually in cavity slides. A drop of water was added and a cover-slip was gently lowered on top to taking care not to cause distortion to its dimensions. Images of the parasites for analysis were captured using techniques described as for light micrographs (see Chapter 2) and saved to file in tiff format. Images of the individual parasites were then measured using image analysis software on the parameters shown in Figure 4.2 to 4.4.

Abbreviations for Figures 4.2 and 4.3

cw	*cephalosome width*
cl	*cephalosome length*
gcl	*genital complex length*
tl	*total length*
trl	*trunk length*
ag	*anterior gap*
cg	*centre gap*
dw	*diverticulum width*
dl	*diverticulum length*
ol	*ovary length*
ow	*ovary width*

Figure 4.2 Diagrammatic representation of adult female *E.sieboldi* showing linear parameters measured using image analysis software.

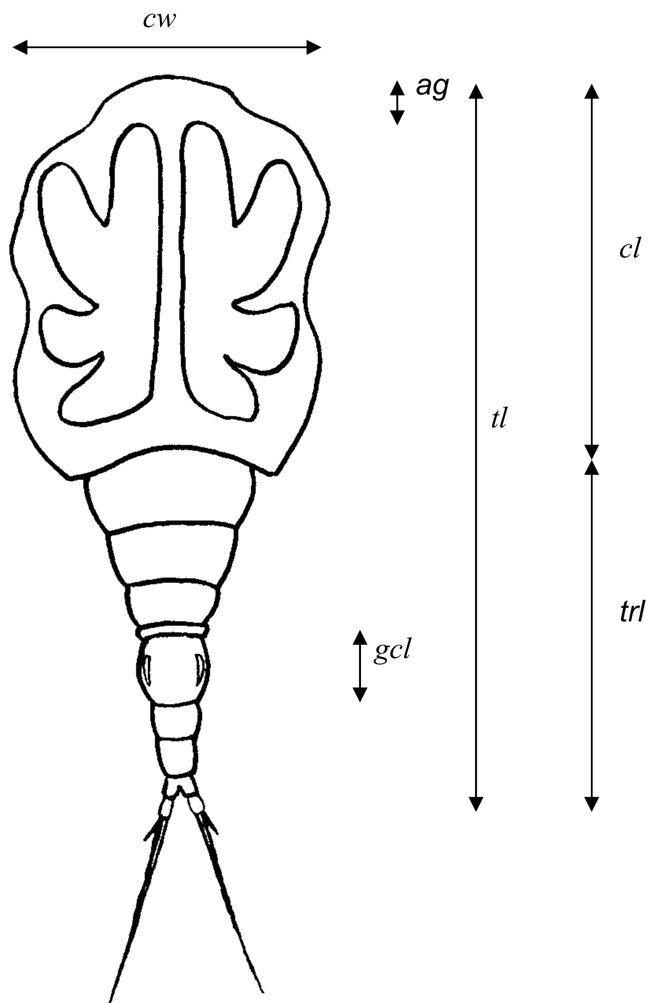


Figure 4.3 Diagrammatic representation of *E.sieboldi* ovary showing linear parameters measured using image analysis software.

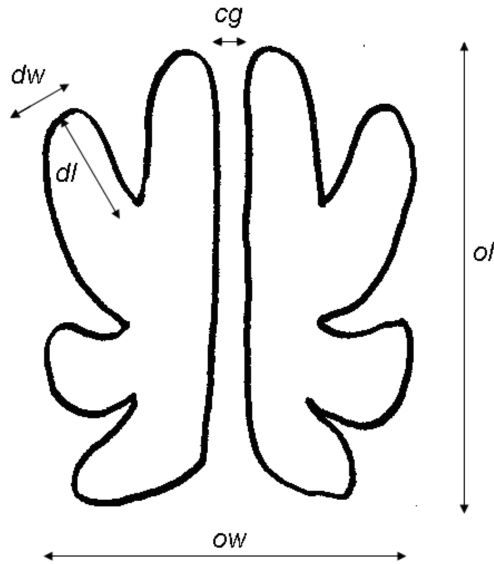
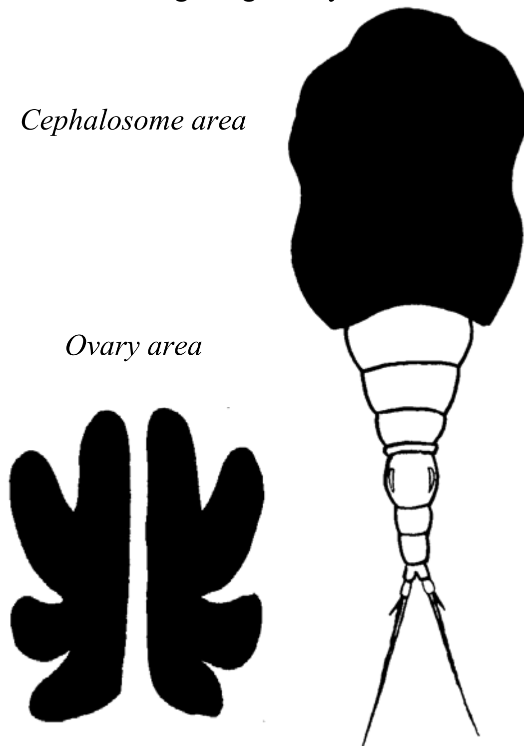


Figure 4.4 Diagrammatic representation of *E.sieboldi* showing area parameters measured using image analysis software.



An image analysis algorithm written by Dr James Bron, Institute of Aquaculture, was used to measure parameters described in Chapter 4. The algorithm was designed using KS 300 version 3.0 (Carl Zeiss Vision GmbH), and run using KSRun 3.0 (Carl Zeiss Vision GmbH). The steps used in processing the algorithm are described below.

1) A database was created to contain the measured parameters and details of the specimen.
2) Measurements were calibrated against a calibration file tiff image of a 1mm slide graticule.
3) An image of the parasite was selected from file.
4) Details of the specimen and host were entered by the user to the database.
5) Interactive point to point measurements of suitable parameters were performed by the user.
6) Area of ovary was separated from the image using a hue saturation and luminance model (HSL).
7) Areas of less than 10000 pixels (non ovary) were removed from the image.
8) Gaps in the ovary image were filled.
9) The perimeter of ovary was smoothed.
10) The user was given the option to manually manipulate the area to be measured to conform to actual observations.
11) Steps 6-10 were repeated for the cephalosome area.
12) All measurements were written to the database and a second image was then processed.

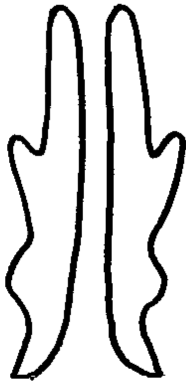
4.2.5.2 Ovarian development during the winter

Nine rainbow trout were caught by angling in November 2005. Three were killed immediately and the adult female *E.sieboldi* were removed using examination needles and fixed in 5% phosphate buffered formalin. The remaining six fish were transported to Stirling University and held in aquarium tanks under ambient light and temperature conditions. Three of these transported fish were killed in January 2006 and their parasites removed and fixed in either 5% phosphate buffered formalin as above or in 2.5 % glutaraldehyde for examination by SEM. Details of SEM preparation and imaging are contained in Chapter 2 General materials and methods.

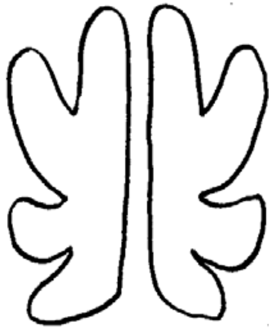
The remaining three fish from the aquarium tanks were killed in March 2006 before oviposition had begun. The parasites from these fish were removed as before and fixed in 5% phosphate buffered formalin.

One hundred parasites from each of the nine fish were examined under an Olympus SZ40 microscope at ×4 magnification using transmitted and incident light sources. The ovarian development of each parasite was classified as stage I,II, III or non-classified. Stages are described in Figure 4.5 below. Non-classified was due to unusual ovary form that did not comply with any of the other classifications. These types formed only a small proportion of the parasites in any given sample.

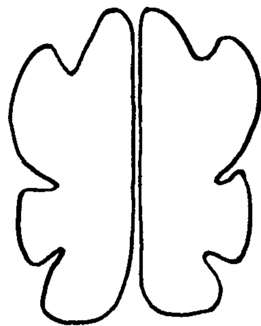
Figure 4.5 Classifications of *E.sieboldi* ovary types.



Type I Small thin paired ovary. Obvious space between paired ovaries for entire length. Lateral diverticula absent or under developed.



Type II Larger developed ovary. Space between ovaries for at least 50% of length. 8 obvious diverticula with gaps between.



Type III Large developed ovary. Space between paired ovaries small or obscured. Enlarged diverticula, gaps between them small. Usually non ovigerous or ovigerous adult parasite with advanced egg sacs.

Proportions of each type were calculated for each fish in each sample.

4.3 Results

4.3.1 Field examination

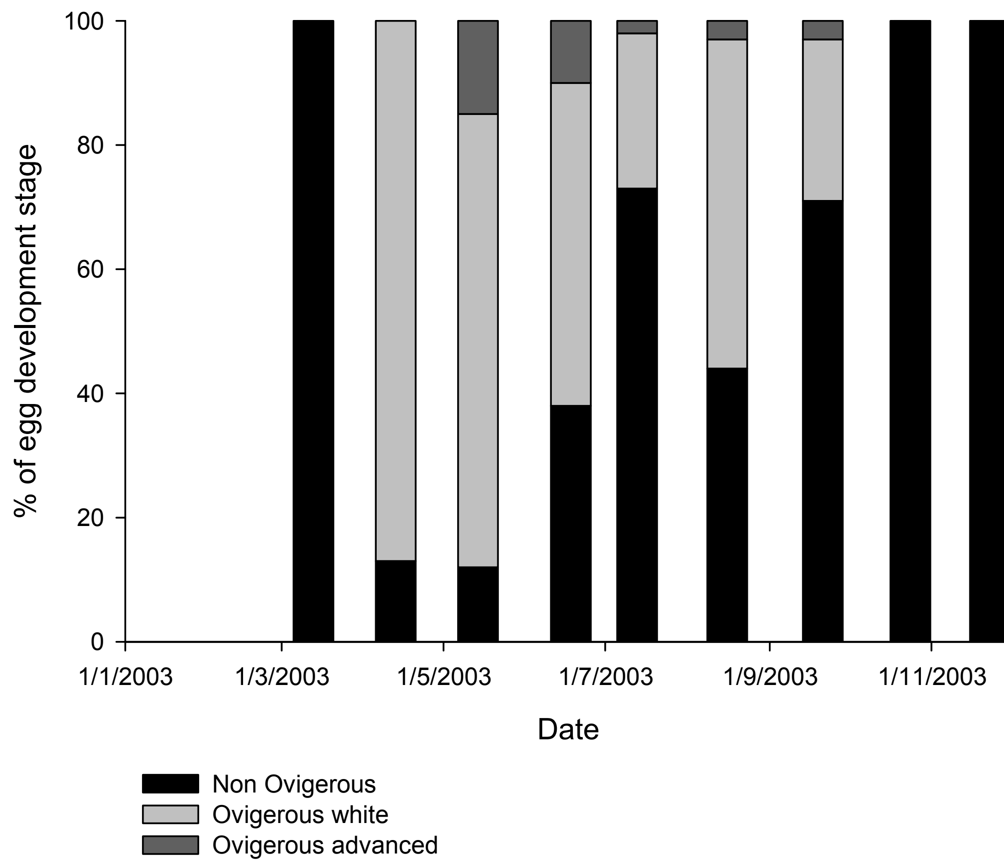
Table 4.3 shows the egg development stages of ovigerous *E.sieboldi* found on angler caught rainbow trout in 2003. Figure 4.6 graphically represents the proportions of non-ovigerous parasites, white and advanced eggs found on these *E.sieboldi*. In total, more than 250,000 parasites were examined in 2003.

Table 4.3 Summary of the *E.sieboldi* egg development stages of the parasites removed from samples of angler-caught rainbow trout in 2003.

Rainbow trout - All fish	Fish examined	Total *E.sieboldi*	Non-ovigerous (%)	Ovigerous white (%)	Ovigerous advanced (%)
13/03/03	2	1916	100	0	0
13/04/03	30	27700	13	87	0
14/05/03	27	29340	12	73	15
18/06/03	11	3971	38	52	10
13/07/03	28	10500	73	25	2
16/08/03	32	16416	44	53	3
21/09/03	29	35467	71	26	3
24/10/03	26	69888	100	0	0
23/11/03	20	48220	100	0	0
27/12/03	4	10120	100	0	0

The overwintered adult females emerged from the winter in a non-ovigerous state, as shown by the March sample comprising entirely non-ovigerous parasites. Oviposition was first seen in the April sample with 87% of the parasites having extruded white opaque egg sacs. The lack of any advanced stage eggs at this time suggests that hatching had yet to take place.

Figure 4.6 The percentage of each egg development stage determined from the parasites removed from samples of anger caught rainbow trout in 2003.



Advanced stage eggs were first seen in the May sample when 15% of the overwintered parasites had egg sacs of this development stage. No sample taken in 2003 contained more than 15% of the egg sacs in an advanced state, suggesting that the advance stage was present for only a short time before hatching. The first nauplii in 2003 hatched from eggs between 13th April and 14th May. These then developed through free living stages before infecting fish from June onwards as the F1 generation.

Tables 4.4 to 4.6 and Figures 4.8 to 4.10 show the proportions of parasites recorded at each of the different developmental stages and are separated into those from overwintered, early stock and stock fish rainbow trout (as described in Chapter 3). It can be seen from these figures that parasites from the overwintered fish dominated the monthly samples until July (denoted as the P generation). Although new infections occurred in June (F1 generation), most of the parasites examined in this month were those that had overwintered due to the higher mean intensity of infection of overwintered fish. New infections occurred until October, and as a result, the proportion of F1+ parasites compared with overwintering P parasites increased as the season progressed. Figure 4.7 shows the proportion of parasites that were examined at each sample point as being recorded from overwintered, early stock or stock rainbow trout. Bias is inevitable when sampling angler caught fish, and small numbers of overwintered fish can dramatically change the cohort of parasites being recorded due to high mean intensities of parasites from the previous year. It should be noted however that during the months when overwintered parasites dominated the samples (April – June), few or no new infections had occurred (see Chapter 3).

Figure 4.7 Total *E.sieboldi* examined in 2003 separated into the host classifications of overwintered, early stock or stock rainbow trout. Three separate rows (different shades) denote the host classification and the height of each bar represents the number of parasites examined in each sample.

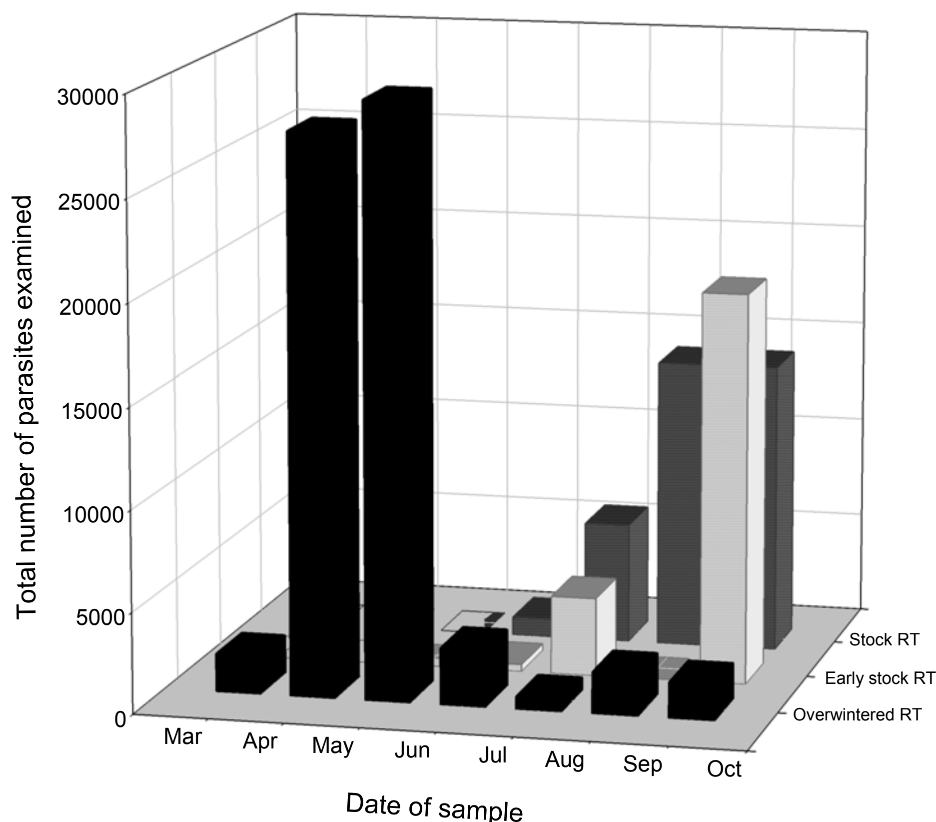
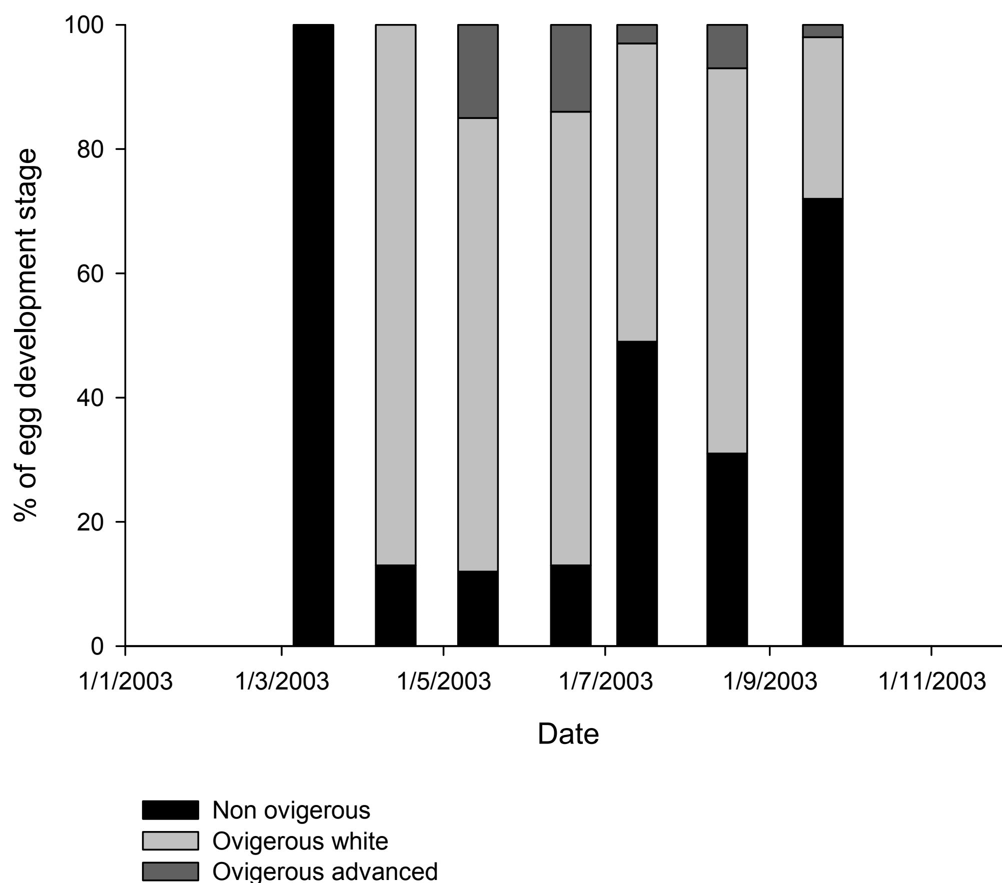


Table 4.4 Summary of the egg development stages recorded on the *E.sieboldi* infecting overwintered rainbow trout sampled in 2003.

Rainbow trout - overwintered	Fish examined	Total *E.sieboldi*	Non-ovigerous (%)	Ovigerous white (%)	Ovigerous advanced (%)
13/03/03	2	1916	100	0	0
13/04/03	10	27700	13	87	0
14/05/03	12	29340	12	73	15
18/06/03	1	2834	13	73	14
13/07/03	1	646	49	48	3
16/08/03	1	2074	31	62	7
21/09/03	2	1788	72	26	2

Figure 4.8 The percentage of each *E.sieboldi* egg development stage determined from the parasites removed from samples of overwintered rainbow trout caught by anglers in 2003.



As new infections occurred from June to October, samples of *E.sieboldi* coming from overwintered fish were made up of an increasingly higher proportion of infections of the new F1+ generation/s. This is seen from the higher proportion of non-ovigerous parasites from July onwards and is due to increasing numbers of new infections and the potential loss of some of the overwintering P generation. As the inter-clutch interval of egg producing females was unknown, this may also have a bearing on the number of non-ovigerous parasites present in each sample and is discussed later in the chapter.

Table 4.5 Summary of the egg development stages recorded on the *E.sieboldi* infecting early stock rainbow trout sampled in 2003.

Rainbow trout - early stock fish	Fish Examined	Total *E.sieboldi*	Non-ovigerous (%)	Ovigerous white (%)	Ovigerous advanced (%)
13/03/03	0	0	-	-	-
13/04/03	0	0	-	-	-
14/05/03	2	0	-	-	-
18/06/03	2	350	100	0	0
13/07/03	6	3888	66	32	2
16/08/03	0	0	-	-	-
21/09/03	13	19344	74	23	3

Figure 4.9 The percentage of each *E.sieboldi* egg development stage determined from the parasites removed from samples of early stock rainbow trout caught by anglers in 2003.

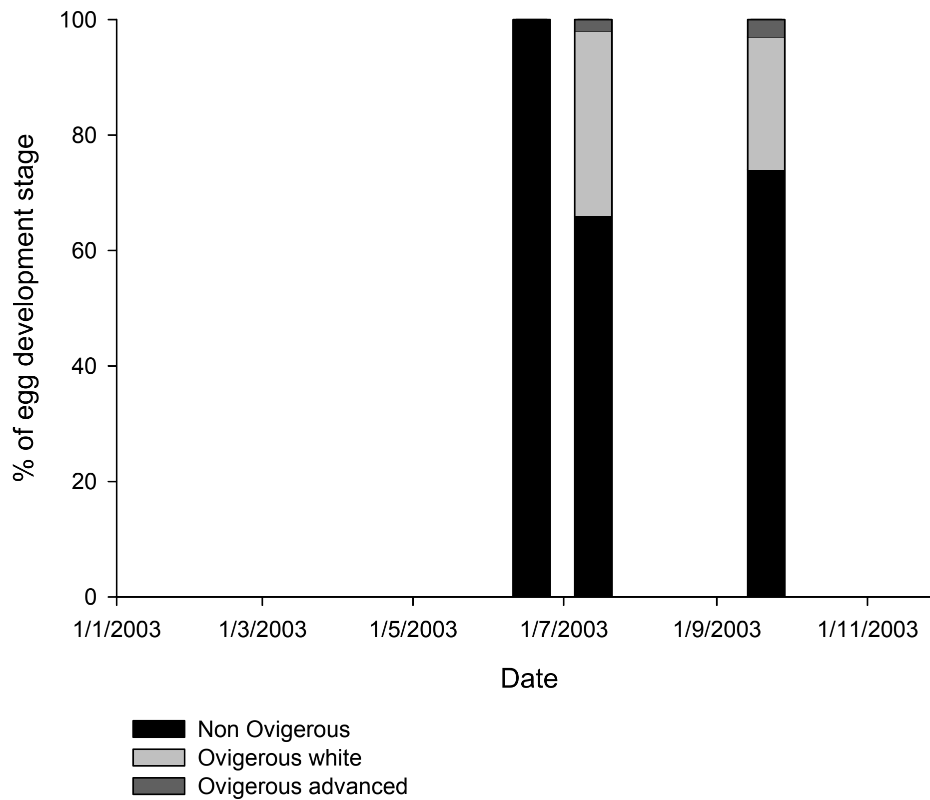
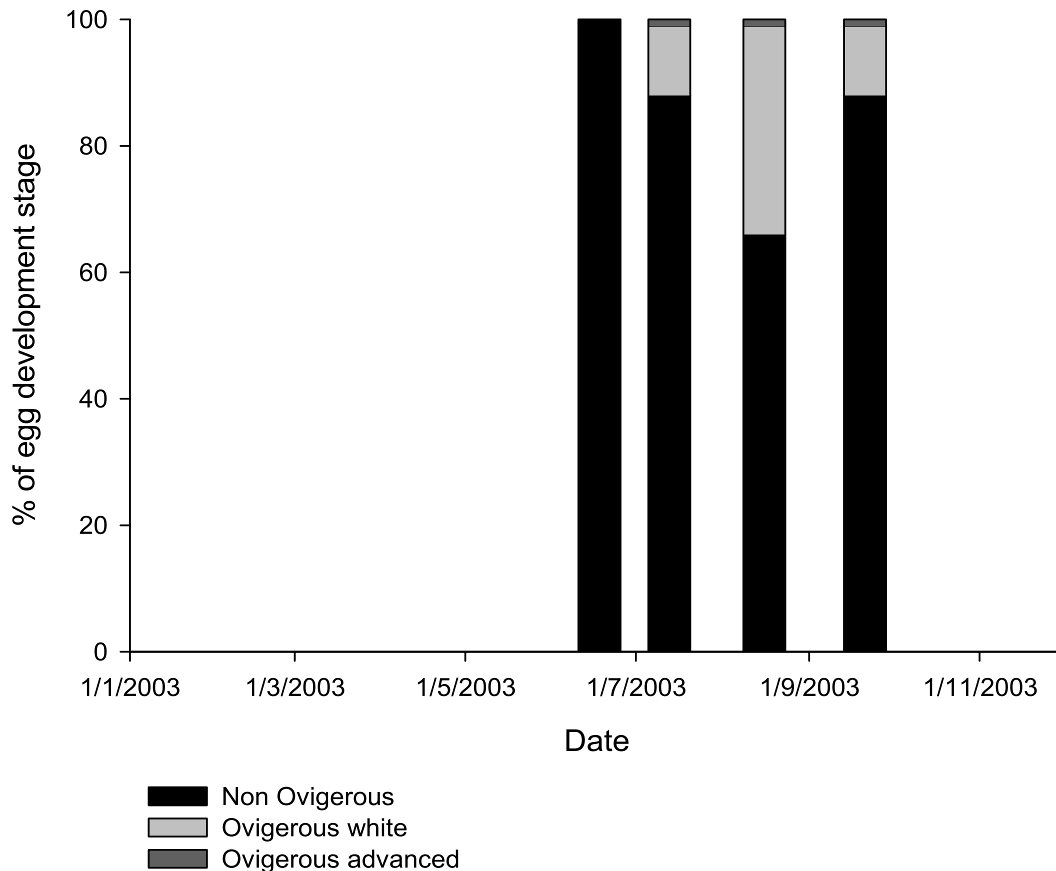


Table 4.6 Summary of the egg development stages recorded on the *E.sieboldi* infecting stock rainbow trout sampled in 2003.

Rainbow trout - stock fish	Fish Examined	Total *E.sieboldi*	Non-ovigerous (%)	Ovigerous white (%)	Ovigerous advanced (%)
13/03/03	0	0	-	-	-
13/04/03	20	0	-	-	-
14/05/03	13	0	-	-	-
18/06/03	8	912	100	0	0
13/07/03	21	5964	88	11	1
16/08/03	31	14322	66	33	1
21/09/03	14	14322	88	11	1

Figure 4.10 The percentage of each *E.sieboldi* egg development stage determined from the parasites removed from samples of stock rainbow trout caught by anglers throughout 2003.



E.sieboldi found on early stock and stock fish in June were entirely non-ovigerous. These were new infections that had not had time to develop egg sacs. One month later in July, there was a difference in the development stage composition of parasites on early stock and recently stocked fish. Approximately 34% of the parasites from early stock fish had egg sacs compared to 12% of the parasites on the recently stocked fish. This supports the assessment of fish as early stock or stock made by the wardens of Rutland Water (see Chapter 3).

Angler caught rainbow trout in 2004 and 2005 were examined for the presence of egg sacs and their development stage was determined in the same way as in 2003. Figures 4.11 and 4.12 graphically represent the proportion of parasites/eggs recorded at the different development stages.

Figure 4.11 The percentage of each *E.sieboldi* egg development stage determined from the parasites removed from the sample of rainbow trout caught by anglers in 2004.

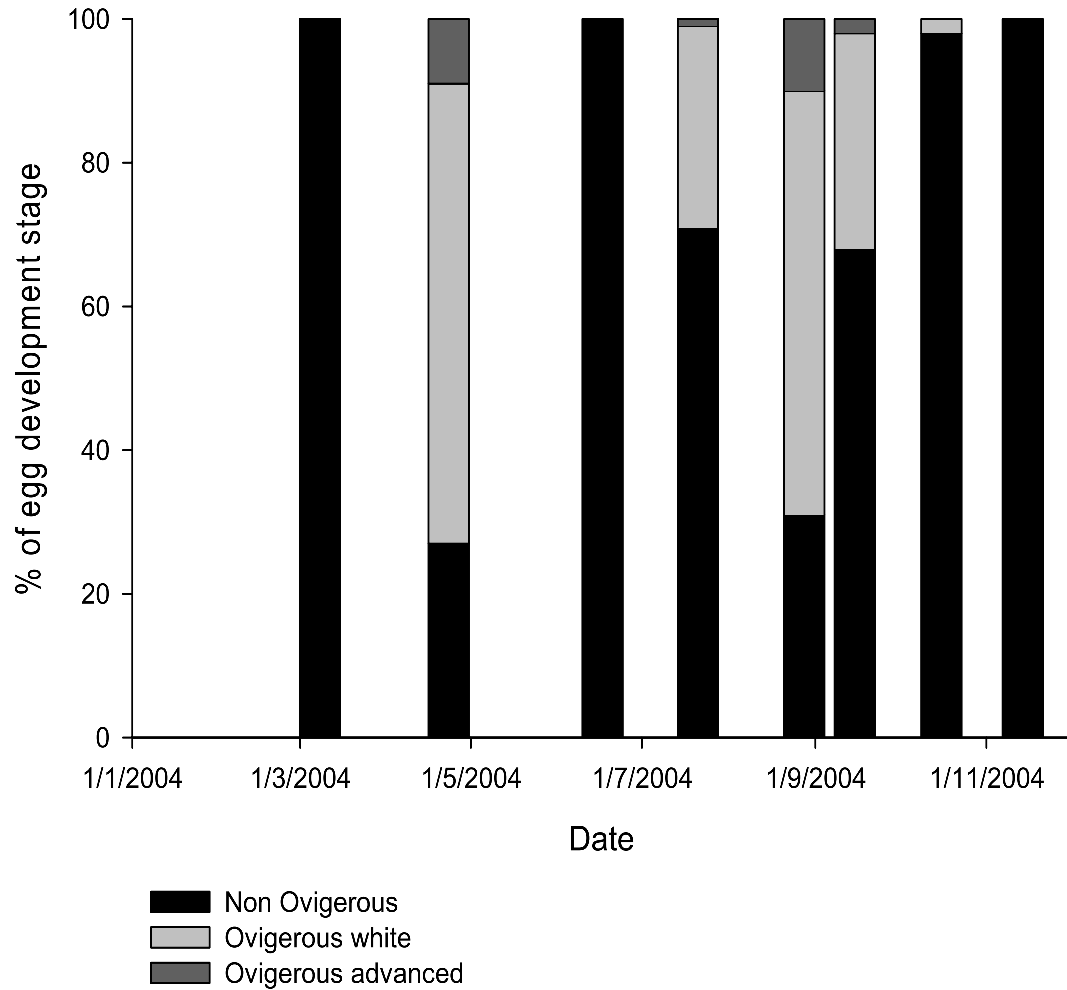
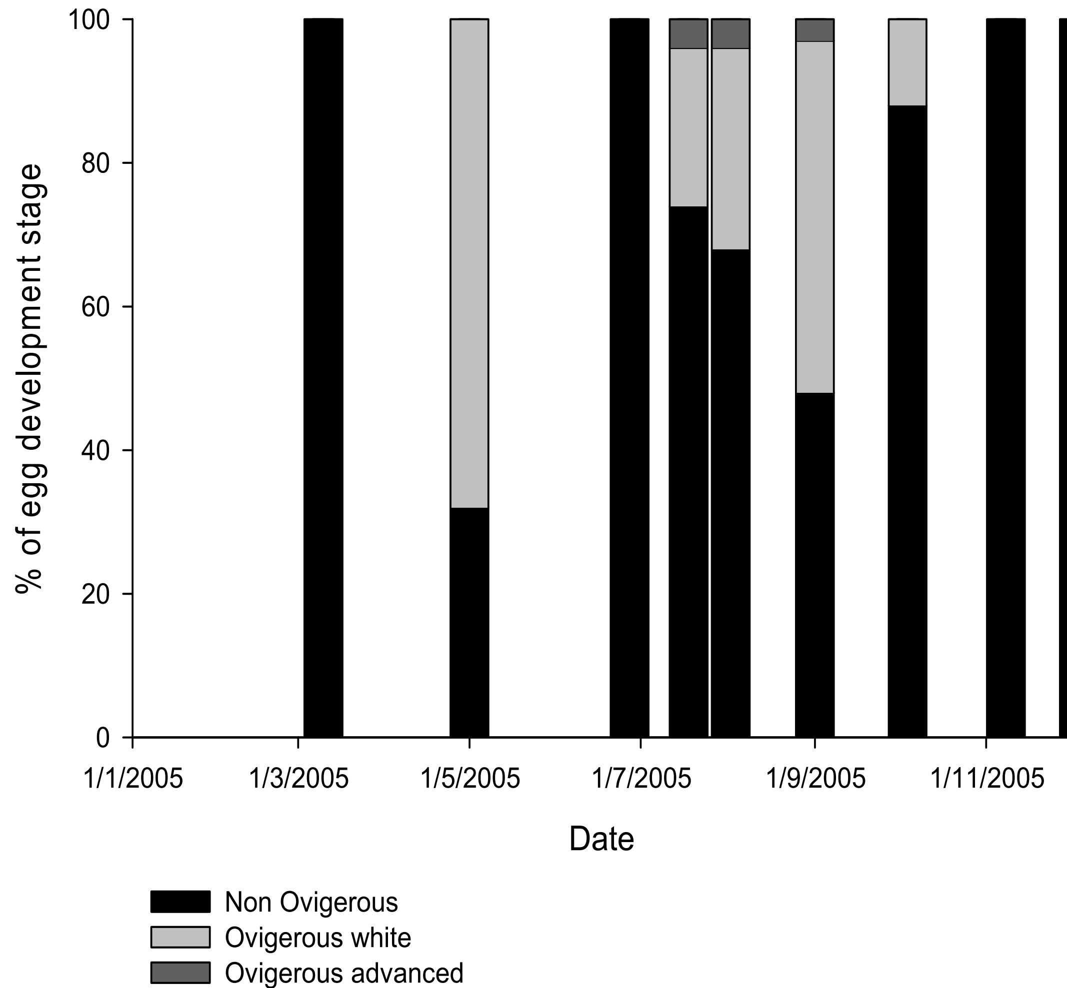
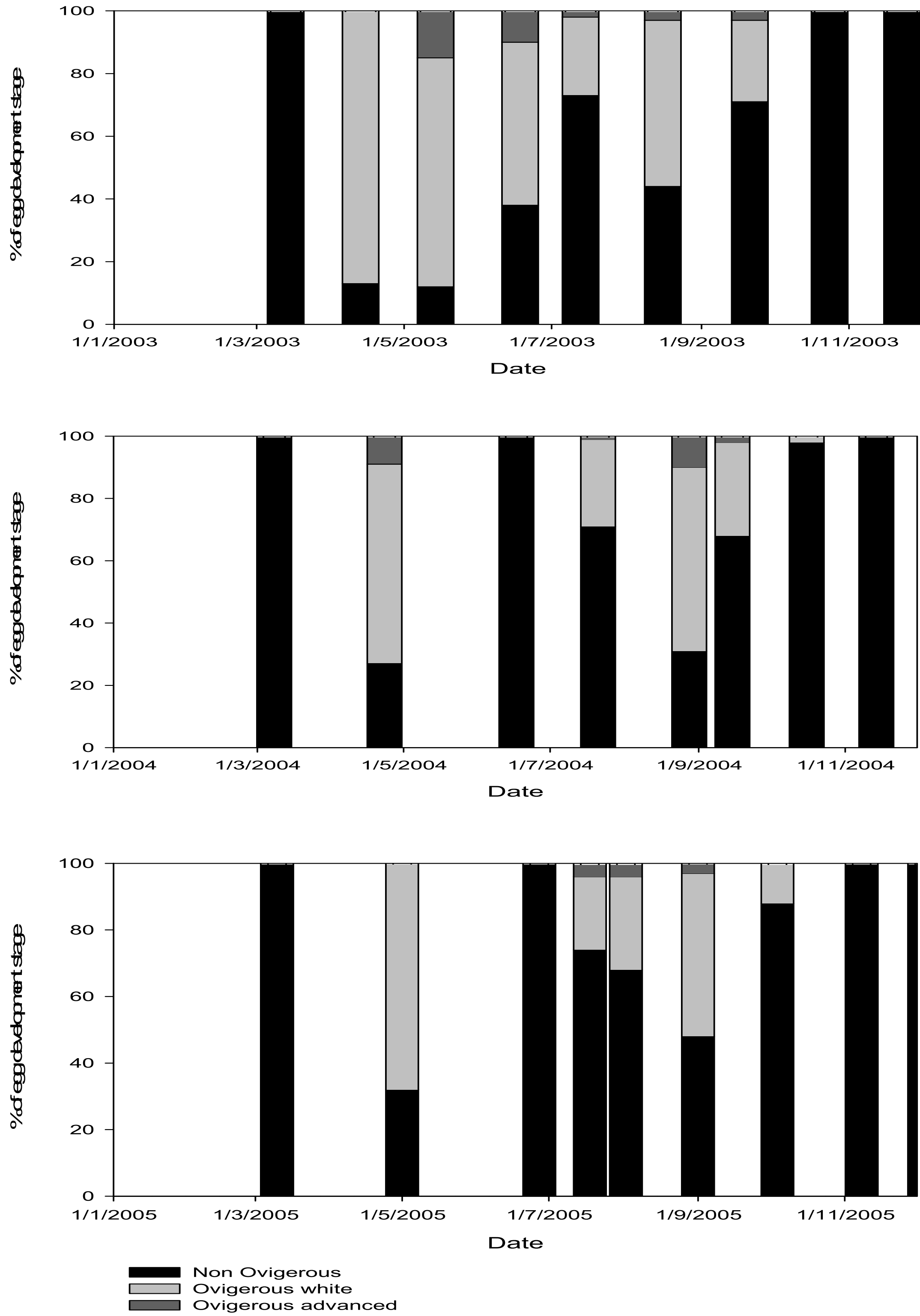


Figure 4.12 The percentage of each *E.sieboldi* egg development stage determined from the parasites removed from the sample of rainbow trout caught by anglers in 2005.



The findings on the parasite development stages for 2003 to 2005 are summarized in Figure 4.13 which compares the proportions of the different stages on all the angler caught rainbow trout that were sampled.

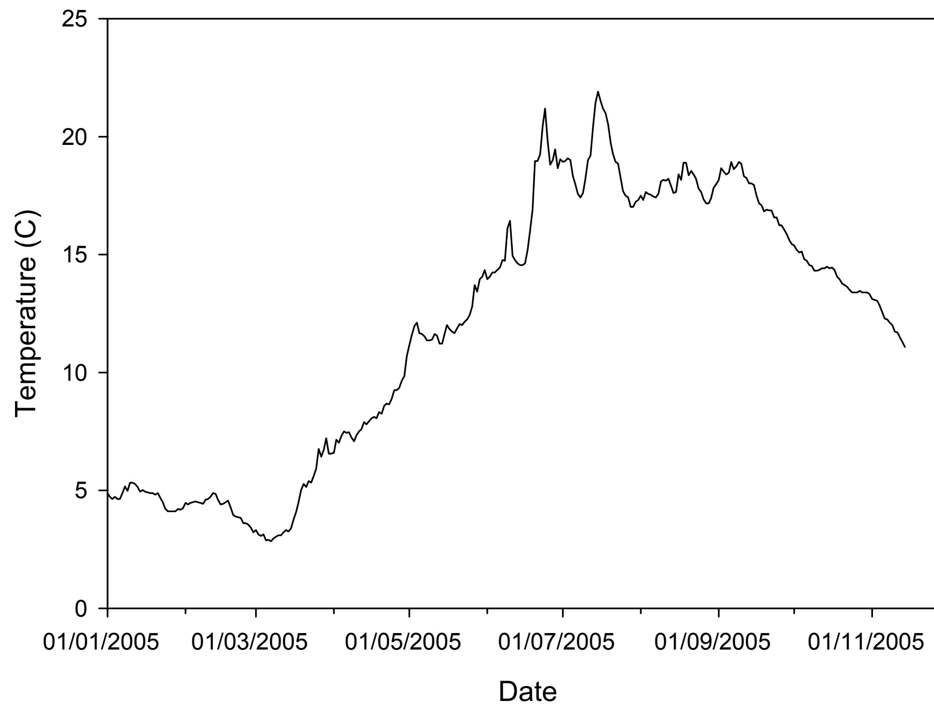
Figure 4.13 The percentage of each *E.sieboldi* egg development stage determined from the parasites removed from the sample of rainbow trout caught by anglers in 2003, 2004 and 2005.



The pattern of development was very similar in all study years. Parasites were non-ovigerous during the winter, and begin to extrude white opaque eggs in April. In 2004, the presence of advanced eggs at the end of April indicated that hatching of eggs may have begun slightly earlier in 2004 than in 2003 or 2005. The proportion of non-ovigerous parasites increased in June as the F1 generation infected the fish and were initially non-ovigerous. The June sample of fish in 2003 contained overwintered fish but the June samples of 2004 and 2005 did not. This accounts for the difference seen in the proportions of development stages recorded for the June sample in the three different years. In July and August, increasingly higher numbers of new infections occurred as described in Chapter 3. However, during this period the proportion of non-ovigerous parasites decreased, possibly indicating that the parasites were producing eggs more quickly after infecting the fish. Between September and October the proportion of non-ovigerous parasites again increased, suggesting that the production of egg sacs was slowing or that the parasites infecting fish at this time were taking longer to start egg production. By mid-October, no ovigerous parasites remained indicating the end of the reproductive season in each of the three years.

From July 2004 to November 2005, a temperature sensor was suspended 1.5m below the surface of the experimental cages as described in Chapter 2 with the temperature being recorded every 3 hours. Figure 4.14 shows the daily water temperature profile recorded in 2005.

Figure 4.14 Water temperature recorded in Rutland Water in 2005 using a sensor suspended 1.5m below the surface of the experimental cages.



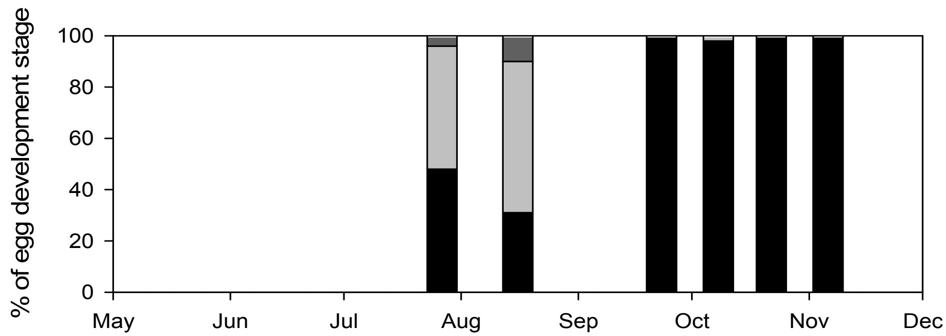
The fish stocked in cage 4 in 2004 and 2005 which were removed / replaced with uninfected fish every 1-2 weeks (as described in Chapter 3) were also examined and the developmental status of their parasites was recorded. Table 4.7 and Figure 4.15 show the reproductive stage of the parasites on the fish removed from this experimental cage. The temperature profile for 2004 and 2005 is plotted below these graphs (see Chapter 2 General material and methods for details on temperature measurement).

Table 4.7 Summary of the *E.sieboldi* egg development stages of the parasites removed from rainbow trout sampled in 2005 from the 2 week recruitment cage

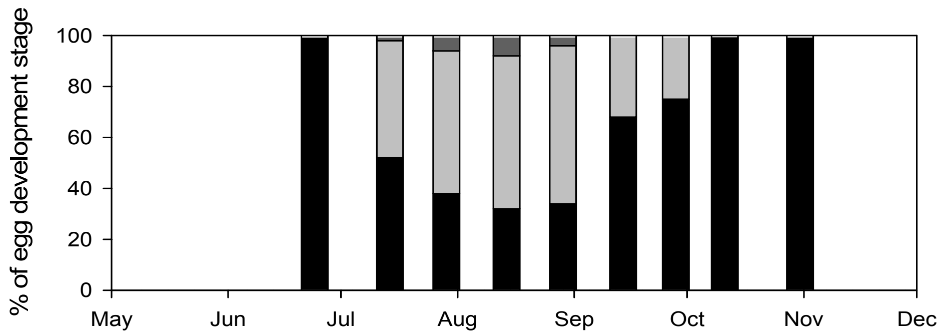
Rainbow trout - 2 week recruitment cage – 2004 and 2005						
Date fish removed	Exposure time (days in water)	Fish Examined	Non-ovigerous (%)	Ovigerous white (%)	Ovigerous advanced (%)	Mean water temperature during sample period (ºC)
27/07/04	14	25	48	48	4	17.2
16/08/04	14	25	31	59	10	18.7
02/09/04	14	30	-	-	-	18.7
23/09/04	14	30	100	0	0	16.4
08/10/04	14	30	98	2	0	14.5
22/10/04	14	30	100	0	0	12.7
06/11/04	14	30	100	0	0	11.0
24/11/04	14	15	-	-	-	8.7
------------	------------	------------	------------	------------	------------	------------
2/06/05	7	20	-	-	-	13.9
10/06/05	7	20	-	-	-	15.0
17/06/05	7	20	-	-	-	14.8
24/06/05	7	20	100	0	0	18.8
14/07/05	14	20	52	46	2	18.8
29/07/05	14	20	38	56	6	19.0
14/08/05	14	20	32	60	8	17.7
29/08/05	14	20	34	62	4	18.0
14/09/05	14	20	68	32	0	18.5
28/09/05	14	20	75	25	0	16.8
11/10/05	14	20	99	1	0	15.0
31/10/05	14	20	100	0	0	13.6
14/11/05	14	20	-	-	-	12.2

Figure 4.15 Percentage egg development stage of parasites sampled from rainbow trout in 2 week recruitment cage in 2004 and 2005 (fish stocked and killed every 2 weeks). 2004 and 2005 temperature profile plotted for reference.

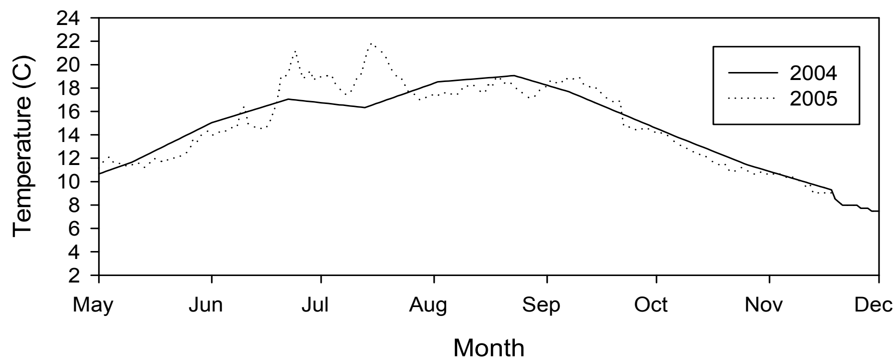
% Egg development stage of *E. sieboldi* from 2 week recruitment cage in 2004



% Egg development stage of *E. sieboldi* from 2 week recruitment cage in 2005



Non ovigerous
Ovigerous white
Ovigerous advanced



In both 2004 and 2005, the data shows that the parasite was able to infect a fish and develop eggs to the advanced stage within a 14 day period between July and the end of August. August was shown to be the time of the most rapid development rate indicated by a greater proportion of white and advanced egg sacs compared to non-ovigerous parasites. This period coincides with the highest water temperatures. However, the rate of development was lower from September when temperatures started to fall indicated by a greater proportion of non-ovigerous parasites and a lack of advanced stage eggs. As discussed in Chapter 3, in 2004 there were no new infections in the last 2 weeks of August. In 2005, there were a reduced number of new infections in the same period. Subsequent to this period, in both years, new infections occurred but egg development rate was less than it had been before this period. This was more pronounced in 2004 as the new infections were not able to extrude egg sacs within a 2 week period of infecting the fish, in 2005 there were ovigerous white parasites but the proportion of these was diminishing from September onwards. The water temperatures at these times were still high at more than 15 ºC but the water temperature was falling. This may suggest that falling temperatures or another environmental cue at this time caused a reduction in the rate of egg production. It may also suggest that the parasites infecting fish after the 2nd week of August were increasingly composed of a later cohort that had a reduced egg production rate than the earlier cohort.

In 2005, egg sacs were first observed on overwintered parasites when the temperature was approximately 9 ºC (angler caught fish 1st May; please see Figure 4.12 and 4.14), but as there was no infected fish sample available in April, the first post winter oviposition could have been earlier. The last egg sacs observed on parasites from angler caught fish were seen in a sample taken on 4th October and from parasites on cage held

fish killed on 11th October when the temperature was approximately 15 ºC. The last new infections to be observed in 2005 were on cage fish (see Table 4.7) in the 14 days prior to 31st October when the temperature was approximately 13.6 ºC but these did not extrude eggs within the 2 weeks before they were killed.

It is interesting to note that the percentage of parasites with egg sacs is higher in the cage held trout in 2005 during the peak infection period than in the recently stocked rainbow trout caught by anglers at the same time in 2003. It is possible that many of the stock fish were in the water for less than 2 weeks before being caught and this would account for more non ovigerous parasites as they had not had time to develop eggs.

4.3.2 Egg production following overwintering- *in vivo experiments*

Individual fish were killed and removed during the experiment when it was believed that to continue to maintain them under experimental conditions would infringe upon their welfare. Gills from these fish were removed and parasites examined as previously described. It was found that approximately 40-50% of the parasites that were present on each fish were visible on the photographs taken during the course of the experiment.

4.3.2.1 Commencement of oviposition following overwintering

An inspection of the gills of the fish upon arrival confirmed that all fish were infected with *E.sieboldi*. All parasites were in a non-ovigerous overwintering state.

Table 4.8 shows the different groups of fish allocated to different holding conditions with the date and number of degree days (threshold 0ºC) that had passed since 1st January until at least 10% of the visible *E.sieboldi* were ovigerous, thus indicating the beginning of the reproductive season.

Table 4.8 Tank held fish groups, holding conditions and the date and degree days accrued when egg sac production began on each fish.

Group	Date moved from ambient	Temp. regime moved to	Light regime moved to	Date when >10% of parasites are ovigerous	Degree days from 1st January until >10% are ovigerous
1	n/a	Ambient	14 light/10 dark	5/04/07	470
1	n/a	Ambient	14 light/10 dark	5/04/07	470
1	n/a	Ambient	14 light/10 dark	5/04/07	470
2	n/a	Ambient	Constant light	5/04/07	470
2	n/a	Ambient	Constant light	5/04/07	470
2	n/a	Ambient	Constant light	5/04/07	470
3a	n/a	Ambient	Dark	5/04/07	470
3a	n/a	Ambient	Dark	4/04/07	462
3a	n/a	Ambient	Dark	5/04/07	470
3b	n/a	Ambient	Dark	5/04/07	470
3b	n/a	Ambient	Dark	5/04/07	470
3b	n/a	Ambient	Dark	4/04/07	462
4	10/01/07	10ºC	Dark	12/02/07	382
4	10/01/07	10ºC	Dark	12/02/07	382
4	10/01/07	10ºC	Dark	12/02/07	382
5	7/02/07	10ºC	Dark	1/03/07	398
5	7/02/07	10ºC	Dark	1/03/07	398
5	7/02/07	10ºC	Dark	1/03/07	398
6	23/02/07	15ºC	Dark	1/03/07	338
7	26/02/07	15ºC	Dark	3/03/07	337
8	21/03/07	15ºC	Dark	26/03/07	465

The data from the tank experiment showed that it was possible to induce the adult female *E.sieboldi* to begin egg production and oviposition during the winter by raising the water temperature.

Different light regimes had no effect on the date of first egg sac production as parasites on rainbow trout in groups 1, 2, 3a and 3b, held at ambient temperature but under different light conditions all began egg production on the 4th or 5th April 2007. Groups 4-8 at raised water temperatures began egg production earlier. The ambient water

temperature when egg sac production began on non-heated tanks was 7 ºC. In some fish, 1 or 2 parasites extruded egg sacs sooner than the bulk of parasites on the gill, but within 3-4 days, the majority of parasites had egg sacs.

Figures 4.16 and 4.17 show the ambient water temperature of non heated tanks during the experiment and also the degree days accumulated over time using 1st January as a start date and 0 ºC as a base threshold.

Figure 4.16 Graph of water temperature (ºC) supplied to ambient tanks.

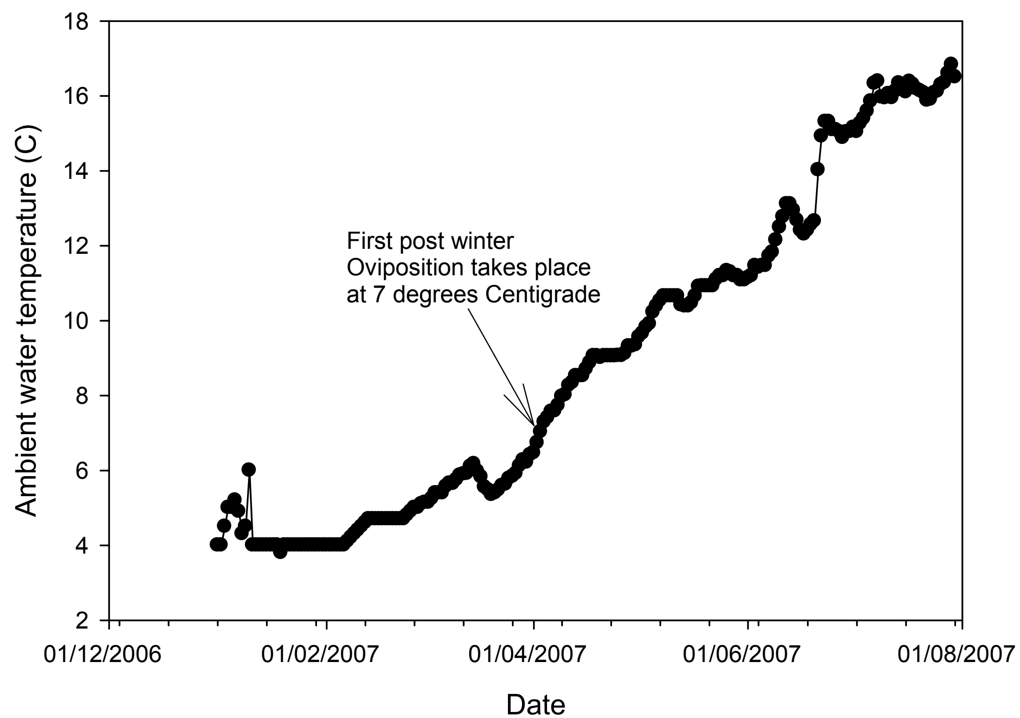
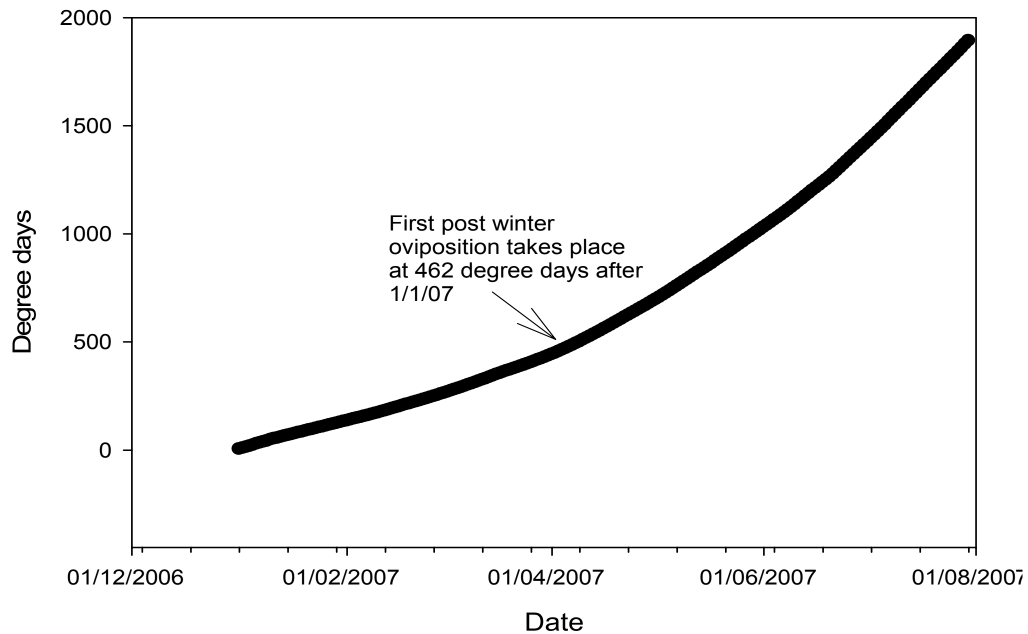


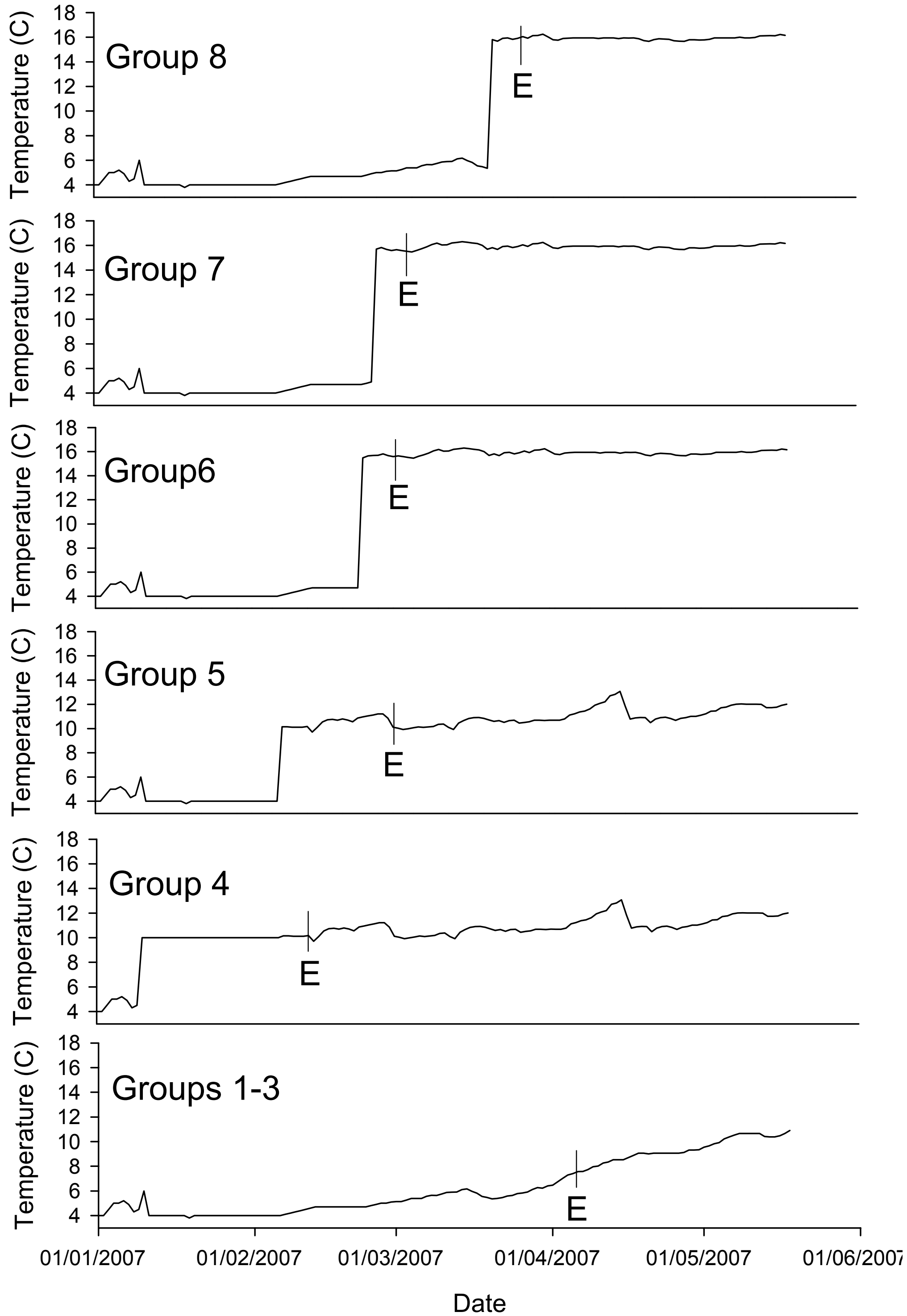
Figure 4.17 Graph of degree days (post 1/1/07 0 ºC threshold) supplied to ambient tanks.



The majority of *E.sieboldi* infecting fish in Groups 1,2,3a and 3b held in ambient conditions were synchronized in that production of egg sacs began at approximately the same time on the 4th or 5th April 2007 regardless of light regime. The individual host fish and intensity of infection, which varied between fish, appeared to have no influence on when egg sacs were first produced. The intensity of infection could only be estimated as a count of all parasites on the gills would have required dissecting the gills from the fish.

Figure 4.18 shows the temperature regimes that groups of fish detailed in Table 4.8 were held in during the course of the experiment. The point at which oviposition was recorded for the first time post – winter phase is plotted on the graph as E for each of the groups.

Figure 4.18 Graphs of temperature regimes for all groups of tank held rainbow trout. Vertical increase in temperature indicates that fish were moved to a tank of heated water from ambient conditions. First post overwintering oviposition is indicated by E for each group.



Analysis of water temperatures experienced by each fish group prior to commencement of oviposition was carried out in order to establish a point at which egg development within the ovaries began. This point could have been when a “trigger” temperature was reached or a date at which development started due to accumulation of degree days. This analysis proved inconclusive in establishing a point that accounted for the observations seen in all the groups. A “trigger” temperature for egg development in the ovaries was not found to be consistent between groups. It may therefore be possible that a continual development of ovaries/eggs had taken place over the winter months starting before January. Unfortunately due to failure of temperature sensors, data for water temperature in Rutland is not available in 2006, and further analysis was not possible.

4.3.2.2 Post overwintering egg clutch production

Records were kept of the reproductive development of eggs from at least five parasites infecting each fish that were held in experimental tanks under different conditions. The data for five of these which were photographed to record development stage are shown in Tables 4.9 to 4.11.

As the gills were not photographed every day, it was likely that some changes in developmental stage of eggs were missed. This is especially true of parasites on fish that were held at higher temperatures where development was faster. However, by photographing fish on 5 consecutive days at certain points of the study, and therefore observing that there was a consistency of developmental changes, it was possible to interpolate the development changes that were occurring throughout the entire period of egg production. Figure 4.19 to 4.21 are Gantt charts that summarise the observed

developmental stages for the 5 parasites for each temperature profile with the estimated egg clutch production highlighted.

Table 4.9 Egg sac stage of five parasites photographed at regular intervals on rainbow trout held at ambient temperatures in dark conditions.

Date	Temp (ºC)	Degree days*	Parasite 1	Parasite 2	Parasite 3	Parasite 4	Parasite 5
5/04/07	7.4	7	W	W	W	W	W
11/04/07	7.7	55	W	W	W	W	W
16/04/07	8.4	97	W	W	W	W	W
17/04/07	8.7	106	W	W	W	W	W
23/04/07	9.0	160	W	W	A	W	W
24/04/07	9.0	169	A	W	A	W	A
25/04/07	9.1	178	A	A	N	A	A
26/04/07	9.0	187	N	A	N	A	N
30/04/07	9.3	225	W	W	W	W	W
1/05/07	9.4	234	W	W	W	W	W
2/05/07	9.6	244	W	W	W	W	W
3/05/07	9.7	254	W	W	W	W	W
7/05/07	10.4	295	W	W	W	W	W
8/05/07	10.5	305	W	W	W	W	W
9/05/07	10.6	316	W	W	W	W	W
10/05/07	10.6	327	W	W	W	W	W
14/05/07	10.3	369	A	A	A	**	N
15/05/08	10.2	379	N	A	N	N	W
16/05/07	10.4	390	N	N	N	W	W
17/05/07	10.4	400	W	N	W	W	W
Total 42 days	Mean 9.3 ºC						

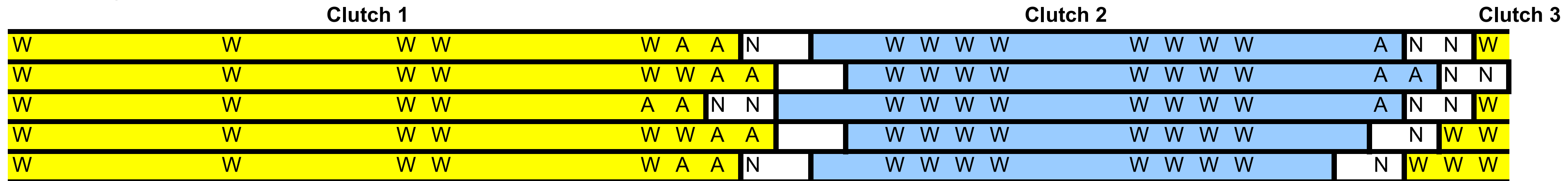
* Degree days calculated from day when >10% of parasites visible had egg sacs
**Unknown as not clearly visible on image
W = Ovigerous with white eggs
A = Ovigerous with advanced dark eggs
N = Non-ovigerous

Table 4.9 above shows an example of the records for five parasites from a single fish in Group 3a, held in ambient water temperatures in the dark. It shows that between 5th April and 16th May, two clutches of eggs had been produced by these parasites and had developed to hatch (see Figure 4.19). A third clutch of eggs was produced shortly

before the end of this monitoring period but had not developed to advanced stage. The ovigerous advanced stage lasted approximately one to two days at temperatures of 7-10ºC. The inter-clutch interval was two days at these temperatures. The ovigerous white stage had the longest duration at approximately 13-15 days. From 5th April to 16th May the temperature increased consistently. As a result the second clutch of eggs were developed and hatched at a higher temperature than the first clutch. Unfortunately as the parasites were ovigerous when photographing began, the time when the first clutch of eggs was extruded was unknown and therefore the time taken to develop was an underestimate. Based on the mean development times of the second clutch of eggs when mean temperature was 9.5 ºC, production of an egg clutch (including one inter clutch interval) to hatching required 171 degree days (threshold 0º C) of which the eggs were white for 14 days (133 ºdays), the eggs were advanced for 2 days (19 ºdays) and there was an inter-clutch interval of 2 days (19 ºdays) for a total of 18 days. This data is summarised in Table 4.12

Figure 4.19 Gantt chart of the observed and estimated developmental stages of 5 *E.sieboldi* held at ambient temperatures under dark conditions. Graph under the Gantt shows water temperature measured hourly by sensor held in same tank as fish

Key
W White eggs
A Advanced eggs
N Non ovigerous



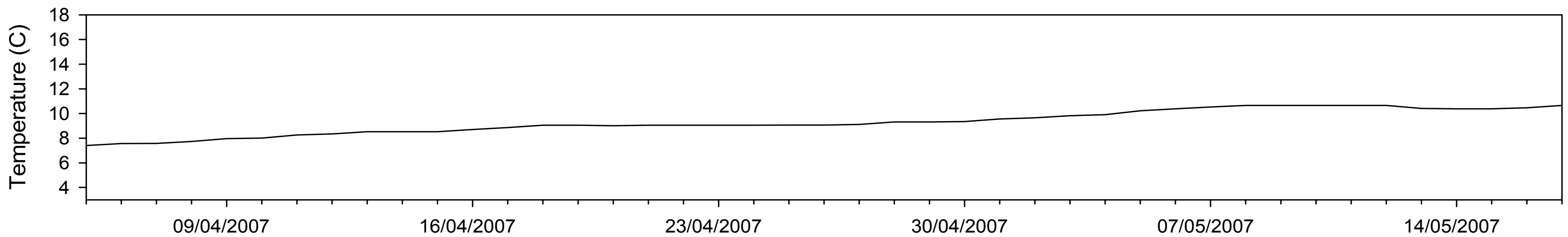


Table 4.10 Egg sac stage of five parasites photographed at regular intervals on rainbow trout held at 10ºC plus temperatures in dark conditions.

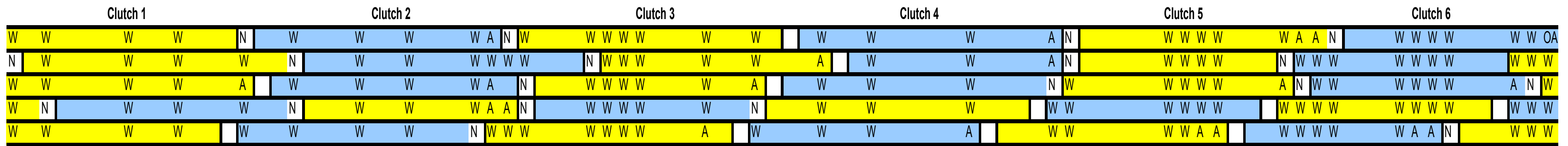
Date	Temp (ºC)	Degree days*	Parasite 1	Parasite 2	Parasite 3	Parasite 4	Parasite 5
12/02/07	10.2	10	W	N	W	W	W
14/02/07	10.1	30	W	W	W	N	W
19/02/07	10.8	84	W	W	W	W	W
22/02/07	10.9	116	W	W	W	W	W
26/02/07	11.2	160	N	W	A	W	W
1/03/07	10.1	192	W	N	W	N	W
5/03/07	10.1	232	W	W	W	W	W
8/03/07	10.1	262	W	W	W	W	W
12/03/07	10.1	304	W	W	W	W	N
13/03/07	9.9	313	A	W	A	A	W
14/03/07	10.4	324	N	W	**	A	W
15/03/07	10.7	335	W	W	N	N	W
19/03/07	10.8	378	W	N	W	W	W
20/03/07	10.7	389	W	W	W	W	W
21/03/07	10.6	399	W	W	W	W	W
22/03/07	10.7	410	W	W	W	W	W
26/03/07	10.4	452	W	W	W	W	A
29/03/07	10.7	484	W	W	A	N	W
2/04/07	10.7	527	W	A	W	W	W
5/04/07	11.1	560	W	W	W	W	W
11/04/07	12.1	629	W	W	W	W	A
16/04/07	11.9	692	A	A	N	W	W
17/04/07	10.8	702	N	N	W	W	W
23/04/07	10.9	767	W	W	W	W	W
24/04/07	10.9	778	W	W	W	W	W
25/04/07	10.8	789	W	W	W	W	A
26/04/07	10.7	800	W	W	W	W	A
30/04/07	11.0	844	W	N	A	W	W
1/05/07	11.1	855	A	W	N	W	W
2/05/07	11.2	866	A	W	W	W	W
3/05/07	11.4	877	N	W	W	W	W
7/05/07	11.9	924	W	W	W	W	W
8/05/07	12.0	936	W	W	W	W	A
9/05/07	12.0	948	W	W	W	W	A
10/05/07	12.0	960	W	W	W	W	N
14/05/07	11.7	1008	W	W	A	W	W
15/05/08	11.7	1020	W	W	N	W	W
16/05/07	11.8	1031	W	W	W	W	W
Total 94 days	Mean 11ºC						

* Degree days calculated from day when >10% of parasites visible had egg sacs
**Unknown as not clearly visible on image
W = Ovigerous with white eggs
A = Ovigerous with advanced dark eggs
N = Non-ovigerous

Table 4.10 above shows the egg developmental stage of five parasites recorded from a fish held in dark conditions with water temperature increased to a minimum of 10ºC. There were six clutches of eggs produced by these parasites held at 10-12ºC over a three month period (see Figure 4.20). Some parasites had begun production of a 7th clutch before the end of the monitoring period. The inter-clutch interval was approximately 1 day and the advanced ovigerous stage lasted 1-2 days. The ovigerous white stage had the longest duration at approximately 11-14 days. Taking the mean results from the five parasites, production of an egg clutch (including one inter clutch interval) at 10.8 ºC lasted 162 degree days (threshold 0º C) of which the eggs were white for 12.5 days (135 ºdays), the eggs were advanced for 1.5 days (16.2 ºdays) and there was an inter-clutch interval of 1 day (10.8 ºdays) for a total of 15 days. This data is summarised in Table 4.12

Figure 4.20 Gantt chart of the observed and estimated developmental stages of 5 *E.sieboldi* held under dark conditions and in water temperatures of at least 10ºC. Graph under the Gantt shows water temperature measured hourly by sensor held in same tank as fish

Key
W White eggs
A Advanced eggs
N Non ovigerous



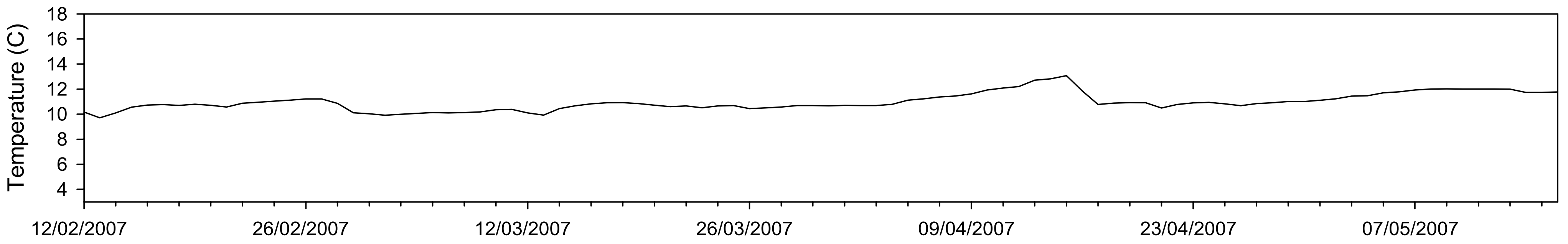


Table 4.11 Egg sac stage of five *E.sieboldi* parasites photographed at regular intervals on a rainbow trout held in dark conditions and in water heated to at least 15ºC.

Date	Temp (ºC)	Degree days*	Parasite 1	Parasite 2	Parasite 3	Parasite 4	Parasite 5
1/03/07	15.7	15.7	W	W	W	W	W
5/03/07	15.5	78	N	A	W	W	W
8/03/07	15.7	125	W	W	W	W	W
12/03/07	16.0	189	W	W	A	A	W
13/03/07	16.0	205	A	W	N	N	A
14/03/07	16.2	221	W	A	N	W	W
15/03/07	16.2	237	W	A	N	W	W
19/03/07	16.1	302	W	W	N	W	A
20/03/07	16.0	318	W	W	N	W	A
21/03/07	15.7	334	A	W	N	A	W
22/03/07	15.8	350	N	W	N	W	W
26/03/07	15.8	413	W	W	N	W	W
29/03/07	15.9	461	W	W	missing	A	N
2/04/07	16.0	526	W	A	missing	W	W
5/04/07	15.9	573	A	W	missing	W	A
11/04/07	16.0	669	W	N	missing	W	W
16/04/07	16.0	748	N	W	missing	A	A
17/04/07	16.0	764	W	W	missing	W	N
23/04/07	15.8	859	W	W	missing	W	W
24/04/07	15.9	875	A	W	missing	W	W
25/04/07	15.8	891	N	A	missing	A	A
26/04/07	15.8	907	W	W	missing	N	A
30/04/07	15.8	970	W	W	missing	W	W
1/05/07	15.8	985	W	W	missing	W	W
2/05/07	15.8	1001	W	A	missing	W	W
3/05/07	15.8	1017	A	W	missing	W	W
7/05/07	16.0	1081	W	W	missing	W	A
8/05/07	16.0	1097	W	W	missing	W	W
9/05/07	16.0	1113	W	A	missing	W	W
10/05/07	16.0	1129	A	N	missing	W	W
14/05/07	16.1	1192	W	W	missing	A	W
15/05/08	16.1	1209	W	W	missing	N	W
16/05/07	16.1	1225	W	W	missing	W	A
Total 77 days	Mean 16ºC						

* Degree days calculated from day when >10% of parasites visible had egg sacs
W = Ovigerous with white eggs
A = Ovigerous with advanced dark eggs
N = Non-ovigerous

Table 4.11 above shows the egg developmental stage of five parasites recorded from a fish held in dark conditions with water temperature increased to a minimum of 15ºC. There were nine clutches of eggs produced by these parasites at 15-16ºC over a two and a half month period (see Figure 4.21). A tenth clutch of eggs had begun developing on some parasites. The inter-clutch interval was 1 day or less and the advanced ovigerous stage lasted 1-2 days. The ovigerous white stage had the longest developmental duration at approximately 5 to 7 days. Taking the mean results, a production of eggs (including one inter clutch interval) at 15.9 ºC lasted 127 degree days (threshold 0º C) of which the eggs were white for 6.5 days (103 ºdays), the eggs were advanced for 1 day (15.9 ºdays) and there was an inter-clutch interval of 0.5 days (8 ºdays) for a total of 8 days. This data is summarised in Table 4.12.

Figure 4.21 Gantt chart of the observed and estimated developmental stages of 5 *E.sieboldi* held under dark conditions and in temperatures of at least 15ºC. Graph under the Gantt shows water temperature measured hourly by sensor held in same tank as fish

Key
W White eggs
A Advanced eggs
N Non ovigerous

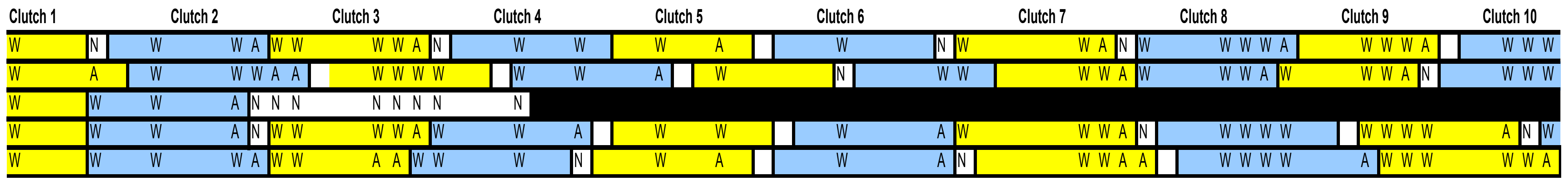


Table 4.12 Time required for egg sac development to hatch at various temperatures as recorded from tank held fish/parasites

Temp. (ºC)	Inter clutch interval (days)	Duration of white egg sac stage (days)	Duration of advanced egg sac stage (days)	Development time of eggs (oviposition to hatch) (days)	Development time of eggs (oviposition to hatch) (degree days threshold 0º C)
9.5	2.0	14.0	2.0	16.0	152
10.8	1.0	12.5	1.5	14.0	151
15.9	0.5	6.5	1.0	7.5	119

In summary, in terms of degree days (threshold 0º C), including an inter-clutch interval, it was recorded that at temperatures of 9.5ºC, a clutch of eggs was produced and hatched every 171 degree days. At temperatures of 10.8ºC, production of a clutch required approximately 162 degree days, and at 15.9 ºC this fell to 127 degree days.

There was some variation amongst egg production and development speed of individual parasites from the same fish as can be seen from the data in Figures 4.19 to 4.21. Some parasites were recorded for several days with no egg sacs and subsequently were missing, presumed dead, as is illustrated in Table 4.11. Also some parasites would disappear when in an ovigerous state, again assumed to have died or fallen from the host.

Most parasites did not move position during the study but there was limited movement observed by some parasites on the gill filaments and between hemibranchs. Over the course of the study movement of parasites was accounted for by regular photographing, allowing individual parasites to be identified. It was observed that when opening the hemibranchs of the gill to allow photographing, parasites could move from the inner hemibranch of a gill to the outer surface of the

next hemibranch in a posterior direction. This was achieved by releasing from one gill surface while adhered in the mucous lying between the gills and reattaching to the other gill surface. This was only observed in the proximal part of filaments where the distance between hemibranchs was minimal.

4.3.2.2.1 Inter clutch interval

Inter clutch interval, at the range of temperatures recorded in Rutland Water when parasites are ovigerous, was estimated from a predictive three parameter exponential decay equation based upon observations of parasites on tank held fish. The predictive equation was:

Inter clutch interval = 0.4929 + 4316.8347*exp(-0.8379*temperature)

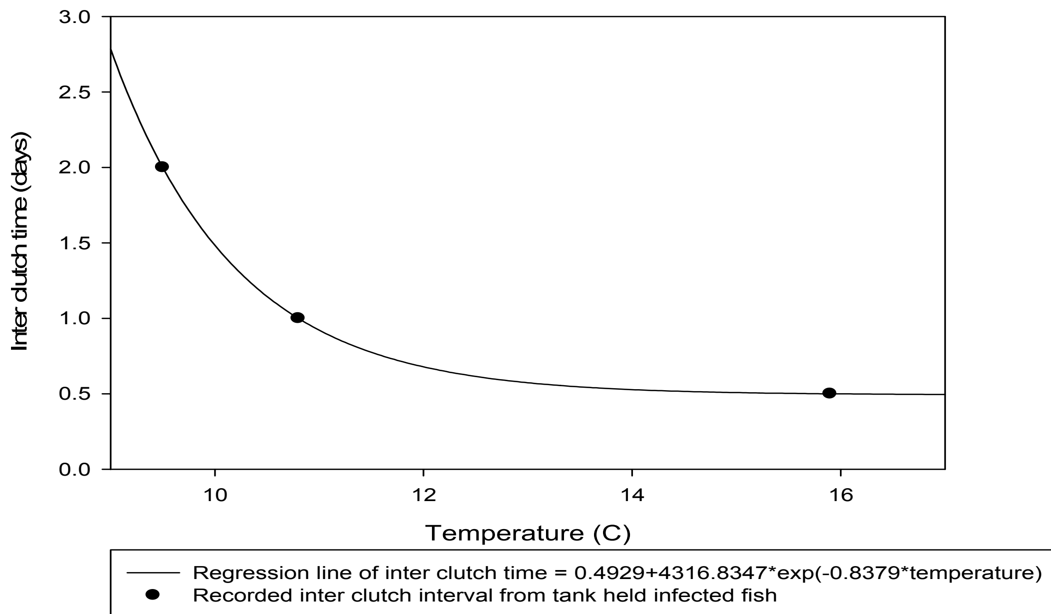
This regression equation has an R^2 value of 99.99%, standard error of estimate of 0.01 and p value of <0.0001. Estimated duration of inter clutch interval based on this equation is detailed in Table 4.13 and plotted in Figure 4.22.

Table 4.13 Recorded and estimated values of inter clutch interval from infected fish held in tank based aquaria at different temperature regimes and dark conditions. Estimates are based on regression equation as detailed.

Temperature (ºC)	Inter clutch interval (Days)	Inter clutch interval (Degree days)
7.0	*12.736*	*89.2*
8.0	*5.790*	*46.3*
9.0	*2.784*	*25.1*
9.5	**2.000**	**19.0**
10.0	*1.484*	*14.8*
10.8	**1.000**	**10.8**
11.0	*0.922*	*10.1*
12.0	*0.678*	*8.1*
13.0	*0.573*	*7.5*
14.0	*0.528*	*7.4*
15.0	*0.508*	*7.6*
15.9	**0.500**	**7.9**
16.0	*0.499*	*8.0*
17.0	*0.496*	*8.4*
18.0	*0.494*	*8.9*
19.0	*0.493*	*9.4*
20.0	*0.493*	*9.9*

Bold indicates recorded values from experimentation
Italics indicates values derived from regression model

Figure 4.22 Recorded and estimated values of inter clutch interval (in days) from infected fish held in tank based aquaria at different temperature regimes and dark conditions. Estimates are based on regression equation as detailed.



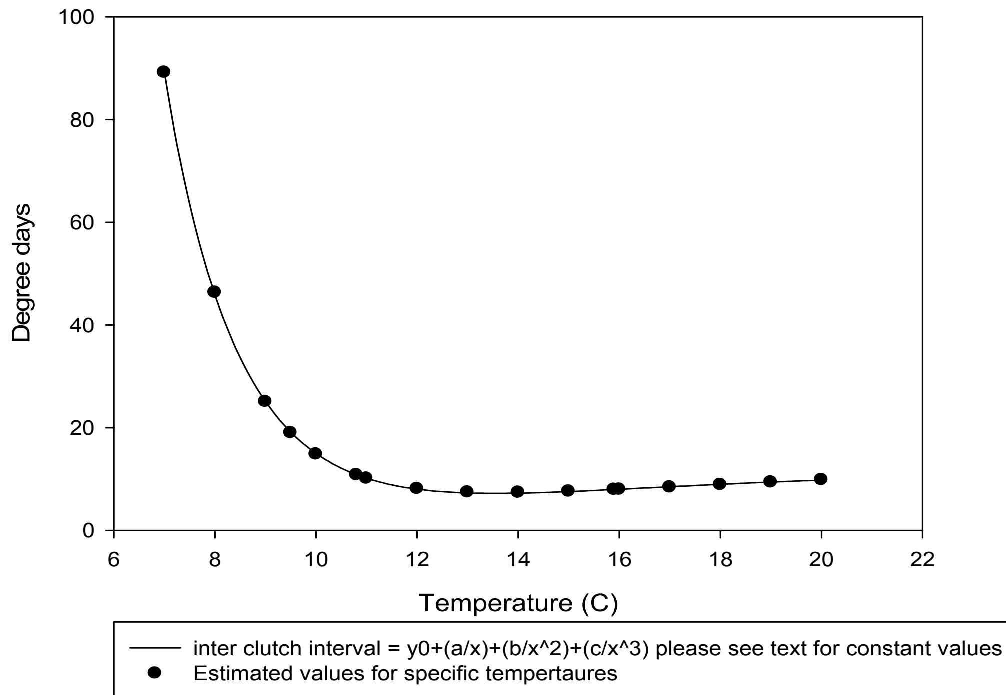
When the duration of inter clutch interval was converted to degree days (using 0 ºC) as base threshold, the data was best explained by the inverse polynomial third order equation

Inter clutch interval =y0+(a/x)+(b/x^2)+(c/x^3), where

y0 = -5.2102
a = 1080.0186
b = -22422.0179
c = 136440.2675

This regression equation has an R^2 value of 99.99%, standard error of estimate of 0.1533 and p value of <0.0001 indicating that the equation was a good predictor of recorded values. This equation that describes inter clutch interval in degree days is plotted in Figure 4.23.

Figure 4.23 Recorded and estimated values of inter clutch interval (in degree days (base threshold 0 ºC)) from infected fish held in tank based aquaria at different temperature regimes and dark conditions. Estimates are based on the regression equation as detailed.



The equations to calculate inter clutch interval are only based upon 3 recorded measurements. Therefore a degree of caution should be applied. The estimated figures for duration of inter clutch interval are also limited to a minimum of 0.5 days as it was not possible to measure a period of less than this in the tank held experiments. Although unlikely, it s possible that extrusion of an egg clutch could take place while a previous clutch is still attached, simply pushing the existing sac until it detaches from the parasite. For this reason the minimum recorded duration of inter clutch interval should be read as 0-0.5 days.

4.3.2.3 Longevity of the post overwintered *E.sieboldi*

The three remaining fish from the tank held experiments were finally killed on the 27th August 2007. These were examined and photographed in the same way as in earlier sample dates with photographs being taken of the outer surface of gill hemibranchs. Table 4.14 shows the number of visible parasites remaining on 3 different fish from Group 3a from 2nd April to the 27th August 2007.

In April, these fish had different intensities of infection with fish B having the largest at 384 visible parasites. It was estimated that the actual intensity of fish 2 was approximately 768-960 parasites at this time (based on 40-50% of parasites present being visible on the digital photographs). The remaining parasites were not visible due to their location at anterior and posterior regions of the gill or on the inner hemibranchs. Fish A had the second largest intensity of infection with 222 visible parasites (estimated intensity 444-555). The least infected fish was Fish C with 94 visible parasites (estimated intensity 188-235). On the last day of sampling, 27th August, the numbers of visible parasites for each fish was very similar to each

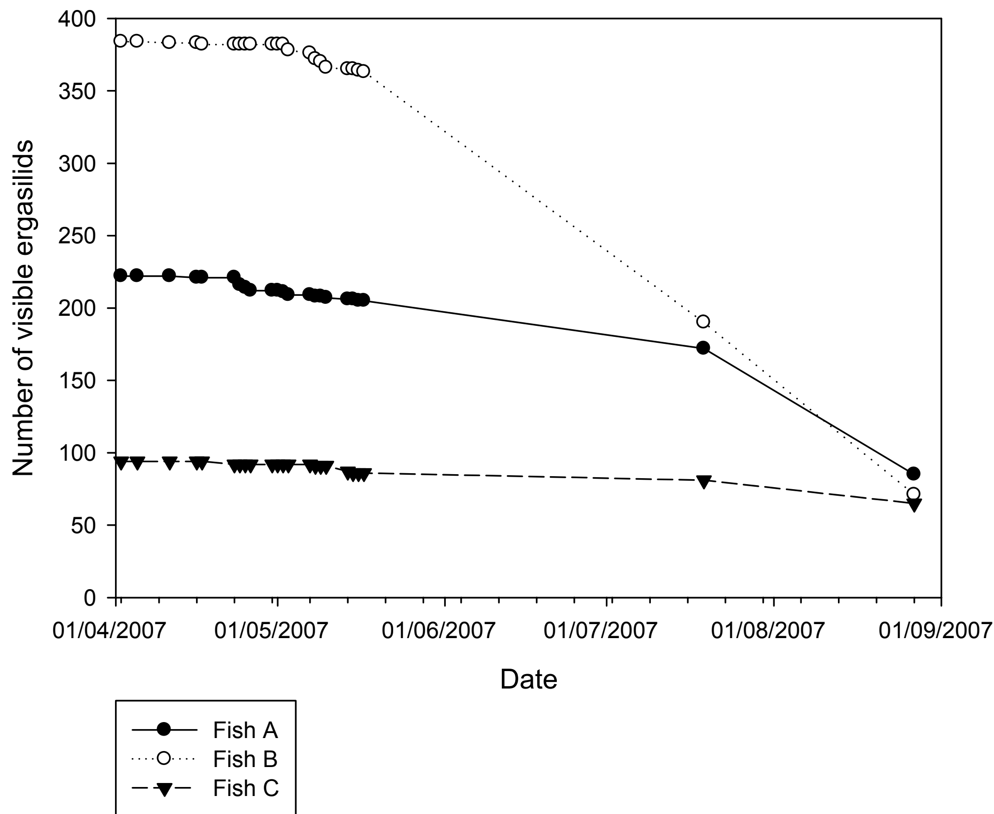
other at 65-85 parasites. The data in Table 4.14 is plotted graphically in Figure 4.24.

Table 4.14 Visible numbers of *E.sieboldi* on the outer surface of the gills of fish from Group 3a (ambient temperature and dark conditions).

Date	Temp (ºC)	Degree days*	Fish A	Fish B	Fish C
2/04/07	7.2	-	222	384	94
5/04/07	7.4	7	222	384	94
11/04/07	7.7	55	222	383	94
16/04/07	8.4	97	221	383	94
17/04/07	8.7	106	221	382	94
23/04/07	9.0	160	221	382	92
24/04/07	9.0	169	216	382	92
25/04/07	9.1	178	214	382	92
26/04/07	9.0	187	212	382	92
30/04/07	9.3	225	212	382	92
1/05/07	9.4	234	212	382	92
2/05/07	9.6	244	211	382	92
3/05/07	9.7	254	209	378	92
7/05/07	10.4	295	209	376	92
8/05/07	10.5	305	208	372	91
9/05/07	10.6	316	208	370	91
10/05/07	10.6	327	207	366	91
14/05/07	10.3	369	206	365	87
15/05/07	10.2	379	206	365	86
16/05/07	10.4	390	205	364	86
17/05/07	10.4	400	205	363	86
19/07/07	16.2	1713	172	190	81
27/08/07	19.2	2402	85	71	65

* Degree days calculated from day when >10% of parasites visible had egg sacs

Figure 4.24 Visible numbers of *E.sieboldi* on the outer surface of gills of fish from Group 3a (ambient temperature and dark conditions)



The data above suggests that the longevity of *E.sieboldi* was approximately 10-12 months. Fish C which had the lowest mean infection, was possibly stocked into the reservoir shortly before the new infections ceased in 2006 (*i.e.* September 2006). As a result, only low numbers of parasites infected this fish. Of the 85 visible parasites that overwintered on this fish from October 2006, 65 were still present at the end of August 2007.

Fish A was potentially present in the reservoir for a longer period of time, possibly since August 2006. The 85 visible parasites remaining at the end of August 2007 were likely to be those that had infected this fish at the end of the season (September onwards) and consequently the numbers are similar to Fish B and C. Between the

19th July and the 27th August, this fish lost 87 of 172 parasites. However, the 172 parasites were the bulk of parasites that had overwintered since October 2006 (222 parasites) indicating minimal loss until July.

It is suggested that fish B was present for the longest time in the reservoir before overwintering, possibly since July 2006 or earlier. The higher mean intensity of infection supports this. Also there was a greater reduction in mean intensity of infection between May 2007 and July 2007 in this fish compared to Fish A and C. This suggests that some of these parasites were older and were dying earlier in 2007.

4.3.3 Egg development and hatching – *in vitro* experiments

4.3.3.1 Experiment 1 and 2. Viability of eggs.

Table 4.15 shows the results of experiments 1 and 2 performed in May 2007 and July 2006.

Table 4.15 Viability of eggs incubated in egg sacs attached to adult parasites, in detached egg sacs and egg removed from egg sacs. All groups incubated at 15 ºC in dark conditions.

		Mean viable eggs from each dish (%)	
Group	Description	Experiment 1 May 2007 (A_1-C_1) P *E.sieboldi*	Experiment 2 July 2006 (A_2-C_2) F1 *E.sieboldi*
A	Eggs in egg sacs attached to adult	67 ± 9	77 ± 4
B	Eggs in egg sacs detached from adult	75 ± 7	85 ± 6
C	Eggs detached from egg sacs	0	0

In Experiment 1 and Experiment 2, the adults with egg sacs attached (Group A) died within 24 hours of being removed from the gill when the temperature was set at

15ºC. The attached egg sacs continued to develop to hatch in most cases, but in these experiments, it was the viability of individual eggs that was recorded. It was noted that advanced eggs would often begin to hatch as the incubation vessel was removed from the dark incubator for examination.

In Experiment 1 in May 2007, using recently attached F1 parasites, the viability of eggs in egg sacs attached to adults (Group A_1) was 67 ± 9%. Eggs within egg sacs which had been detached (Group B_1) and allowed to develop independently achieved a success rate of 75 ± 7%. Egg sacs that had been detached from the adult parasite were significantly more viable (tested using students t test at 95% confidence ($p = 0.03$). Eggs that had been removed from the white egg sac (Group C_1) failed to develop further.

In Experiment 2 in July 2006, using overwintered P parasites that had started to produce a new clutch of eggs, viability of eggs in egg sacs attached to adults (Group A_2) was 77 ± 4%. Eggs within egg sacs which had been detached (Group B_2) and allowed to develop independently achieved a success rate of 85 ± 6%. Egg sacs that had been removed from the adult parasite were significantly more viable (tested using students t test at 95% confidence ($p = 0.01$). Again, eggs that had been removed from the white egg sac (Group C_2) failed to develop further.

In both experiments, eggs took a maximum of 8 days to hatch. Petri dishes which contained adults attached to the egg sacs (A_1 and A_2) tended to accrue more fungal growth than detached egg sacs which may account for a difference in viability. However, it demonstrates that eggs within sacs attached to ovigerous adult females

that had become dislodged from the host could still develop eggs to hatching even if the adult itself had died.

The viability of eggs from F1 parasites was significantly better than overwintered parasites when incubated at 15 ºC (Groups A_1 and B_1 *vs.* A_2 and B_2, using t test, $p = 0.01$).

An incidental observation was that adult female parasites, ovigerous or non-ovigerous can be kept alive for several days if kept in clean water at low temperatures or on moist gills at low temperatures. It was found that if non-ovigerous parasites were removed from the gill, they would not extrude egg sacs. Also if the fish host was killed and gills removed and kept in chilled reservoir water, non-ovigerous parasites would not extrude egg sacs. It was possible that when the host was killed, the parasite stopped feeding and this arrested further ovarian development / oviposition. This hypothesis was not tested further.

4.3.3.2 Experiment 3 and 4. Development rate of egg sacs

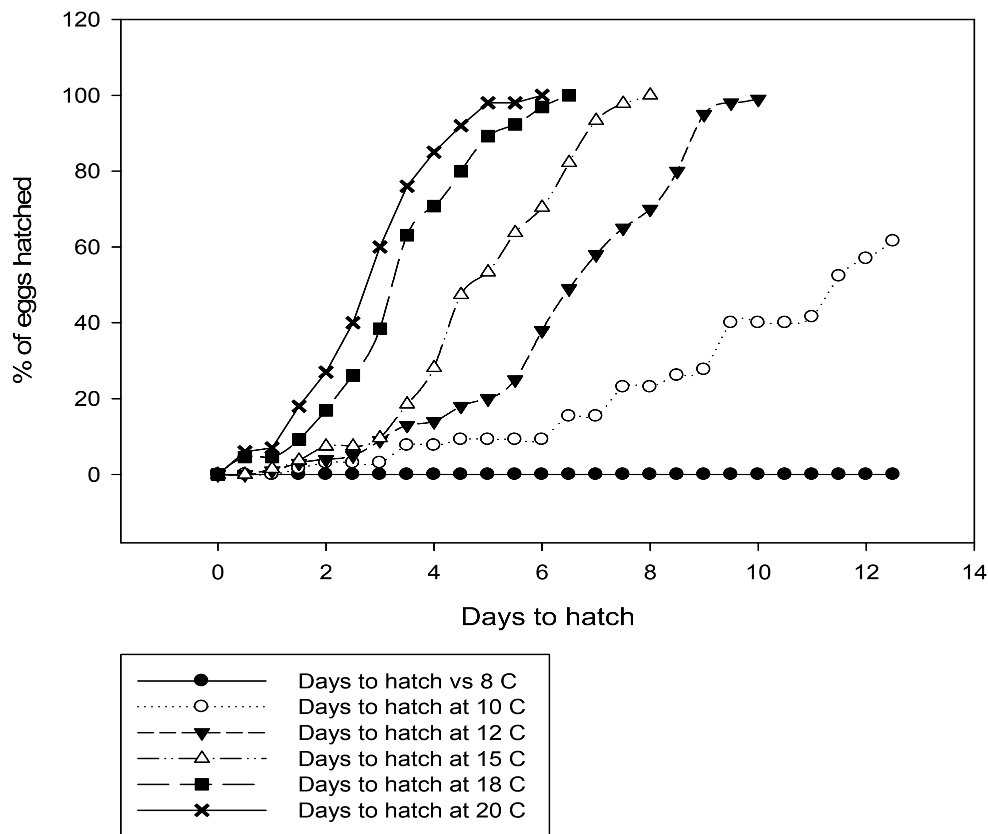
Figure 4.25 and Table 4.16 show the percentage of 96 egg sacs that hatched when held at different temperatures in dark incubators over fourteen days. As there was no way of knowing when precisely an egg sac was extruded from an adult female *E.sieboldi* removed from a gill, the maximum time taken to hatch was assumed to be the development time of egg sacs at a given temperature. This assumes that the egg sac that took the longest time to develop to hatching was the one that was most recently extruded. This was likely to be an overestimate as time for an egg clutch

production on tank held fish/parasites showed some variation between individual parasites.

Table 4.16 Days and degree days required for *E.sieboldi* egg sacs to hatch at various temperatures (ºC) as recorded from *in vitro* development of detached egg sacs.

Temperature (ºC)	Time for 50% to hatch (days)	Maximum time to hatch (days)	% of egg sacs that hatched	Maximum degree days to hatch (threshold 0º C)
8	n/a	n/a	0	n/a
10	11.4	12.5	62	125
12	7.5	10	99	120
15	4.7	8.0	100	120
18	3.4	6.5	100	117
20	3.1	6.0	100	120

Figure 4.25 Time for detached *E.sieboldi* egg sacs to hatch at various temperatures (ºC) when incubated in dark conditions.



Egg sacs incubated at 8ºC failed to hatch. Epibionts and fungal growth were observed on the egg sacs after 10 days and no development was apparent.

At 10 ºC, 62% of the incubated egg sacs hatched. At 12 ºC, 99% hatched and at higher temperatures, all the egg sacs hatched. Figure 4.26 shows the maximum times to hatch at each temperature tested for detached egg sacs.

The duration of time at the advanced stage with blue / black eggs was a mean of 1.9 days at 10 ºC, 1.5 days at 12 ºC, 1.0 day at 15 ºC, 1.0 day at 18 ºC and 0.9 days at 20 ºC.

The experiment was repeated using overwintered parasites in May 2007. The results for the maximum hatch times for each temperature were the same as for F1 parasites in July of 2006. The results for 50% of eggs to hatch were also approximately the same.

Figure 4.26 Graph showing the maximum time to hatch of 96 egg sacs developed and hatched at given temperatures under dark conditions. The plotted line is of a predictive three parameter exponential decay equation.

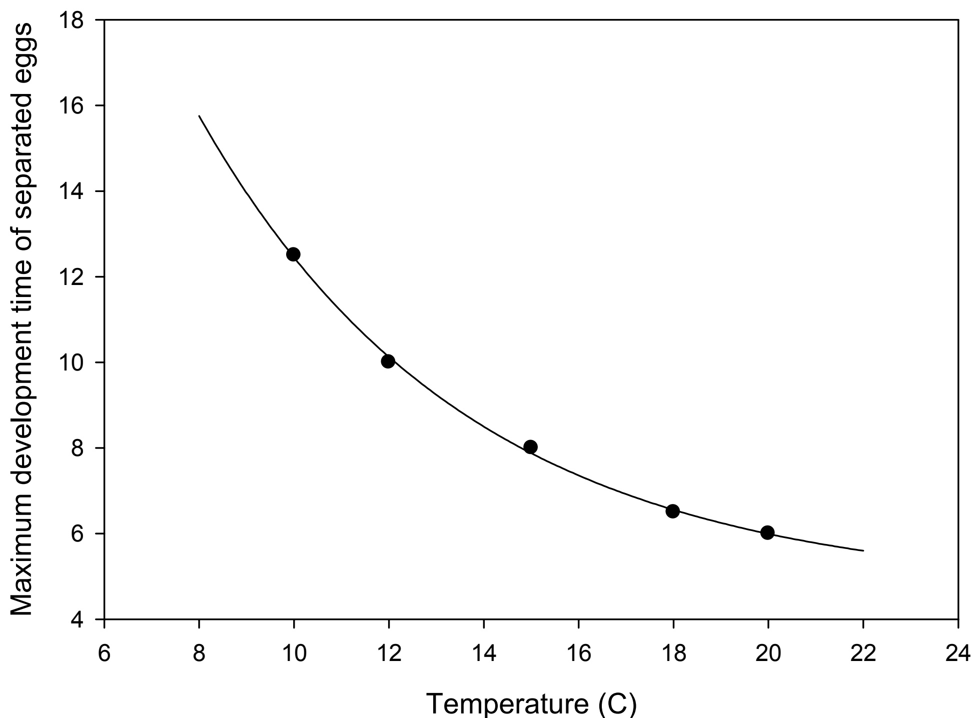


Figure 4.26, shows that there was a negative non-linear relationship between temperature and development time of detached egg sacs. Using a non-linear regression model of time to develop to hatch as a function of temperature, a three parameter exponential decay equation was effective at predicting the data:

Development time (days)=y0+a*exp(-b*x) where

y0 = 4.6701,
a = 45.7319,
b = 0.1772,
x = temperature.

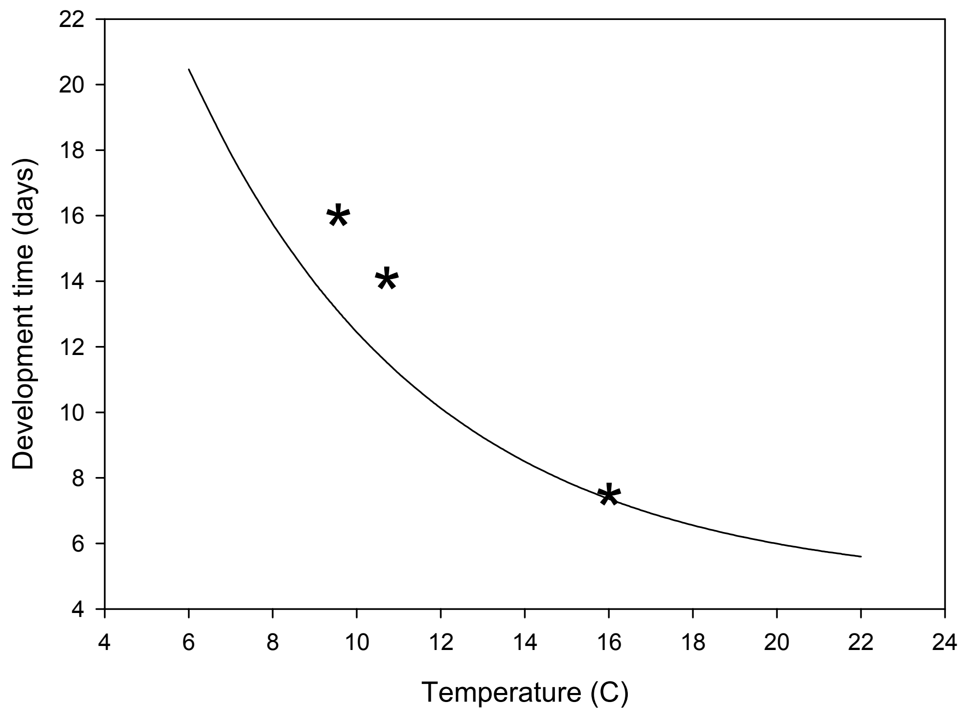
This regression equation has an R^2 value of 99.86%, standard error of estimate of 0.0458 and p value of 0.0014 indicating temperature as a significant predictor of development time.

Table 4.17 Estimated egg development time based on regression equation at the range of temperatures recorded at Rutland Water during the reproductive season.

Temperature (ºC)	Recorded development time (days)	Estimated development from regression equation D=4.6701+45.7319*exp(-0.1772*temperature)
6		20.5
7		17.9
8		15.8
9		14.0
10	12.5	12.4
11		11.2
12	10.0	10.1
13		9.2
14		8.5
15	8.0	7.9
16		7.4
17		6.9
18	6.5	6.6
19		6.2
20	6.0	6.0
21		5.8
22		5.6

Table 4.17 estimates the development time in days of detached eggs based on the regression equation detailed above. The estimated times are also plotted in Figure 4.27 as an equation line. The asterisks on this figure indicate the development times of eggs as observed attached to parasites in the tank held fish experiment for comparison.

Figure 4.27 Estimated development time (days) for detached *E.sieboldi* egg sacs based on an exponential decay regression equation D = 4.6701 + 45.7319*exp(-0.1772*temperature). Asterisks mark the observed developmental time (days) recorded from tank held infected fish for comparison.



Egg development times when egg sacs are detached from the adult female parasite under *in vitro* conditions were consistently shorter than when egg sacs were attached to the parasite under *in vivo* conditions. The difference in rate of development was greater at lower temperatures. The difference in development times for white and

advanced stages for the detached and attached eggs as calculated using the regression equations are shown in Table 4.18.

Table 4.18 Comparison of development times for different egg stages and for different temperatures *in vitro* (detached egg sacs incubated in micro well plates) and *in vivo* (attached egg sacs observed on parasites attached to host in tank experiments).

	In vivo (from tank held infected fish)			*In vitro* (from detached egg sacs)		
Temperature (ºC)	Duration of white egg stage (days)	Duration of advanced egg stage (days)	Total time for clutch to be hatched (days)	Duration of white egg stage (days)	Duration of advanced egg stage (days)	Total time for clutch to be hatched (days)
7.0	*26.1*	*4.6*	*30.7*	*14.3*	*3.6*	*17.9*
8.0	*20.6*	*3.2*	*23.8*	*12.9*	*2.9*	*15.8*
9.0	*16.6*	*2.3*	*18.9*	*11.7*	*2.3*	*14.0*
9.5	**14.0**	**2.0**	**16**	*11.1*	*2.1*	*13.2*
10.0	*13.6*	*1.8*	*15.4*	**10.6**	**1.9**	**12.4**
10.8	**12.5**	**1.5**	**14**	*9.3*	*1.7*	*11.0*
11.0	*11.4*	*1.4*	*12.8*	*9.6*	*1.6*	*11.2*
12.0	*9.7*	*1.3*	*11*	8.5	**1.5**	10.1
13.0	*8.5*	*1.1*	*9.6*	*7.9*	*1.3*	*9.2*
14.0	*7.7*	*1.1*	*8.8*	*7.3*	*1.2*	*8.5*
15.0	*7.0*	*1.0*	*8*	**7.0**	**1.0**	**7.9**
15.9	**6.5**	**1.0**	**7.5**	*6.3*	*1.0*	*7.3*
16.0	*6.5*	*1.0*	*7.5*	*6.4*	*1.0*	*7.4*
17.0	*6.2*	*1.0*	*7.2*	*5.9*	*1.0*	*6.9*
18.0	*5.9*	*1.0*	*6.9*	**5.5**	**1.0**	**6.6**
19.0	*5.7*	*1.0*	*6.7*	5.3	*0.9*	*6.2*
20.0	*5.6*	*1.0*	*6.6*	**5.1**	**0.9**	**6.0**

Bold indicates recorded values from experimentation
Italics indicates values derived from regression models

As already seen, the development times for egg sacs detached from the adult are shorter than when the egg sacs are left attached. The difference in times was mainly due to a more protracted period of development in the white egg stage when attached to the parasite. The duration of time spent at the advanced stage of development was similar when egg sacs were attached or detached from adult parasites. At higher temperatures (above 13 ºC) the difference in development

times between attached and detached development is small (*e.g*. at 14 ºC 8.8 days attached *vs*. 8.5 days detached). At lower temperatures when development period is longer in both conditions, the difference between them is magnified (*e.g*. at 8 ºC 23.8 days attached *vs*. 15.8 days detached). This suggests that when eggs are attached to the adult parasite, there is a mechanism that inhibits the development of eggs, particularly at the earlier white egg stage which, when is not in operation when the egg sacs are detached from the adult.

Although Table 4.18 and Figure 4.27 above show predicted development times based on the regression equation of detached egg hatching experiments at temperatures ranging from 6-22 ºC, it is very unlikely that development of eggs in Rutland Water would ever take place at a constant temperature, especially at low temperatures such as 6 or 7 ºC, as these would occur in the spring when temperatures are rising rapidly. Therefore it is useful to express a model of development times in terms of degree days, as this can be applied to a varying temperature regime over time.

To use a model of degree days it was necessary to establish a theoretical low threshold of temperature below which, no development occurs. This threshold can then be used as the baseline for calculating degree days in further models.

4.3.4 Analysis of laboratory, tank and field data

4.3.4.1 Estimation of theoretical low threshold temperature for egg development

The *in vitro* development and hatching of detached egg sacs failed to identify the low temperature threshold, below which development will not take place. Although egg sacs incubated at 8 ºC failed to develop, the onset of fungal growth was possibly the cause rather than exceeding the tolerance limits for development. Given that ovigerous parasites were observed when the water temperature rose to 7 ºC, it is likely that egg development can occur at this temperature. As the analysis of commencement of post winter oviposition was inconclusive in determining if it was triggered by a certain water temperature or an accumulation of degree days, it may be possible that oviposition could take place when the temperature is below 7 ºC.

Due to the fluctuating temperature during a reproductive season, a model that is based upon degree days is necessary to estimate egg production throughout the year. This requires the estimation of the theoretical low threshold temperature. Data collected in the study that could be used to calculate this figure is either: a) development rate of eggs attached to parasites in tanks held conditions; b) maximum development times of detached egg sacs; or c) time for 50% of detached egg sacs to develop.

None of these measures are ideal as tank held development are only based on 3 measurements, and the detached egg sacs have been shown to develop faster than egg sacs attached to the adult parasite. However, the lower limit for development should be the same for *in vitro* or *in vivo* incubation, and therefore for statistical

robustness, the theoretical low temperature threshold was calculated for detached egg sacs based on maximum time to hatch and on 50% time to hatch using reciprocals of development times as detailed below.

Figure 4.28 Regression plot of reciprocal of development time against temperature for values of maximum development times of detached egg sacs incubated at different temperatures in dark conditions

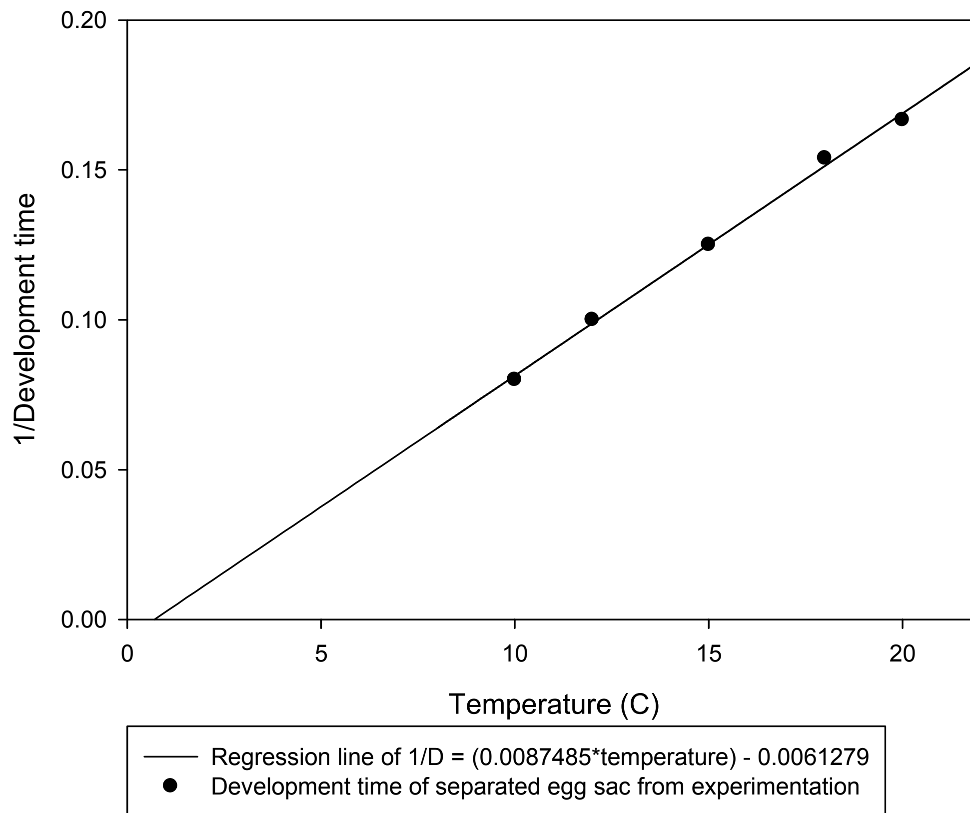


Figure 4.28 plots the regression of the equation formulated as:

$$1/\text{development time} = (0.0087485 * \text{temperature}) - 0.0061279$$

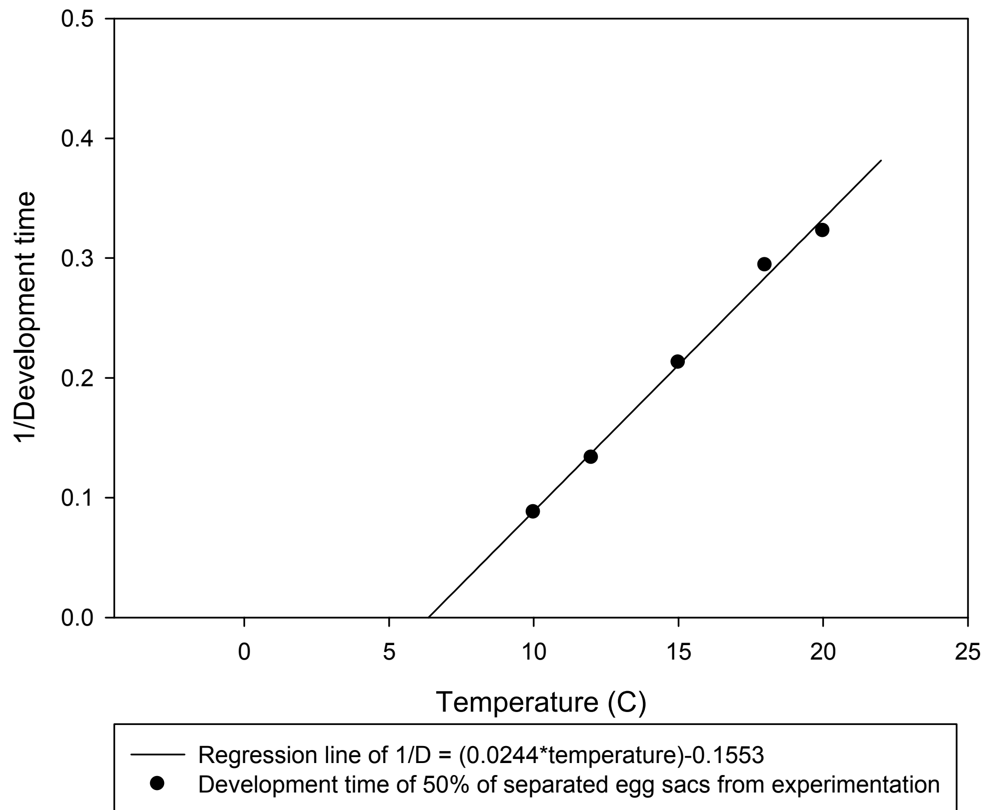
This regression equation has an R^2 value of 99.6%, standard error of estimate of 0.04 and p value of <0.001.

From the regression analysis detailed above, the theoretical low temperature threshold for development was approximately 0.7 ºC. This is the figure based upon the egg sac that took the longest to hatch at each temperature tested. Figure 4.29 plots the regression analysis for the time at which 50% of eggs hatched at each incubation temperature. Regression equation for 50% of eggs developed to hatching is:

$$1/\text{development time} = (0.0244*\text{temperature}) - 0.1553$$

This regression equation has an R^2 value of 99.4%, a standard error of estimate of 0.009 and a p value of 0.0002.

Figure 4.29 Regression plot of reciprocal of development time against temperature for values of when 50% of detached egg sacs incubated at different temperatures in dark conditions had hatched.



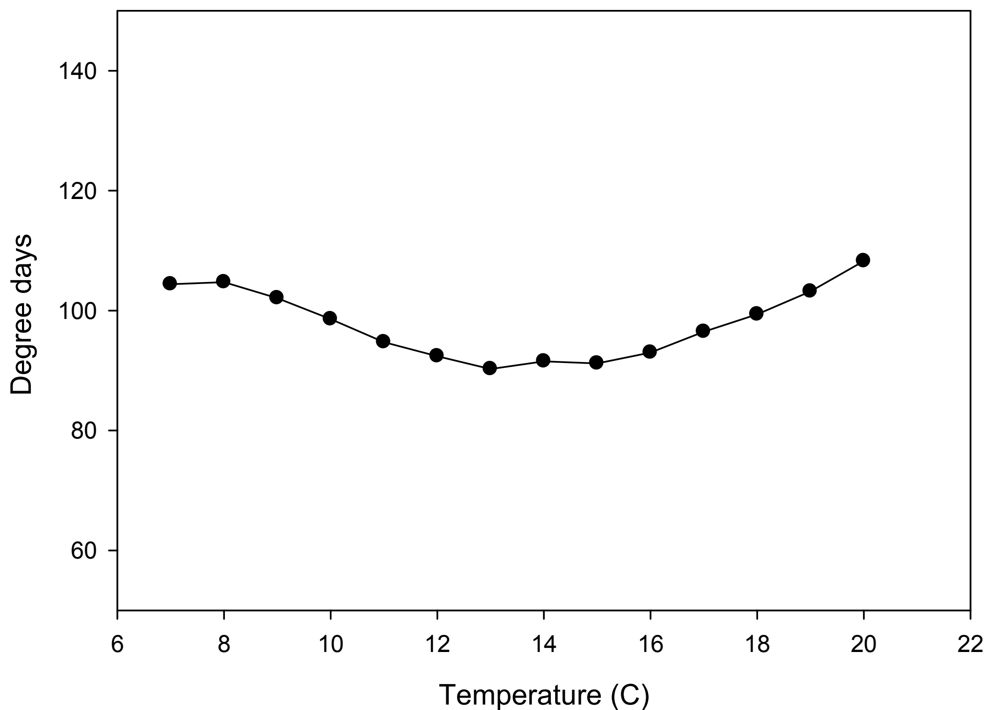
From the regression analysis detailed above, the theoretical low temperature threshold for development was approximately 6.4 ºC. This is the figure based upon the time point when 50% of egg sacs developed to hatching at each temperature tested.

The results from hatching of detached egg sacs suggest that the low temperature threshold for development was between 0.7 and 6.4 ºC. Linear regression of the reciprocal of development times observed on the tank held infected fish suggested that the lower threshold for development was 4.2 ºC. However although this falls in the range identified by hatching of detached egg sacs, this was only based on three observed temperatures, and therefore the requirements for regression were violated. For the purpose of later modeling of egg clutch production, a mean of the results from hatching of detached egg sacs will be used to minimize the error incurred. The figure used is 3.6 ºC.

In order to estimate the reproductive development and number of egg clutches produced by post overwintered *E.sieboldi* in Rutland Water, a model is proposed based on the findings of the tank held (*in vivo*) infected fish egg sac development times as this more accurately reflects normal development under natural conditions. However, the model uses the theoretical low temperature for development estimated from hatching of detached egg sacs for reasons of statistical robustness detailed above. Also, the point at which development does not occur due to low temperature should be the same for egg sacs under both types of incubation.

The data for clutch production of tank held fish (see Table 4.18) was converted to degree days using a lower development threshold of 3.6ºC. A mean requirement of 97.5 ºdays (standard deviation 5.61 ºdays) was estimated as necessary for the development of eggs from oviposition to hatching. The estimated degree days required for each temperature are plotted in Figure 4.30.

Figure 4.30 Degree days required for development of extruded eggs to hatching based on development times modeled from observations of infections of tank held fish.



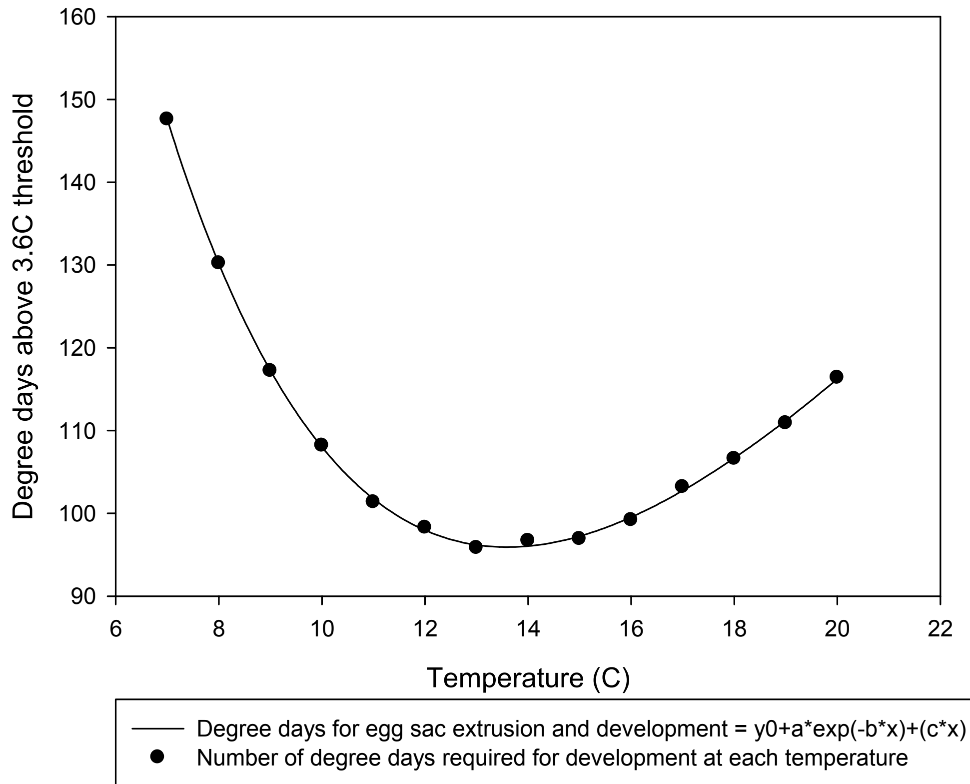
In terms of degree days, there was a lower requirement to produce a clutch of eggs when the temperature was between 12 and 16 ºC. Although development rate continued to increase at higher temperatures, it suggests that production of eggs was most efficient in *E.sieboldi* during this temperature window.

Completion of a production of an egg clutch also requires an inter clutch period. Using the calculated and estimated figures from Table 4.13, the degree day requirements of egg clutch production have been recalculated in Table 4.19 and plotted in Figure 4.31 to include 1 inter clutch period at each given temperature by adding degree days for development from oviposition to hatch to degree days for inter clutch period at each given temperature.

Table 4.19 Time requirements for egg clutch production in terms of days and degree days at given temperatures to include egg development time and inter clutch interval.

Temp. (º C)	Days required to develop	Degree days above 3.6 º C required to develop	Inter clutch interval (days)	Inter clutch interval (degree days above threshold 3.6ºC)	Requirement for clutch production (egg development + inter clutch interval) (degree days above 3.6C)
7.0	*30.7*	*104.4*	*12.7*	*43.18*	*147.58*
8.0	*23.8*	*104.7*	*5.8*	*25.52*	*130.22*
9.0	*18.9*	*102.1*	*2.8*	*15.12*	*117.22*
10.0	*15.4*	*98.6*	*1.5*	*9.60*	*108.20*
11.0	*12.8*	*94.7*	*0.9*	*6.66*	*101.36*
12.0	*11.0*	*92.4*	*0.7*	*5.88*	*98.28*
13.0	*9.6*	*90.2*	*0.6*	*5.64*	*95.84*
14.0	*8.8*	*91.5*	*0.5*	*5.20*	*96.70*
15.0	*8.0*	*91.2*	*0.5*	*5.70*	*96.90*
16.0	*7.5*	*93.0*	*0.5*	*6.20*	*99.20*
17.0	*7.2*	*96.5*	*0.5*	*6.70*	*103.20*
18.0	*6.9*	*99.4*	*0.5*	*7.20*	*106.60*
19.0	*6.7*	*103.2*	*0.5*	*7.70*	*110.90*
20.0	*6.6*	*108.2*	*0.5*	*8.20*	*116.40*

Figure 4.31 Required degree days for development from oviposition to hatching plus 1 inter clutch interval at each given temperature.



The curve of the requirements in degree days for egg clutch production was best predicted by an exponential linear combination decay equation of the form:

Required degree days = $y0+a*exp(-b*x)+c*x$ where

a= 552.4886
b= 0.2009
c= 7.2389
$y0$= -38.4991
x= temperature

This regression equation has an R^2 value of 99.9%, standard error of estimate of 0.3771 and p value of <0.0001.

The start date of egg production in 2005 was unknown as there was no infected fish available in the April sample. Egg production began in tank held fish when the temperature rose above 7 ºC, and for the purposes of further analysis it was assumed that egg production of *E.sieboldi* in Rutland Water during 2005 also began when the temperature reached this point. The water temperature in Rutland first reached 7 ºC on the 2nd April 2005. Oviposition had effectively ceased by 29th September 2005 (see Table 4.7). Cage held fish in the 2 weeks subsequent to this were infected with *E.sieboldi* but only 1% were ovigerous.

To calculate the number of egg clutches produced in 2005, it was not possible to divide the total number of degree days above the lower development threshold by a constant number of degree days, as the equation that describes degree day requirements for egg production is not linear, and varies according to water temperature at any given time point. The most accurate estimate is found by calculating the number of egg clutches produced in a short period of time using the mean water temperature in that period. Therefore it was proposed to calculate the number of egg clutches (or fraction of development of a clutch) produced each day using the daily mean water temperature in the equation:

$$\text{Number of clutches produced in a day} = \frac{(mdt-3.6)}{y0+a*\exp(-b*mdt)+(c*mdt)}$$

where *mdt* = mean daily water temperature

$a = 2250.5896$

$b = 0.4053$

$c = 4.2421$

$y0 = 31.0418$

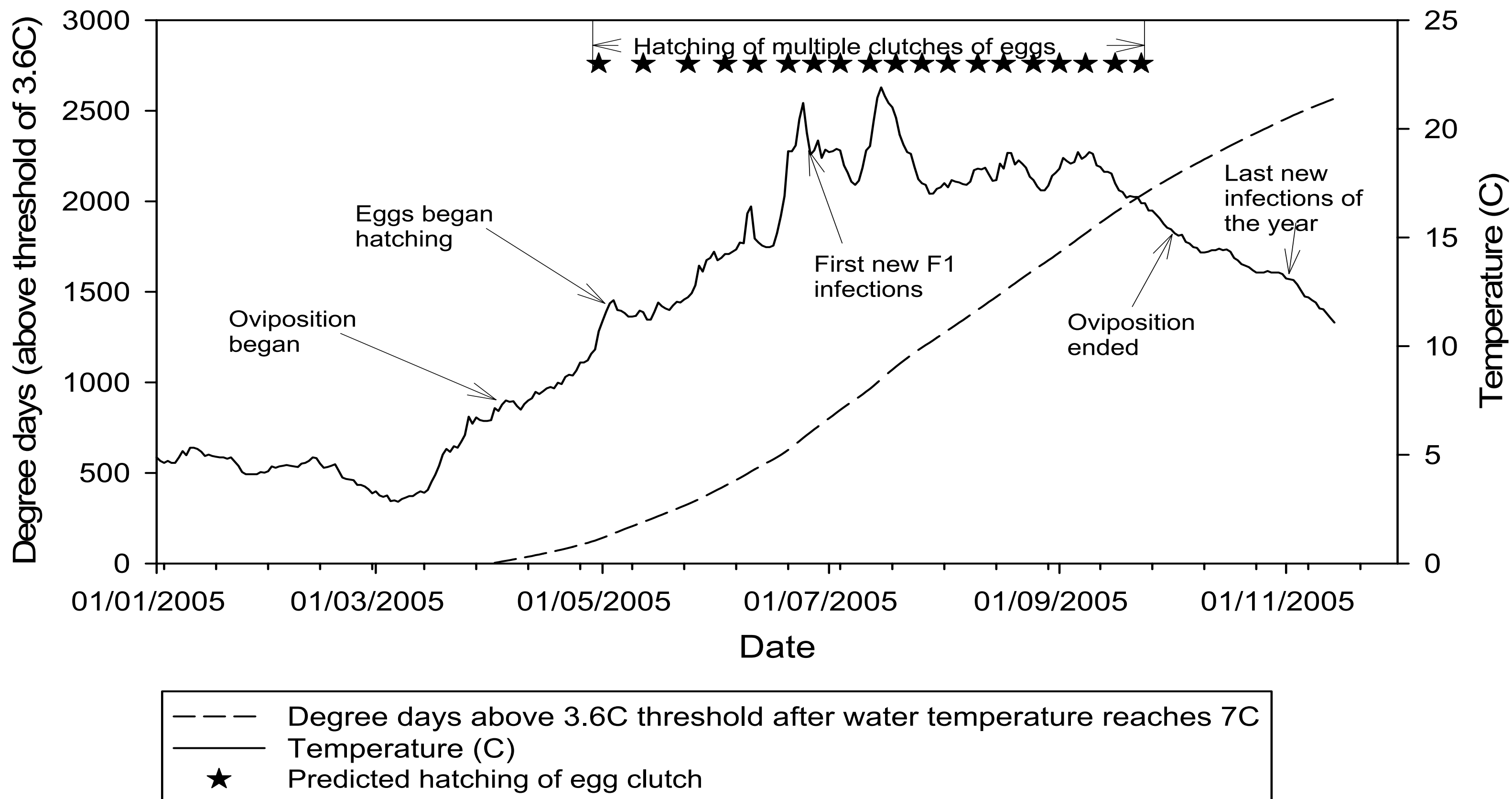
Therefore, the number of clutches of eggs produced in 2005 was:

$$\sum_{Os}^{Oe}(mdt - 3.6)/(y0 + a * \exp(-b * mdt) + (c * mdt))$$

Where *Os* was the first day of oviposition and *Oe* was the last day of oviposition.

Using this equation for the field data collected in 2005, up to 19 clutches of eggs were produced and hatched by parasites that had overwintered from 2004 and had begun oviposition on the 2nd April 2005 and were still producing egg clutches at the same frequency when oviposition ended 29th September 2005. Figure 4.32 shows the point of hatching for each of these egg clutches on a graph of mean water temperature and degree days accumulated during the period of oviposition. Events in the reproductive development of *E.sieboldi* and infection of the fish of Rutland Water are also annotated.

Figure 4.32 Mean daily water temperature of Rutland Water from in 2005. The dashed line indicates the calculated degree days accumulated above the threshold of 3.6 ºC after oviposition is believed to have commenced (2nd April when the temperature rose above 7 ºC). The star symbol identifies the point at which hatching of egg clutch by overwintered parasites is predicted from egg clutch model. Infection and reproduction events are annotated.



4.3.4.2 Fecundity at different temperatures

As previously described, the model for egg clutch production has been applied to 2005 data as this was recorded by a sensor that recorded temperature every three hours. The temperature in 2003 and the first seven months of 2004, was recorded during monthly visits using a thermometer. As such it is not accurate enough to apply to the model and thereby address the question of whether different temperature profiles in the different study years could result in the observed different intensities of infection (see Chapter 3) due to a change in reproductive activity.

To simulate whether a potential increase in temperature in 2003 may have been responsible for increased egg production and subsequently greater infection levels, the 2005 temperature profile was applied to the egg clutch model with a daily mean temperature of the corresponding 2005 temperature daily +1 ºC to simulate a warmer year. Using the same parameters of post winter oviposition beginning when temperature increased to 7 ºC and ending when temperatures fell below 15 ºC, the model predicted that a total of 21 clutches were produced between 26th March and 18th October. If the temperature was 2 ºC warmer, the model predicted 22 clutches produced between 20th March and 3rd November. In contrast, if the water temperature was 1 ºC cooler than the 2005 profile, the model estimated that there would still be 19 clutches of eggs produced between 16th April and 27th September.

4.3.5 Ovarian development

4.3.5.1 Ovarian structure

The microscopic examination of the ovary of *E.sieboldi* confirmed that described for the generalized ergasilid ovary described by Wilson (1911). *E.sieboldi* has a paired ovary located dorsally to the gut in the cephalosome. The structure comprises 4 diverticula on each half of the ovary (Plate 17). The posterior diverticula are connected to oviducts that carry the eggs during oviposition (Plates 18 and 19). Ova develop at the posterior, free end of the ovary before passing into the ovarian diverticula, collecting yolk granules as they increase in size.

As the eggs within the ovary of *E.sieboldi* developed, the cephalosome was seen to expand dorso-laterally to accommodate the enlarged organs (Plate 20). As the diverticula continued to expand to accommodate the growing oocytes, the distal ends of the diverticula curved towards the ventral surface following the contour of the dorsal surface of the cephalosome (Plate 21). In parasites immediately prior to oviposition, the ovarian diverticula appeared to fill the entire cephalosome when viewed from a dorsal position. The cuticular membranes between the first pedigerous somite and adjacent somites became enlarged under the pressure of the growing diverticula. This gave the impression of a lack of articulation and fusing of somites at this point as noted by Abdelhalim (1990).

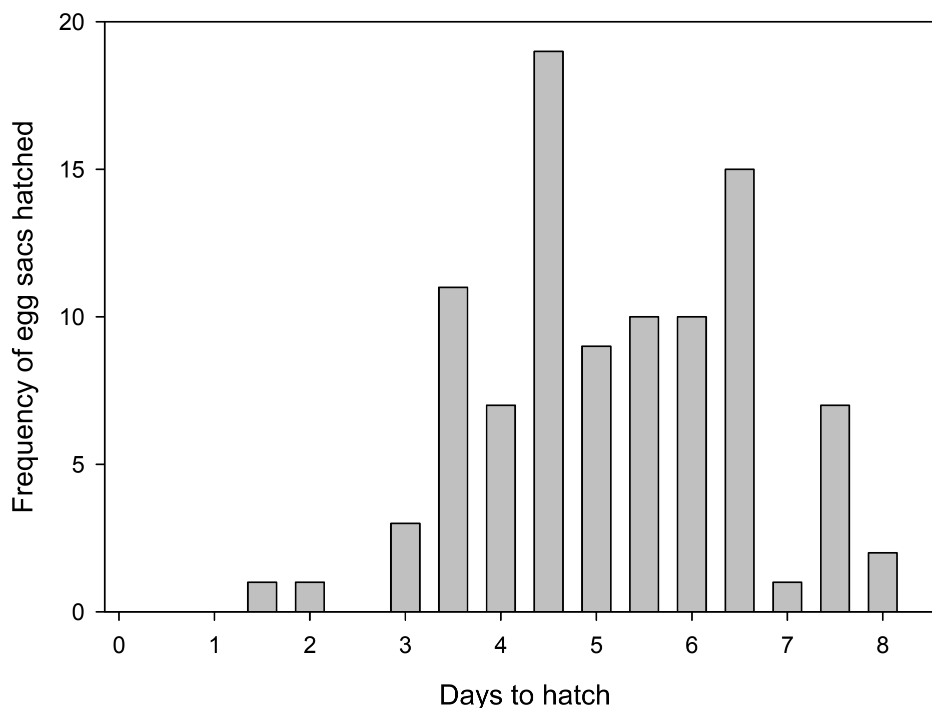
The general pattern of ovary shape accounted for most of the parasites examined. However, some ovaries showed a very different characteristic in development of the diverticula. Plate 22 shows some of the variation observed. In some cases the

diverticula were foreshortened in the anterior aspect. In others the lateral diverticula appeared to have ruptured.

4.3.5.2 Comparison of ovarian development stage with time to hatch of previously extruded egg sacs

Single egg sacs were detached from ovigerous female *E.sieboldi* in April 2006. This was the first egg clutch produced after overwintering. The adult parasite that had extruded each of the egg sacs hatched above was fixed in 5% phosphate buffered formalin at the time the egg sac was detached. Egg sacs were incubated at 15ºC in dark conditions, and checked every 12 hours for signs of hatching. Figure 4.33 shows the frequency of 96 egg sacs that hatched every half day during the experiment.

Figure 4.33 Histogram showing frequency of 96 egg sacs hatched at different times when incubated at 15 ºC in dark conditions.



All incubated egg sacs hatched during the experiment. Maximum time to hatch was 8 days. 50% had hatched at 5 days. More than 80% of the egg sacs hatched between 4 and 7 days. This indicates that the first post-winter oviposition is synchronized in that most of the parasites begin extruding eggs within a few days of each other. Due the slight variation in hatching times as recorded for some parasites in the tank held infected fish experiment, there were a few egg sacs that took slightly longer to hatch. It may be that yolk content of these eggs was less and as a result, development rate was reduced or simply that the ovarian development of these parasites prior to the winter was less advanced than the majority of parasites. This may be as a result of when they infected the host or when or if they last produced an egg clutch.

Measurements were then taken of various parameters of the parasite and ovary. The hypothesis and rationale for this experiment was that if an egg sac took a long time to hatch, it had been extruded recently and the ovary of the parasite would be less developed as a new clutch of oocytes began to be formed. Conversely, an egg sac that hatched quickly would have had more time to develop since being extruded and the ovary of the adult should be more developed as oocyte production was more advanced.

The analysis of data from the ovary size and shape parameters described in 4.2.5 highlighted a number of problems with the methodology. Firstly, there was a variation in ovary shape and size and many of the parameters bore no correlation with the estimated time elapsed since extrusion of egg sacs had taken place.

Secondly, a 2 dimensional image of the dorsal surface is limited in explaining the 3 dimensional structure of the ovary, with diverticula increasingly bending in a dorsal to ventral direction as extension occurs during development of oocytes . In the larger ovaries, the ends of the diverticula may not have been visible from 2D dorsal view of the parasite. Also, extension of the cephalosome in the ventro-dorsal plane would not be recorded by the parameters of the image analysis programme. To do so would require lateral images to be taken and 3 dimensional modeling of the interior of the cephalosome.

Finally, the curvature of the parasite presented some difficulties in obtaining images directly above the dorsal surface as they had to be manipulated between the cavity slide and cover slip to sit flat without distorting the body shape. In many cases the antennae had to be removed in order to allow the parasite to be positioned flat in the cavity slide. This was a time consuming operation that reduced the number of parasites that could be analysed by this technique.

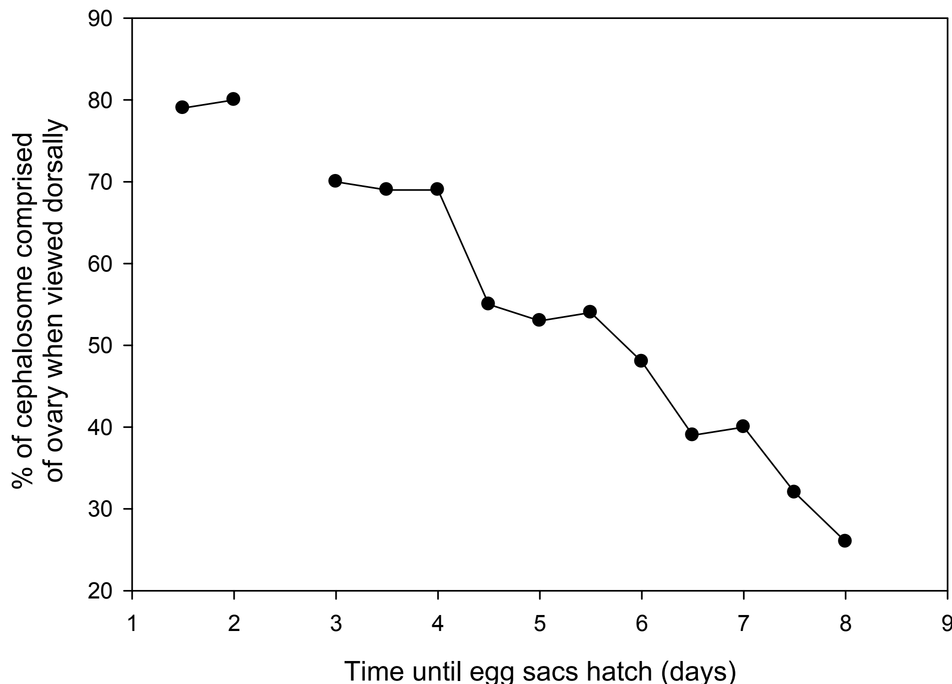
Of all the parameters recorded, the function of parameters that had the best correlation with time since egg extrusion was the ratio of ovary area : cephalosome area. This function expressed as a percentage shows the proportion of the cephalosome that was comprised of ovary when viewed dorsally in a 2D plane. Table 4.20 shows the relationship between this proportion and the time to hatch of extruded egg sacs.

Table 4.20 Summary of ovary development compared to development times of last extruded egg clutch for 96 parasites and egg sacs incubated at 15 ºC in dark conditions.

Time to hatch (days)	n	Mean (ovary area/ cephalosome area *100)	Minimum (ovary area/ cephalosome area *100)	Maximum (ovary area/ cephalosome area *100)
0.5	0	-	-	-
1.0	0	-	-	-
1.5	1	79	79	79
2.0	1	80	80	80
2.5	0	-	-	-
3.0	3	70	59	80
3.5	11	69	55	79
4.0	7	69	59	75
4.5	19	55	43	72
5.0	9	53	44	65
5.5	10	54	48	69
6.0	10	48	40	55
6.5	15	39	30	52
7.0	1	40	40	40
7.5	7	32	20	44
8.0	2	26	22	30

The mean area of cephalosome that was dorsally comprised of ovary is plotted in Figure 4.34 against time for last clutch of eggs to develop.

Figure 4.34 Ovary development compared to development times of last extruded egg clutch for 96 parasites and egg sacs incubated at 15 ºC in dark conditions.



Although there were relatively few egg sacs that hatched at certain time points, (*i.e.* only 2 egg sacs hatched at 2 days or less), there was clearly a negative correlation between size of ovary (compared to cephalosome) and time to hatch of the last egg clutch. This demonstrates that after extruding a clutch of eggs, the adult female was immediately developing oocytes for the next clutch while the existing clutch was still attached to the parasite in egg sacs. It was of course possible that more than one clutch of oocytes was being developed in the ovary at the same time.

It is unclear if the relationship between ovary size and egg clutch development time was linear or occurred in stepped stages. This may be due to the ovary membrane expanding in stages to accommodate growing oocytes or perhaps due to increase in

size of the oocytes as they developed from one stage to the next. Alternatively, it may be an artifact of analysis and the relationship is a linear progression

4.3.5.3 Ovarian development during the winter

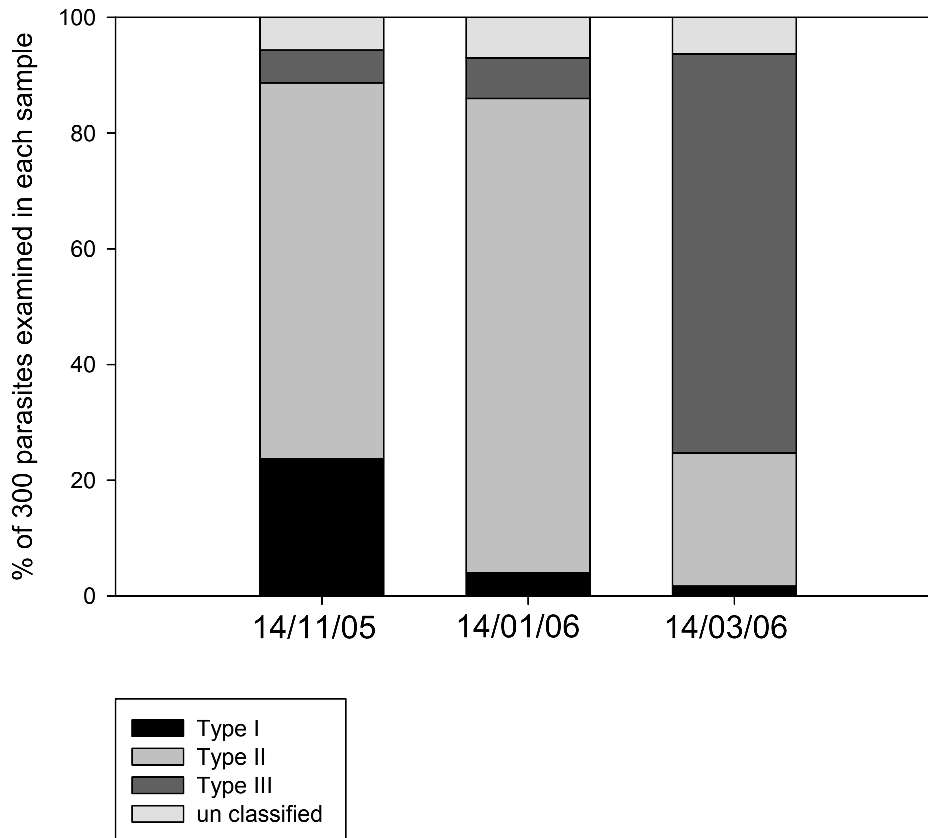
Overwintering parasites were examined for ovary development in November 2005, January 2006 and March 2006. All parasites were in a non-ovigerous state and were removed from three fish in each sample (100 parasites per fish). Ovary size and shape were classified as Type I, II, III or unclassified according to criteria described in 4.2.5.2. Table 4.21 summarises the number of parasites with each ovary development type in each sample.

Table 4.21 Summary of 300 *E.sieboldi* examined from each sample date that had Type I, II, III or unclassified ovary types. Sample dates were in November 2005, January 2006 and March 2005.

Sample date	Fish number	Type I	Type II	Type III	Unclassified
14/11/05	1	30	61	5	4
	2	22	68	7	3
	3	19	66	5	10
14/01/06	4	5	80	7	8
	5	2	86	6	6
	6	5	80	8	7
14/03/06	7	3	26	65	6
	8	1	21	72	6
	9	1	22	70	7

The data from Table 4.21 is plotted graphically in Figure 4.35 as a percentage of each sample of 300 parasites, pooling the parasites by ovary type from all the fish examined on each of the sample dates.

Figure 4.35 The percentage of 300 *E.sieboldi* examined in each sample that had Type I, II, III or unclassified ovary types. Sample dates were in November 2005, January 2006 and March 2005.



The November sample was taken soon after oviposition had ended in 2005. All ovary types were represented in the parasites examined from this sample. This suggests that ovarian development was arrested in all parasites simultaneously, despite the extent of development already attained. There were more parasites with ovaries of Type I undeveloped ovaries in November than in later samples. Some of these were possibly recent infections that had not developed more advanced ovaries before overwintering began. However, the majority of parasites in this sample were Type II with medium developed ovaries. A small proportion were Type III

suggesting that they were about to extrude a clutch of eggs but that this process was arrested by the falling temperatures or onset of the overwintering process.
In January 2006, the proportion of Type I parasites had decreased since November. This suggests that the early ovarian development occurs throughout the winter. The proportion of Type II parasites had increased reflecting the transition from Type I. The proportion of Type III parasites was similar to the January sample 2 months earlier. This suggests that the final developmental process of the ovary had not occurred during the early winter months.

In March 2006, 2-3 weeks before oviposition began, there was still a small proportion of parasites in the Type I stage. These parasites may have failed to develop ovaries, and form the proportion of parasites that remain non-ovigerous in the spring that had been recorded in field observations in each study year. Type II parasites were fewer compared to two months earlier. Many of these parasites had developed to type III with larger ovaries in preparation for oviposition.
There was a similar proportion of parasites in all samples that had un-classified ovaries. Many had discontinuous diverticula, or were deformed in some way. It was unknown how many of these would successfully extrude egg sacs.

4.4 Discussion

This chapter has examined the reproductive development of *E.sieboldi* under field conditions in Rutland Water and under controlled laboratory conditions. A sampling programme over three consecutive years has led to an understanding of the reproductive activity of the parasite and how this relates to the population size of the parasite described in Chapter 3. Although previous studies have recorded the seasonality of reproductive development, few have had the opportunity to observe this over a prolonged period and to be able to record population changes.

All the results in this chapter are based on observations of parasites from rainbow trout. Other host species were encountered too infrequently to determine if reproductive development of *E.sieboldi* differed depending on host.

The seasonality of reproduction of *E.sieboldi* in Rutland Water was similar to that recorded by previous studies in temperate conditions (Gnadeberg 1948; Zmerzlaya 1972; Pojmanska 1984; Grabda-Kazubska *et al.*1987; Abdelhalim 1990). The adult female overwinters in a non-ovigerous state, begins oviposition in the spring, continues to produce eggs during the summer and begins to overwinter again in the late autumn. The consistency of this pattern over the three years of the study, allied with reports of the same occurrence from water bodies in different countries, confirms the strategy as successful in a wide range of conditions.

4.4.1 Development during the winter

Ovarian development during the winter was confirmed by analysis of ovary size and shape using criteria suggested in this study. In the examination of parasites in November and January, most parasites had developed from Stage I to Stage II ovary types as the oocytes in the ovary increased in size and the ovarian diverticula increased in size to accommodate them. Between January and March when water temperatures were at their lowest (3-6 ºC), development of ovaries continued with cephalosomes becoming distended due to the mass of oocytes developing in the ovary (Stage III). Throughout the winter period, a small proportion of ovaries remained undeveloped. In the April and May samples of infected angler caught fish for each of the study years, there was also a small proportion of non-ovigerous parasites present when the majority had developed egg sacs. This would suggest that a small proportion (<10%) of adult females fail to produce eggs after overwintering. Internal herniation of the diverticula is possible to explain some of the ovaries observed. This may be due to poor handling of the specimen or as a result of the rapidly expanding diverticula. In the free living copepod, *Pseudocalanus acsuspes*, collapsed ovaries were observed that may be due to senescence (Norrbin 1994). As the *E.sieboldi* being examined were overwintered, it is possible that they also were senescent.

There were also a small proportion of parasites that overwintered in an advanced ovarian Stage III. It was assumed that ovarian development was arrested in these parasites at the same time point in October but they already had fully formed ovaries in preparation for egg clutch extrusion. This would agree with the findings of Alston (1994) who recorded different stages of development during the winter in

Ergasilus briani and (Paperna & Zwerner 1976), studying *Ergasilus labracis*, who considered ovarian development to have slowed during the winter but not arrested. In contrast, Zmerzlaya (1972), studying *E.sieboldi,* reported that ovaries did not develop during the winter and that developed uterine processes were only observed in the spring, before oviposition took place.

4.4.2 Commencement of oviposition.

In this study, post-winter oviposition (more than 10% of parasites were ovigerous) was first observed when the water temperature reached 7 ºC. In Rutland Water this was in early April. Difficulties in acquiring infected fish at this time in each of the study years led to a degree of inaccuracy in confirming the exact start time but close observation of tank held fish confirmed the commencement of oviposition. Studies on *E.sieboldi* in the lakes of the Konin region of Poland that were subject to thermal pollution showed that oviposition occurred earlier in the warmest lakes (Pojmanska 1984). Sustained cold winter temperatures in central Finland resulted in oviposition first being reported in June when temperatures rose above 10 ºC (Tuuha, Valtonen, & Taskinen 1992). The results of this study combined with the others described, suggests that the temperature at which oviposition begins was not the "trigger" temperature to stimulate production but that the start of oviposition depends on the water temperatures, and therefore development taking place, in the weeks or months before oviposition is first observed. It had previously been suggested that photoperiod may be a control mechanism affecting egg production (Kuperman & Shulman 1977), however results from tank trials indicated that different light regimes had no effect on commencement of oviposition.

The infected trout held in tank systems showed that oviposition could be induced by raising the water temperature. This confirms the findings of Alston (1994), who raised the water temperature of infected tench (*Tinca tinca*) in tanks during February and observed egg sacs on parasites a few days later. In this study, tank held fish were subjected to increased temperatures at different times during the winter and at different temperatures. Analysis of the data was inconclusive in determining a minimum temperature threshold or date at which development of the first post winter clutch of eggs in the ovary began. This may have been because the development of eggs and ovaries began at a point before temperature was recorded. Due to failure of the temperature sensor on the cage system in 2006, temperature data was only available after the fish had been transferred to the aquarium systems at the Institute of Aquaculture (from 1st January 2006). As a result, the degree days of development that may have occurred prior to the 1st of January were not recorded and consequently the analysis of development at this time is difficult. This experiment should be repeated in order to understand the relationship between degree days and development at this crucial time. More replicates, and the use of constant temperatures from a time point before the start of overwintering may be more revealing about relationship of temperature and egg production.

4.4.3 Minimum temperature threshold for development

The minimum temperature at which development of ovaries and eggs is possible is an important parameter. An accurate estimate of these parameters allowed further modelling of reproductive activity to be based on degree days rather than

temperature. This permitted the application of laboratory findings to observations from the field.

Ovarian development was observed in *E.sieboldi* during the winter, when the water temperatures were 3 to 6 ºC. The minimum temperature for development of eggs in the ovary may be different from the minimum temperature for egg development within extruded egg sacs as the two are separate biological processes.

The most satisfactory means of measuring this threshold for egg development would be to perform laboratory studies to determine the minimum temperature at which development occurred. However, in this study, this was not possible due to invasive attack of eggs by epibionts during the prolonged period of development at low temperatures. Other studies were more successful in observing egg development at low temperatures. Alston (1994) successfully incubated egg sacs at 8 ºC but not at 6.3 ºC. Gnadeberg (1948) observed egg development at 4 ºC but found that viability was reduced. In field and tank observations, egg sacs had been extruded when the temperature was 7 ºC, and therefore it is assumed that development at this temperature would be successful.

Using the 2 statistical approaches described in 4.2.4, lower development threshold for egg development in this study was estimated to be 0.7 ºC (based on maximum development time of detached eggs) and 6.4 ºC (based on time for 50% of eggs to hatch). Neither of these methods of deducing temperature threshold was entirely satisfactory. The mean value of the two methods of determining threshold was therefore taken as a theoretical measure (3.6 ºC). Although this may have limited biological significance as egg sacs would not normally be extruded at this

temperature, it did allow a threshold for further modelling of development data in degree days.

4.4.4 Inter clutch interval

The period of time between egg extrusions was estimated by observing *E.sieboldi* on tank held rainbow trout. Recent advances in the development of digital cameras allowed multiple photographs to be taken at high resolution of the gill surface. Without the need to develop photographs, digital imaging proved to be a cost effective and efficient way of monitoring the development of eggs and parasites. In total, more than 50,000 images were captured and examined to chart the development of individual parasites during the experiment. Prior to this, the only method of estimating inter clutch interval would be from field observations of the proportion of parasites with or without egg sacs. This is an inaccurate measure as results are distorted by recent infections of non-ovigerous parasites that have yet to produce an egg clutch and also senile or barren parasites that are not capable of egg extrusion.

Urawa (1990) used observations of tank held infected fish to monitor the reproductive development of *Neoergasilus japonicus*. This species is predominantly found attached to fins and therefore it is simple to observe on captive fish without the need for photography. His findings for inter clutch interval were greater than was found with *E.sieboldi* in this study. At 15 ºC, inter clutch interval for *N. japonicus* was approximately 8 days. At the same temperature, inter clutch interval for *E.sieboldi* was only half a day. However the development rate of eggs following extrusion in *N. japonicus* was faster than *E.sieboldi*, suggesting that the

oocytes developing in the ovary were more advanced before extrusion into egg sacs than *E.sieboldi.*

4.4.5 Egg clutch production

In the field observations, of each of the study years, egg production was most synchronised in the first clutch production after overwintering. This was also observed in parasites infecting fish held in aquarium tanks. Due to small variations between parasites in time taken to produce and develop a clutch, there was less synchronicity between parasites with each subsequent clutch. This study did not identify the reasons for variability in development rate, but it is postulated that it is related to age of the parasite. Previous studies have shown that young parasites and old parasites produce fewer eggs in a clutch (Abdelhalim 1990). A difference in reproductive performance in these parasites may therefore explain variation in development times as overwintered parasites will be of different ages. Alternatively, this may form part of a strategy to ensure a constant supply of eggs from the population to ensure that the free living stages can benefit from times of optimal conditions. ‘Bet-hedging’ strategies have previously been described in *Argulus coregoni* (Fenton *et al.* 2006), where the egg hatching rate shows great variability at an intraspecific level. This mechanism, it is thought, has been selected to ensure successful transmission of the parasite in a highly variable environment.

The white opaque egg stage was shown to be the longest in duration once eggs have been extruded. The advanced blue/black stage had a much shorter duration and once egg sacs take on this appearance, hatching of nauplii soon follows. This was suggested by the proportion of these stages present in samples of angler caught fish.

Advanced stage of development was never more than 15% of the total number of *E.sieboldi* examined in a sample (14th May 2003). The short duration of the advanced stage was also confirmed by observations of egg development on infected tank held fish. Duration of both stages was negatively correlated with temperature in a non-linear pattern.

Production of egg clutches requires increased metabolic activity from the parasite as energy is required to produce and develop oocytes in addition to normal maintenance functions. Although this study did not study pathological effects of the parasite on the host, it was noted that during the period of egg clutch production, mucus production from the gill surface was greatly increased. This is illustrated in Plates 11 and 12 which show 2 un-cleaned gills that are infected with a similar number of *E.sieboldi*, one in March prior to the onset of egg clutch production, and one from July when clutch production was at a high rate. However, this may be due to the effects of temperature and oxygen saturation which would also have an effect on gill function at these times.

In this study it was demonstrated that *E.sieboldi* continually extrude egg sacs with inter clutch intervals from shortly after infecting the host. The period before egg clutch production begins in a newly attached parasite was estimated from a cage trial where fish were stocked and removed every 1 or 2 weeks in 2005. It was shown that in the months of July and August 2005, parasites could infect fish, produce eggs and develop them to advanced stage within a 2 week period. The mean temperature in this period was 18 ºC. From a model of egg development time, it would take 6.9 days from extrusion to hatch at this temperature. Therefore the first clutches of eggs were extruded less than 7 days after the parasite infected the

host. Between 17th and 24th June, parasites had infected fish but they had not extruded eggs before being removed. The mean temperature in this period was 16 ºC. This demonstrates that the pre-clutch interval was of a longer duration than the inter-clutch interval, and it is surmised that the adult female requires the nutritional requirements from feeding on the host in order to develop the ovary.

Most of the previous work on egg clutch production in ergasilids was based on field observations. As fish were killed before examination, estimates of number of egg clutches was based on the proportions of different development stages in each subsequent sample. This was shown by this study to result in a gross underestimate of the number of clutches produced by *E.sieboldi*. Abdelhalim (1990), suggested that 3-5 clutches of eggs were produced by *E.sieboldi* during the course of its life time. Alston (1994), working on *Ergasilus briani*, estimated that 4 clutches of eggs were produced by parasites infecting tench in 1992, although he recognised that this was probably an under estimate.

In this study, *E.sieboldi* infecting tank held fish were recorded as producing 10 clutches of eggs in two and a half months when temperature was raised to 15 ºC. Regression analysis of data from tank held fish and experimental egg development rates suggested that it was possible for overwintered parasites to produce 19 clutches of eggs between April and September 2005 in Rutland water. Urawa (1990), calculated from his tank observations that *N.japonicus* produced between 10 and 29 clutches in its life span. These results suggest that egg production in *E.sieboldi* and the close related *N.japonicus* are more similar in terms of clutch number than had been originally suggested. It also suggests that *E.sieboldi* has much greater fecundity than has previously been reported.

The effects of increasing the mean daily water temperature appeared to have a surprisingly small effect on the total number of egg clutches produced. The model of egg clutch production estimates 19 clutches produced in 2005 but increasing the temperature by 1 ºC increases the number of clutches produced to only 21 clutches. A 2 ºC increase results in a prediction of 22 clutches.

Rutland Water is a very large and deep (up to 40m) water body. In the spring, the water temperatures increase much more slowly than in shallower, smaller lakes in the same region. Increasing the temperature at this time may permit *E.sieboldi* to begin oviposition earlier, but water temperatures are still low and egg development is also prolonged.

An increased reproductive season due to higher temperatures at the end of the season may be more critical for Rutland Water. As the stocking of trout ends in September, and fewer anglers visit at this time of year, the stock turnover of trout is likely to be slower. A prolonged reproductive period for the parasite may lead to increased infection levels in fish that will overwinter to the following year. This will be discussed further in Chapter 6.

The equation to predict number of egg clutches was based on a number of assumptions with potential errors. Firstly the data that was the basis for the equation comes from the post overwintered population of *E.sieboldi*. The development rate of the parasite at different temperatures prior to overwintering is unknown. However, detached egg sacs required the same development time when derived from F1 newly attached parasites or from overwintered parasites. Secondly, the parasites that provided the data for the equation were artificially heated out of season in order to induce egg production. This may not translate to

the same developmental patterns under natural conditions. Thirdly, there was evidence from field observations that during September there was a reduction in the rate of egg development. As the tank held fish/parasite experiment was ended before development rates had slowed (if indeed it was the case), the equation was based on egg development and inter clutch intervals that may be faster than occurs in Rutland Water during a full year. Finally, the data was based on a limited range of recorded temperatures with the model based in tiers on estimations and therefore results for important parameters such as inter clutch interval and lower threshold of developmental temperature, if inaccurate, could have a larger effect on the outcome of the equation.

This study examined rate of egg development using three different methods: field observations; observations of parasites infecting trout held in tanks systems; and *in vitro* hatching of detached egg sacs. It was found that egg sacs that had been detached from the adult female developed faster than those that were still attached. This was more apparent at low incubation temperatures. A mechanism of maternal inhibition of hatching had previously been described in free living broadcast spawning calanoid copepods (Marcus 1982); (Kahan, Berman, & Bar-el 1988). Kahan (1988), was the first to describe it in a copepod that lays egg sacs, the harpacticoid *Tigriopus japonicus*. He considered the mechanism to be advantageous to the copepod in times when densities of nauplii were high, and that delaying egg development would be a strategy to ensure hatching at the most favourable conditions. In *T.japonicus*, an umbilical chord type structure was identified between the egg sac and the adult that would transmit an inhibition signal. It is unknown if such a mechanism exists in ergasilids, but would be worthy of further investigation.

Egg sacs can become detached from the adult at any stage of development, and as they are negatively buoyant, they would fall to the benthos and be as risk of predation. Therefore faster hatching of the eggs contained would be a useful strategy to avoid predation. Alston (1990) observed that advanced egg sacs when removed from a dark incubator would often start hatching as a result of being placed under an examination light. The same occurrence was noted in this study. This would also prove a useful strategy to avoid predation as the eggs would be subject to more light as they fell from the dark opercular cavity of the host into the open environment and thereby hatch before being at risk of predation. Free swimming nauplii were observed to be positively phototaxic in this study (see Chapter 5). It may be that at the most advanced stage of egg development, the inherent drive to move towards a light source assists the nauplii in hatching.

4.4.7 End of reproductive season

Some authors have referred to ergasilids entering a stage of diapause during the winter period (Baud et al. 2004;Kashkovsky & Kashkovskayasolomatova 1985;Kuperman & Shulman 1977;Pojmanska & Dzika 1987;Urawa, Muroga, & Kasahara 1991). Although diapause is a term often associated with an obligate arrest of development, some physiological processes may remain active in a state of facultative diapause (Hahn & Denlinger 2007). The findings of this study would indicate that the winter may be seen as a period of quiescence, but as egg production was easily induced by raising water temperature it should not be described as diapause.

The possible cues for entering this period are a period of reducing water temperature, a theoretical temperature threshold, changes in photoperiod, changes in host physiology or behaviour or an inherent biological mechanism that is independent of environment. In Rutland Water, oviposition was recorded in the spring when water temperatures were rising and ≈ 7 ºC. The observations of angler caught fish in 2003 to 2005 suggest that oviposition had ended during October when water temperatures fell below 14-15 ºC. The observations of a number of other studies support the findings that the reproductive season ends at a higher temperature than it began. Zmerzlaya (1972) showed oviposition began at 5.7-7 ºC and ended at 11-12 ºC. It therefore seems unlikely that a trigger temperature is the cue for cessation of oviposition.

A change in host behaviour as a potential cue is unlikely to be the trigger for cessation of oviposition. *E.sieboldi* infects a wide range of hosts, some of which may change behaviour in terms of the environment they inhabit at this time of the year, but many would not.

The effect of declining photoperiod was ascribed to the cessation of oviposition by Kuperman and Shulman (1977). In this study the effects of photoperiod were tested using tank held infected fish. Although photoperiod appeared to have no effect on commencement of oviposition in post overwintered parasites, the effects on cessation were not studied due to the premature end to the experiment. To test photoperiod effects it is recommended to repeat the tank held experiments with overwintered parasites and with parasites that had infected the fish in the summer before temperatures fall.

Prior to the end of oviposition in October, there was evidence from the field observations of *E.sieboldi* that rate of egg production had slowed during the month of September. This was indicated by an increased proportion of non-ovigerous parasites in the sample at this time. However, this was also the time of the greatest increase of new infections (see Chapter 3). Therefore, an increased number of new infections, combined with an increased pre-clutch interval as temperatures fall would account for the proportions of development stages of parasites observed on angler caught fish and fish held in the 2 week experimental cage (see figure 4.15).

An inherent biological mechanism in the parasite was possibly partly responsible for the change in egg production rates in the population during September and October. The reduced numbers of new infections occurring in late August suggests that when large numbers of new infections again infected fish in September, the majority of new infections were of a new cohort. This cohort may form the bulk of parasites that overwinter on the fish and the environmental cues for development of eggs may in part be different for these parasites than for the earlier cohort.

The strategy of slowing or arresting further egg production before temperatures fall below 14-15 ºC may be related to the success of the free living stages. As water temperature falls increasingly rapidly from October to January, conservation of energy reserves in preparation for the overwintering process may serve the population better than production of more nauplii which may be less successful in developing to adults in the cooler temperatures. In free living copepods, egg production rates have been related to food availability for the nauplii produced. The fastest egg production rates in *Calanus helgolandicus* have been associated with the period immediately before and during the spring phytoplankton bloom, the food

source for nauplii (Ceballos *et al*. 2006). In lecithotrophic nauplii such as those produced by *Lepeophtheirus salmonis,* nauplii food is not required and as a consequence, maximum hatching coincides with host availability (Tucker *et al*. 2000).

4.4.8 Generations, cohorts and longevity of *E.sieboldi*

A substantial difference between this study and previous studies is that here, the host population is directly managed. In Rutland Water, trout are stocked every week between March and September. Trout are caught by anglers and removed from the system every day between April 1st and December 31st. As a result of this stock turnover, the parasites on the fish have been attached for a shorter period of time than in a study of fish from an unmanaged lake. Some fish will be present in the system uncaught and therefore the parasite will complete its life as it would in a more natural environment, but in most cases the fish and the parasites attached to it are removed from the system before this would naturally occur.

This presents difficulties in estimating the longevity of the parasite as few fish were sampled with parasites that were old enough to be dying due to old age.

Observations on fish with different intensities of infection that were removed from the reservoir in December 2006 to aquarium tanks where no new infections occurred did indicate the life span of parasites that had overwintered. In the fish with the largest number of parasites, that was assumed to have been present in the reservoir for the longest period of time, *E.sieboldi* started to die off from May 2007. The fish with the medium level of infection started to lose parasites from July 2007, and the

fish with the lightest infection level began to lose parasites in August 2007. This suggested a life span of 10-12 months for parasites that had infected fish from late summer onwards. This would agree with the findings of Pojmanska (1984). However, Pojmanska indicated that the first new infections of the year did not survive long enough to overwinter. This cannot be confirmed or challenged by this study as there were not sufficient overwintered fish available in the summer to monitor a possible decrease in parasite abundance.

The examination of the development stage of parasites from rainbow trout caught by anglers and those held in experimental cages has provided data on the proportionality of parasites at any given stage of the reproductive season. With 2 large peaks of non-ovigerous parasites occurring during the infective period (June/July and then again in September) (see Chapter 3), it could be proposed that there are 2 cohorts of infection in each season. This would agree with the findings of similar studies of *E.sieboldi* in temperate lakes (Abdelhalim, Lewis, & Boxshall 1991;Alston 1994;Kashkovsky & Kashkovskayasolomatova 1985;Pojmanska 1984).

In this study, the two cohorts are separated by a reduction in new infections occurring at the end of August, and also by different egg production activities observed in the population before and after this period as discussed earlier.

The composition of these cohorts is more complex, as they do not relate to single generations. The post-overwintered generation in the spring was defined as the P generation. In April this generation began oviposition, and the findings of this study indicated that oviposition continued until the parasite died 10-12 months after it first infected the host. The first progeny of this P generation (F1) were hatched in late

April/early May and infected fish from mid June onwards. As the P generation continued to produce egg clutches, the F1 generation increased in size throughout the summer and is regulated in size by the size of the P generation and the success of the free living stages. Due the increasing numbers of the parasites in the P generation dying as the summer progressed, the rate of increase of the size of the F1 generation would also fall.

From late June, the F1 generation began to hatch a new F2 generation. This generation developed through the free living stages and infected fish from August onwards, and in combination with the continued production of F1 parasites, is responsible for the exponential increase in the *E.sieboldi* population seen in 2003.

In the Rutland Water trout fishery, the P generation were present on a limited number of fish. Only those that had overwintered at the end of the season are hosts for *E.sieboldi*. From these, the F1 generation infected large numbers of fish very rapidly (see Chapter 3 for increase in prevalence at this time). The size of the P generation, the successful hatching of eggs, the availability of hosts and the success of the free living stages are the three key elements to the success of the population.

Chapter 5 will examine another of these elements, the success of the free living stages.

Chapter 5: Feeding and development of *Ergasilus sieboldi* nauplii

5.1 Introduction

5.1.1 The laboratory culture of ergasilid free living stages

Several authors have attempted to culture ergasilids from egg to adult under experimental conditions (Alston, Boxshall, & Lewis 1996;Ben Hassine & Raibaut 1981;Mirzoeva 1972;Paperna & Zwerner 1976;Urawa et al. 1980;Urawa, Muroga, & Kasahara 1980;Wilson 1911). In all cases, the investigators have been unable to complete the life cycle under laboratory controlled conditions. Successful completion of the life cycle has only been achieved in a semi-natural culture system where water conditions and food availability was not controlled and was largely unknown.

The life cycle of *E.sieboldi* was first successfully observed by Zmerzlaya (1972) by hatching eggs into plankton net cages submerged in a water body where there was a population of *E.sieboldi* on the fish. In 1991 Abdelhalim obtained all the free living stages of *E.sieboldi* from outside experimental tanks that contained infected fish and a considerable population of *flora* and *fauna* that had developed naturally. The purpose of both these studies was to obtain free living stages of the parasite for morphological descriptions of the stages of the life cycle. However, Abdelhalim (1991) using SEM techniques, described the occurrence of six naupliar stages whereas Zmerzlaya (1972) only recognised three separate stages using conventional light microscopy. Both studies confirmed that five copepodid stages exist for this species.

5.1.2 The feeding activities of the free living ergasilids

Little attention thus far has been given to the feeding of the free living stages beyond that necessary to provide a culture of specimens for morphological studies. When

attempts have been made to culture the free living stage in controlled conditions, little success has been achieved. Zmerzlaya (1972) and Alston (1994) were not able to develop *E.sieboldi* nauplii past the first stage when cultured in the presence of unicellular algae. Ben Hassine (1981) observed development of the euryhaline ergasilid *E.lizae* with the addition of unicellular algae but also in the absence of food which led her to believe that earlier naupliar stages of this species were lecithotrophic. Paperna & Zwerner (1976), working with *E.labracis,* observed some development in the naupliar stages with the addition of nanoplankton, but the species or mixtures of species used was not reported and neither was the number of stages of development.

Other authors have suggested that some of the naupliar stages of ergasilids may be lecithotrophic (Ben Hassine & Raibaut 1981;Paperna & Zwerner 1976;Wilson 1911). However Alston (1996) and Urawa (1980a), showed that all the naupliar stages of the freshwater ergasilids *E.briani* and *N.japonicus* were at least capable of feeding as they had functional mouth parts, gut and anus from the first naupliar stage. Alston (1990) also attempted the culture of *E.sieboldi,* in vessels that allowed the passage of phytoplankton smaller than the mesh sized used, into an inner chamber containing the nauplii. In these experiments he observed a 'green amorphous mass' in the gut of the nauplii that he attributed to small algal cells. When food particles were limited to <5µm, the nauplii apparently died of starvation. When large mixed particles of up to 40 µm were introduced, feeding and development took place. The first stage nauplii also produced faecal casts but it was not possible to identify the species of algae consumed. In 3rd stage nauplii (NIII), Alston recorded the presence of the algae *Scenedesmus* spp. in the gut. His attempts at culturing nauplii in monocultures of different green and

brown algal species and a monoculture of ciliates all failed with all nauplii dying within 3 days with no food present in the gut.

Due to the difficulties in culturing ergasilid nauplii, there have been few copepodids cultures. Consequently, there have been few reports on the feeding of the copepodid stages of ergasilids. Urawa (1980), who cultured *N.japonicus* to the first copepodid stage in pond water, found that copepodids died quickly after emerging from the naupliar stage. Ben Hassine (1983, cited in Alston 1994) had more success with *E.lizae* when the culture tank contained a benthic substrate of mud and macrophytes taken from a water body where the parasite existed. She observed the copepodid and adult stages staying close to this benthos suggested that feeding took place here. The change in behaviour, coupled with the development of functional adult mouth parts in the first copepodid of ergasilids (Fryer 1978) and the failure for further development in planktonic cultures led these authors to believe that development to copepodid stages required different nutritional sources from nauplii for further development to take place.

5.1.3 The development of *E.sieboldi* from nauplii to adult

The results of the field examination of rainbow trout in Chapter 4 suggested that *E.sieboldi* eggs were first extruded in early April, nauplii were hatching from egg sacs in late April before re-infecting fish in mid June. This suggested a development time of 10-11 weeks (egg to adult) or 7-8 weeks from nauplius to adult (including time to mate and find a host) at temperatures of 9-16 ºC.

In this study, observations were of trout regularly stocked and removed from a trout fishery. Due to the overlapping development of eggs and free living stages during the

summer it was not possible to estimate the development times of the free living stages of the parasite after the initial infections were recorded. However, if it is assumed that nauplii development rate, like egg development rate, is related to temperature (in the absence of limiting factors such as food availability), nauplii hatching later in the summer would develop substantially faster. The work of Zmerzlaya (1972) who studied *E.sieboldi* in Alol Lake in Russia with a similar summer temperature profile to Rutland Water, suggested that the first generation of *E.sieboldi* of the year require 9-10 weeks development time from egg to adult and the when the temperature reached 17.7-20.1 ºC, the development time fell to 22 days. However if the food source of the nauplii does become a limiting or inhibiting factor due to quantity or type available, it is possible that development rate of the free living stages will extended, and consequently, the success of the *E.sieboldi* population will be reduced.

5.1.4 The aims of this study

The success of the *E.sieboldi* population in Rutland Water is dependent upon several factors, the understanding of which is important in modelling the infection in a fishery. The population dynamics of the adult female parasite were reported in Chapter 3. Consideration of the reproductive success of the population was presented in Chapter 4. This chapter will examine the development and feeding of the free living naupliar stages of *E.sieboldi*, and its potential role in regulating the population changes that have been recorded.

As a drinking water reservoir, Rutland Water has statutory obligations to regularly sample the water body for a number of parameters including the quantity and types of algae present. This has generated a data set of algal cell counts for many years,

including monthly samples during the study years of 2003-2005. Anglian Water has kindly allowed access to this data set in order to try to relate the data to the findings of this study, and to determine if it may be related to the success of the *E.sieboldi* population.

The aims of this study were therefore firstly, to develop a successful methodology for the controlled development of free living stages of *E.sieboldi* following hatching from the egg. Secondly, by exposing the nauplii to monocultures and defined mixtures of algae from cultures of species representative of different types present in Rutland Water, to determine which species were palatable to the nauplii, and, if possible, which would provide a good nutritional supply that would allow development through different stages of the parasite. The findings would then be compared to the field data from the algal sampling in Rutland Water to determine if the presence of algal species or types may in part be responsible for the higher levels of transmission seen in 2003 compared to 2004 and 2005.

The results from the field and cage trial observations discussed in Chapter 4 appeared to suggest that egg development rate was slower in September when the adult population was of a second cohort that were preparing to overwinter. The development rate and conditions of nauplii produced from eggs extruded at this time were compared to eggs developed earlier in the season.

5.2 Materials and methods

5.2.1 Hatching of *E.sieboldi* eggs

The hatching of *E.sieboldi* eggs was observed by removing advanced stage egg sacs from adult female parasites using mounted needles and transferring them with a glass Pasteur pipette into a Petri dish containing water from the tank that held the fish. Videos of nauplii hatching from *E.sieboldi* eggs were recorded using a JVC TK-CI480B camera attached to an Olympus SZ40 dissecting microscope with an Olympus SZ-CTV mount. Video was recorded in mpeg format and plates were captured using screen grab to tiff format.

5.2.2 Culturing methods for *E.sieboldi*

5.2.2.1 Progeny of the F1 generation of *E.sieboldi*

A number of different methods were tested for the culturing of *E.sieboldi* in laboratory conditions. Vessel volume, temperature of culture, light conditions, aeration and proportion of water changes were tested to find best practice.

From July 2006 to August 2006, a number of different culturing methodologies were used to try and develop nauplii of *E.sieboldi*. Table 5.1 is a summary of the different conditions for used. Egg sacs were removed from F1+ ovigerous females and incubated and hatched as described in Chapter 4. All nauplii hatching on a single day were then transferred to a culturing vessel until each vessel contained approximately 600 nauplii per litre. The vessels were 1 litre glass beakers, 10 litre buckets or a 100 litre black bin with sides and base covered in black electrical tape to ensure uni-directional light from above. One litre and 10 litre vessels were held in a Binder environmental chamber. One hundred litre bins were held in a compound in the grounds of the University of Stirling

with a Gemini data probe in the bottom to record water temperature at 1 hour intervals. Water was collected daily from Airthrey Loch on the campus of the University of Stirling and filtered at 53 µm using a nylon mesh.

The conditions for each group are detailed in Table 5.1. Each group was repeated in triplicate. Daily quantities of nauplii remaining in each vessel were estimated by taking four aliquots of 5ml each using a Stempel pipette sequentially at four different depths from top of the vessel to the bottom and placing the 20ml in a Petri dish for examination and counting under an Olympus SZ40 dissecting microscope (if large quantities of nauplii were present, a Bogorov counting chamber was used). Daily examination of the development of nauplii took place by removing 5 specimens from the Petri dish to a cavity slide, measuring maximum body length of nauplii (not including caudal knob) and identifying stage based on the descriptions given by (Abdelhalim, Lewis, & Boxshall 1991) using an Olympus BH2 compound microscope and an eye piece graticule. Any remaining nauplii in the Petri dish were returned to the vessel as soon as possible.

Water was filtered prior to being added to each vessel by passing through a 53 µm nylon mesh. Water was changed each day by partially submerging a glass cylinder with a 30 µm mesh at the lower end into the vessel. The water that flowed into the cylinder from the vessel via the mesh was pipetted out and replaced with freshly collected water that had been filtered with a 53 µm nylon mesh and allowed to equilibrate to the temperature of the vessel. When aeration was added it was by means of compressed air through a 1” air stone at the bottom of the vessel. The aeration was sufficient to provide movement of water within the vessel but gentle to avoid damage to the nauplii.

Table 5.1 Summary of conditions under which *E.sieboldi* nauplii from F1 generation were cultured in July to September 2006.

Group	Size of vessel	Temp(°C)	Light conditions (hours per day)	Aeration	Water changes (per day)
A	1 l	10	Dark	Yes	30%
B	1 l	15	Dark	Yes	30%
C	1 l	10	Constant light	Yes	30%
D	1 l	15	Constant light	Yes	30%
E	1 l	10	16 light/ 8 dark	Yes	30%
F	1 l	15	16 light/ 8 dark	Yes	30%
G	1 l	10	16 light/ 8 dark	No	50%
H	1 l	15	16 light/ 8 dark	No	50%
I	1 l	10	16 light/ 8 dark	Yes	100%
J	1 l	15	16 light/ 8 dark	Yes	100%
K	10 l	10	16 light/ 8 dark	Yes	30%
L	10 l	15	16 light/ 8 dark	Yes	30%
M	10 l	20	16 light/ 8 dark	Yes	30%
N	10 l	11 (light) 9 (dark)	16 light/ 8 dark	Yes	30%
O	10 l	16 (light) 14 (dark)	16 light/ 8 dark	Yes	30%
P	1 l	Outdoors, 10-22	12-17 light per day	Yes	30%
Q	100 l	Outdoors, 10-22	12-17 light per day	Yes	none

5.2.2.2 Progeny of the P generation of *E.sieboldi*

From April to May 2007, nauplii development studies were carried out on the progeny of *E.sieboldi* that had overwintered (P generation parasites). The techniques for hatching were the same as detailed above and the groups tested are detailed in Table 5.2.

Table 5.2 Summary of conditions under which *E.sieboldi* nauplii from the P generation adults were cultured in April to May 2007.

Group	Size of vessel	Temp(°C)	Light conditions (hours per day)	Aeration	Water changes (per day)
R	1 l	10	16 light/ 8 dark	Yes	30%
S	1 l	15	16 light/ 8 dark	Yes	30%
T	1 l	20	16 light/ 8 dark	Yes	30%
U	1 l	Outdoors, 6-15	13-17 light per day	Yes	30%
V	100 l	Outdoors, 6-15	13-17 light per day	Yes	none

5.2.3 Culturing of nauplii in algal monocultures and controlled polycultures

E.sieboldi nauplii were cultured in the presence of a single species of algae to determine if the algae were palatable to the nauplii, and if growth, development or increase in longevity would be achieved through the consumption of the algae. Mixed cultures of specified algae were also tested against the same parameters and to determine if *E.sieboldi* had a preference for, and was able to select different types of algae.

Batch cultures of single celled algae were cultured using 2 litre glass conical flasks containing Jaworski's media (JM). JM was prepared by adding 1ml of each of 9 stock solutions to 1 litre of distilled water which was then autoclaved at 15 PSI for 15 minutes. Each stock solution was prepared in 200ml distilled water according to the quantities below and were used once cooled. Fresh batches were prepared for new cultures.

1) $Ca(NO_3)_2.4H_2O$ 4.0 g
2) KH_2PO_4 2.48 g
3) $MgSO_4.7H_2O$ 10.0 g
4) $NaHCO_3$ 3.18 g
5) EDTAFeNa 0.45 g + $EDTANa_2$ 0.45 g
6) H_3BO_3 0.496 g + $MnCl_2.4H_2O$ 0.278 g + $(NH_4)_6Mo7O_{24}.4H_2O$ 0.20 g
7) Cyanocobalamin 0.008 g + Thiamine HCl 0.008 g + Biotin 0.008 g
8) $NaNO_3$ 16.0 g
9) $Na_2HPO_4.12H_2O$ 7.2 g

Each conical flask was inoculated with the 3-4mls of relevant algae stock culture obtained from Sciento Ltd, Manchester, UK. Gentle aeration was supplied to each flask through a drawn out glass pipette through a cotton wool bung in the top of each flask. Cultures were kept in a Binder environmental cabinet under constant light conditions until algal had reached the exponential phase of growth. Algae were decanted as necessary from the flasks to sterile bacteriophage tubes and cell counts measured using a haemocytometer. A Gilson pipette was then used to allocate the relevant quantities of

algal cells to 1 litre glass beakers containing loch water that had been double filtered to 0.2 µm using a Whatman syringe filter.

Egg sacs were removed from P generation adult parasites from overwintered rainbow trout and hatched using methods described above and in Chapter 4. Approximately 200-250 nauplii were added to each culture and beakers were placed in a Binder environmental chamber set at 16 hours light (16 ºC) and 8 hours dark (14 ºC). The groups of monocultures and polycultures used are detailed in Table 5.3.

Table 5.3 Summary of monocultures and polycultures used to determine nauplii feeding.

Group	Algae	Cell count (ml^{-1})
Control	None	None
MonoA	*Cryptomonas* sp.	5000
MonoB	*Cryptomonas* sp.	10000
MonoC	*Cryptomonas* sp.	20000
MonoD	*Haematococcus* sp.	5000
MonoE	*Haematococcus* sp.	10000
MonoF	*Haematococcus* sp.	20000
MonoG	*Euglena* sp.	5000
MonoH	*Euglena* sp.	10000
MonoI	*Euglena* sp.	20000
MonoJ	*Chlorella* sp.	5000
MonoK	*Chlorella* sp.	10000
MonoL	*Chlorella* sp.	20000
PolyA	*Cryptomonas : Haematococcus* (50:50)	10000
PolyB	*Cryptomonas : Euglena* (50:50)	10000
PolyC	*Cryptomonas : Chlorella* (50:50)	10000
PolyD	*Haematococcus : Euglena* (50:50)	10000
PolyE	*Haematococcus : Chlorella* (50:50)	10000
PolyF	*Euglena : Chlorella* (50:50)	10000
PolyG	*Cryptomonas : Haematococcus* (10:90)	10000
PolyH	*Cryptomonas : Haematococcus : Euglena* (33 :33 :33)	10000
PolyI	*Cryptomonas : Haematococcus ; Chlorella* (33 :33 :33)	10000
PolyJ	*Cryptomonas : Euglena : Chlorella* (33 :33 :33)	10000
PolyK	*Haematococcus : Euglena : Chlorella* (33 :33 :33)	10000
PolyL	*Cryptomonas : Haematococcus : Euglena: Chlorella* (25:25:25:25)	10000

Ten nauplii were removed each day from each of the cultures and examined live in a cavity slide under an Olympus BX51 microscope with a JVC TK-CI480B camera and photographs were taken as detailed in Chapter 2. Nauplii length and development stage were recorded as before. Nauplii were then fixed in 4% phosphate buffered formalin. 300mls of water (30%) was changed in each culture vessel everyday through a 30µm sieve as before to avoid loss of nauplii from the system. Additional algae were added to restore the approximate cell count for each group.

5.2.4.1 Confocal imaging of lipids in nauplii.

Eggs from P generation adult *E.sieboldi* were hatched in 0.2µm loch water in a 35mm Petri dish. Five nauplii were collected immediately after hatching in a finely drawn out glass pipette and fixed in 4% phosphate buffered formalin for 24 hours. The remaining nauplii were left for 48 hours at 15°C in a Binder environmental cabinet with light regime 16 light: 8 dark.

Fixed nauplii were then removed from formalin and immersed in 80µM solution of Bodipy 505/515 (non polar stain for lipids) in DMSO and distilled water for 1 hour in dark conditions. The individual nauplii were transferred to Iwaki 35mm Glass Base Dishes (Iwaki, Asahi Techno Glass, Japan). Imaging was carried out using sequential slice methodology with a Leica TCS SP2 AOBS Confocal Scanning Laser Microscope (CSLM) and viewed with Leica Confocal Software version 6.21.

Nauplii 48 hours post- hatch were then fixed in 4% phosphate buffered formalin for 24 hours and processed as above. The procedure above was repeated with naupliar progeny of F1 adult *E.sieboldi*.

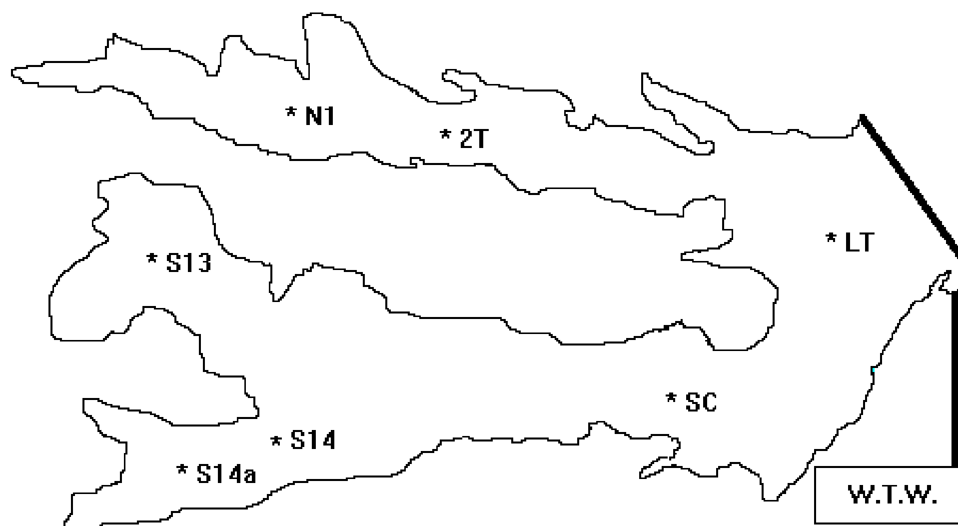
5.2.4.2 Confocal imaging of nauplii structure

General imaging of nauplii structure was obtained by staining formalin fixed nauplii in Blankophor solution (1µl in 1ml distilled water) for 30-40 minutes. Imaging was carried out using 3 dimensional reconstruction of sequential slice images using equipment and software described in 5.2.4.1. Five nauplii from eggs extruded by P were fixed within 1 hour

5.2.5 Analysis of historic algal counts from Anglian Water

Records of algal counts from water samples collected from seven sites in Rutland Water (see Figure 5.1) between 2003 and 2005 were provided by Anglian Water Central Laboratories, Huntingdon. Algae were identified to class, genus or species level and analysis was performed by the author using MS Excel. Algal quantities were in terms of cells per ml. As it was unknown which areas of the reservoir may serve as a nursery for the free living stages of *E.sieboldi*, the results from all 7 monitoring sites were totalled for each monthly sample.

Figure 5.1 Location of sampling sites for algae analysis at Rutland Water. Code numbers for each site are those designated by Anglian Water.



5.3.1 Hatching of *E.sieboldi* eggs

The hatching of advanced eggs within a sac was usually accomplished within 20 minutes of the first egg beginning to hatch. Occasionally the process could take up to an hour. In the first instance, the egg sac was seen to rupture, usually in the proximal part of the egg sac close to where it attaches to the adult (see Plate 23). In most cases this was followed by multiple ruptures in several parts of the egg sac and some eggs on the outer margins of the sac appeared to move away from the bulk of eggs, possibly due to water flowing into the sac and a release of pressure rather than movement of nauplii within the eggs. In the next stage, nauplii were observed to increase in length due a slight extension of the caudal knob and possibly also straightening in the dorso-lateral plane. The nauplii then breached the thin single layered egg capsule at the anterior end with the naupliar rostrum (see Plate 24). Antenna and antennules of the nauplii then moved laterally to be in contact with the surface of the egg capsule causing it to expand its width. This had the effect of pulling the egg capsule further over the rostrum and increasing the size of the breach (see Plate 25). When the breach reached the joint between the antennules and the body, the nauplii rapidly moved the antennules while the antenna directed more laterally moved in an opposite direction to draw back the capsule which by now was breached to the full width of the nauplii (see Plate 26). Further agitation of the antenna and antennules then allowed the nauplii to swim free of the capsule (see Plates 27 and 28), at which point the antennules moved forward into the normal resting and swimming position (see Plate 29). The discarded egg capsule remains visible after the nauplius has hatched and moved away (see Plate 30).

5.3.2 Culturing methods for *E.sieboldi*

5.3.2.1 Progeny of F1 generation of *E.sieboldi*

A number of different culture methods were tested to determine the best method for developing *E.sieboldi* nauplii under controlled conditions (see 5.2.1). The maximum stage of nauplii developed in any of the cultures was NV. The longest duration before a culture failed was 19 days.

Table 5.4 shows the results for each of the groups tested in terms of duration of culture before the number of nauplii per litre of water fell below 5 (at which point the culture was classed as failed). The results are for the most successful of each of the three replicates for each group. Means were not calculated as individual crashes in populations would skew results and may not be due to the parameters being tested.

Table 5.4 Summary results of the culture of the progeny of F1 *E.sieboldi*. Performance of each culture group is assessed in terms of most advanced developmental stage of *E.sieboldi* reached, and the duration of the culture before numbers of nauplii dropped below 5 l^{-1}.

Group	Most advanced nauplii stage (in any of the three replicates for each group)	Number of days before failure of culture (maximum of the three replicates for each group)
A	I	3
B	I	2
C	I	6
D	I	8
E	IV	14
F	IV	12
G	I	6
H	I	5
I	I	6
J	II	6
K	III	14
L	V	13
M	II	6
N	III	19
O	V	17
P	IV	14
Q	V	19

The results of the various culture methods suggest the following;

1) Culture vessel volume appeared to have little effect on the development rate or the duration of the culture. The lack of aeration did reduce the success of the cultures and was not helped by increased volume of water change.

2) Constant dark cultures failed within 2 days. No food matter was observed in the guts of the nauplii cultured in dark conditions. Green or olive to brown matter was seen in the gut of nauplii from all other groups (see Plates 31 and 32).

3) Constant light cultures lasted longer but nauplii failed to develop.

In most cases, cultures lasted slightly longer at 10 ºC than at 15 ºC, but less development took place within this time. At 20 ºC, culture duration was reduced further but development was also reduced. Reducing the temperature by 2 ºC during the dark cycle appeared to increase the duration of the culture. However, development rate was also slightly reduced.

Nauplii were positively phototaxic in all cultures. However, as cultures began to fail and numbers of nauplii were reducing, an incidental observation was that the taxis response to light was diminished in most nauplii. This effect was not as apparent in the cultures maintained outdoors as nauplii examined appeared healthy and phototaxic on every day they were examined. Unfortunately, after approx ten days, nauplii and subsequently copepodids of *Cyclops* sp. appeared in these cultures and numbers of *E.sieboldi* declined rapidly.

The gut contents of nauplii were examined using standard light microscopy of the intact specimen or by extruding the contents with gentle pressure on the cover slip to rupture

the specimen. Gut contents were green or olive and algal cells were either too damaged or digested to permit identification. In the water used, there were many types of algae present including blue greens (Cyanophyta) *Nostoc* sp. and *Anabaena* sp., greens (Chlorophyta) *Chlorella* sp., *Chlamydomonas* sp., *Scenedesmus* sp., dinoflagellates *Ceratium* sp., diatoms *Asterionella* sp., and Cryptophyta *Cryptomonas* sp. and *Rhodomonas* sp.

5.3.2.2 Progeny of the P generation of *E.sieboldi*

Having determined the most successful methods of culturing the progeny of the F1 generation of *E.sieboldi*, the progeny of overwintered parasites were hatched and developed in culture conditions outlined in 5.2.1.2. The results for these cultures are summarised in Table 5.5.

Table 5.5 Summary results of the culture of the progeny of P generation *E.sieboldi*. Performance of each culture group is assessed in terms of most advanced developmental stage of *E.sieboldi* reached, and the duration of the culture before numbers of nauplii dropped below 5 l^{-1}.

Group	Most advanced nauplii stage (in any of the three replicates for each group)	Number of days before failure of culture (maximum of the three replicates for each group)
R	II	18
S	I	6
T	I	4
U	II	16
V	II	22

The most advanced stage of nauplii examined in the cultures of P generation progeny was stage NII. The longest duration of a culture was 22 days. Although development stages reached were less than with the progeny of F1 parasites, P generation nauplii performed better in terms of duration at the lowest temperature tested (10 ºC).

Conversely, they performed less well than F1 generation nauplii at the higher temperatures of 15 and 20 ºC.

5.3.2.3 Size of nauplii.

Measurements were taken of the length (not including caudal knob) of 5 nauplii examined each day in each of the cultures. The mean, standard deviation and number of specimens examined is reported in Table 5.6 for nauplii hatched from F1 and P generation *E.sieboldi.*

Table 5.6 Summary data of the mean length of *E.sieboldi* naupliar stages NI to NV measured from live specimens in water. Results for progeny of F1 and P generation of adult parasites removed from angler caught rainbow trout in 2006 and 2007.

	Progeny of F1 generation			Progeny of P generation		
Stage	Mean length (µm)	Standard deviation	N	Mean length (µm)	Standard deviation	N
NI	81.2	4.7	100	90.3	8.2	100
NII	106.8	6.2	50	116.5	9.1	31
NIII	148.7	9.6	22	-	-	-
NIV	165.2	9.4	25	-	-	-
NV	198.4	19.1	10	-	-	-

The results for measurements from the progeny of F1 parasites in Table 5.6 are graphically illustrated in Figure 5.2. A comparison of the sizes of NI and NII stage nauplii from F1 and P generation *E.sieboldi* is made graphically in Figure 5.3.

A statistical comparison of the mean lengths of NI and NII nauplii from F1 and P generation adult *E.sieboldi* was made using t test with Welch's correction to account for differences in standard deviation. All groups were normally distributed as confirmed by Kolmogorov and Smirnov statistic.

NI mean length (F1) *vs.* NI mean length (P), $t = 9.628$, $p = <0.0001$

NII length (F1) *vs.* NII mean length (P), $t = 5.230$, $p = <0.0001$

In both cases, the NI and NII nauplii progeny of the P generation are significantly (95% confidence) larger than the same stages as progeny of the F1 generation.

Figure 5.2 Bar chart of mean length of *E.sieboldi* naupliar stages hatched from F1 generation adult parasites removed from rainbow trout. Error bars indicate standard deviation.

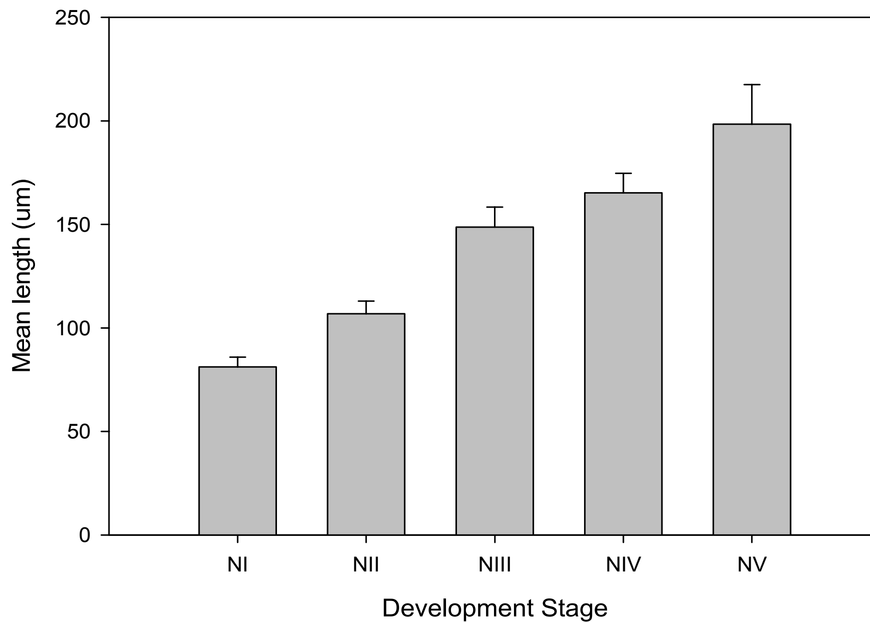
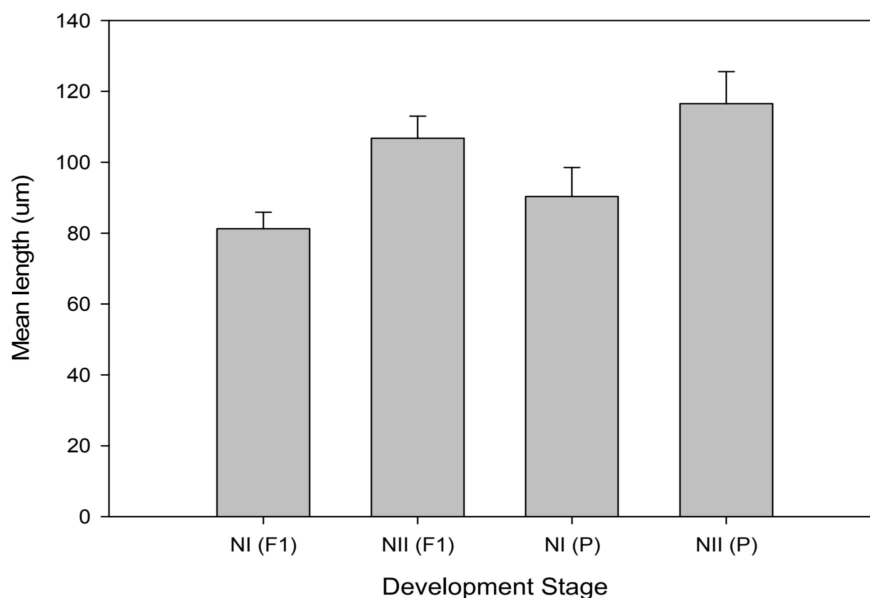


Figure 5.3 Bar chart of mean length of *E.sieboldi* naupliar stages I and II comparing those hatched from F1 and P generation adult parasites removed from rainbow trout. Error bars indicate standard deviation.



5.3.3 Culturing of nauplii in algal monocultures and controlled polycultures

5.3.3.1 Monocultures

The results of the culturing of nauplii in cultures of a single algal genus are summarised in Table 5.7.

Starved nauplii in the control groups died within 3 days of hatching. No development to stage II nauplii occurred in any of the monocultures. Green or olive food, presumably algae was observed in the gut of nauplii of all groups except the starved control group. The nauplii fed on *Cryptomonas* sp. (see Plates 33 to 35) had the longest duration before numbers fell to less than 5 l^{-1}. Nauplii fed on *Chlorella* sp. (see Plate 36) were the second best performers in terms of duration of culture. Performance of nauplii in cultures of *Haematococcus* sp. (see Plates 37 and 38) and *Euglena* sp. was the same as cultures lasted for 6-7 days.

Table 5.7 Summary results of the culture of the progeny of P generation *E.sieboldi* in monocultures of single algal types. Performance of each culture group is assessed in terms of most advanced developmental stage of *E.sieboldi* reached, and the duration of the culture before numbers of nauplii dropped below 5 l^{-1}.

Group	Algae	Cell count (ml^{-1})	Most advanced nauplii stage (in any of the three replicates for each group)	Number of days before failure of culture (maximum of the three replicates for each group)
Control	None	None	I	3
MonoA	*Cryptomonas* sp.	5000	I	9
MonoB	*Cryptomonas* sp.	10000	I	10
MonoC	*Cryptomonas* sp.	20000	I	4
MonoD	*Haematococcus* sp.	5000	I	7
MonoE	*Haematococcus* sp.	10000	I	7
MonoF	*Haematococcus* sp.	20000	I	6
MonoG	*Euglena* sp.	5000	I	7
MonoH	*Euglena* sp.	10000	I	7
MonoI	*Euglena* sp.	20000	I	6
MonoJ	*Chlorella* sp.	5000	I	8
MonoK	*Chlorella* sp.	10000	I	9
MonoL	*Chlorella* sp.	20000	I	8

It was observed that nauplii in all cultures with algal cell counts of 20000 ml^{-1} did not perform any better than in cultures of 10000 ml^{-1} in terms of duration. In fact, the cultures with 20000 ml^{-1} *Cryptomonas* sp. cells performed less well. It was observed that at higher densities these cells became colonial and nauplii were caught in the colonies and immobilised. Shortly after this occurred, the nauplii would die. For the controlled polyculture experiments, all cell counts were to be performed at 10000 ml^{-1}.

5.3.3.2 Controlled polycultures

The results of the culturing of nauplii in cultures of a single algal genus are summarised in Table 5.8.

The results of the controlled polyculture experiments suggested that development of the nauplii to stage II only occurred when the three algal genera *Cryptomonas, Haematococcus* and *Chlorella*, were available (see Plate 39). When the same cultures were used, but *Haematococcus was* replaced with *Euglena*, development did not occur *Cryptomonas* sp. and *Chlorella* sp. were favoured algal cells and were observed more often in the gut of the nauplii than *Haematococcus* sp. or *Euglena* sp. This was the case even in cultures such as PolyG where only 10% of the cells were *Cryptomonas* sp. The nauplii had appeared to select this species in preference to *Haematococcus* sp. In Group PolyJ (*Cryptomonas : Euglena : Chlorella* (33 :33 :33)), the maximum duration of the culture was 16 days. In this time, development had failed to take place in the nauplii examined. This was the longest duration of any culture where development did not take place.

Table 5.8 Summary results of the culture of the progeny of P generation *E.sieboldi* in monocultures of single algal types. Performance of each culture group is assessed in terms of most advanced developmental stage of *E.sieboldi* reached, and the duration of the culture before numbers of nauplii dropped below 5 l^{-1}.

Group	Algae	Most advanced nauplius stage (in any of the three replicates for each group)	Number of days before failure of culture (maximum of the three replicates for each group)
Control	None	I	3
PolyA	*Cryptomonas : Haematococcus* (50:50)	I	12
PolyB	*Cryptomonas : Euglena* (50:50)	I	11
PolyC	*Cryptomonas : Chlorella* (50:50)	I	17
PolyD	*Haematococcus : Euglena* (50:50)	I	7
PolyE	*Haematococcus : Chlorella* (50:50)	I	9
PolyF	*Euglena : Chlorella* (50:50)	I	10
PolyG	*Cryptomonas : Haematococcus* (10:90)	I	12
PolyH	*Cryptomonas : Haematococcus : Euglena* (33 :33 :33)	I	13
PolyI	*Cryptomonas : Haematococcus ; Chlorella* (33 :33 :33)	II	18
PolyJ	*Cryptomonas : Euglena : Chlorella* (33 :33 :33)	I	16
PolyK	*Haematococcus : Euglena : Chlorella* (33 :33 :33)	I	10
PolyL	*Cryptomonas : Haematococcus : Euglena: Chlorella* (25:25:25:25)	II	18

5.3.4 Confocal imaging of lipid droplets in nauplii progeny of P and F1 *E.sieboldi*

Confocal images of starved nauplii from P generation adult females, immediately after hatching showed scattered lipid droplets lining the gut when stained with Bodipy (see Plate 40). After 48 hours of starvation, no lipid droplets were seen. Stained nauplii

from the F1 generation adults, examined in the same way did not have obvious lipid reserves immediately after hatching or after 48 hours starvation.

5.3.5 The feeding and digestive mechanism of *E.sieboldi*

Confocal images of starved nauplii from P generation adult females, after hatching showed scattered lipid droplets lining the gut when stained with Bodipy (see Plate 40). After 48 hours of starvation, no lipid droplets were seen. Stained nauplii from the F1 generation adults, examined in the same way showed no evident lipid reserves.

The actual ingestion of algal or any food type was not fully observed in this study. After nauplii were moved to examination vessels under a microscope, algal cells were occasionally observed between the seta of the antenna or mandible (small algal cells such as *Chlorella* sp). Algae were also observed between the mouth and gut, in the fore gut and hind gut of the nauplii. The gut of all developmental stages of nauplii examined (NI to NV) was a large structure lined by blue / black pigmented cells (see Plate 41). The intensity and presumably number of pigmented cells increased as development stage increase (see Plates 41 and 42). Frequently seen in nauplii that had recently fed was a large bolus of material in the hind gut (see Plate 43). This was assumed to be undigested algal matter in preparation for deposit as a faecal pellet (see Plate 44). In cultures of some algal types, the bolus appeared to be comprised of an entire and undamaged algal cell. This appeared to stay in the hind gut for some time as the nauplii ingested further algae. The additional algae were moved forwards and backwards through the entire gut by muscular contractions. When the hind gut algal cell was ruptured it was passed as a faecal pellet from the anus. This mechanism was most apparent in the large *Haematococcus* sp. cells which appeared to be more resistant to

breaking down than cells of the brown algae *Cryptomonas* sp. Confocal imaging of nauplii in a culture of *Cryptomonas* sp. shows the large undamaged cell in the hind gut, with a partially damaged cell anterior to it (see Plate 45). All algal cells including other cells in the culture surrounding the nauplii are distinctively bright red under the laser illumination.

A functional anus was confirmed in all stages of nauplii examined. Examination of the hind gut area with confocal microscopy identified two structures located laterally either side of the hind gut. These may have been a primitive form of gastric mill or glands involved in the digestion of food matter (see Plate 46).

5.3.6 Analysis of historic algal counts from Anglian Water

The historic algal data held by Anglian Water has been compiled through the observations of several different employees over many years. This has resulted in a data set that has been difficult to interpret due to different classifications being made of the same material. For instance, in some cases, species of algae have been identified, whereas in other samples, only identifications to genus level have been recorded. In some cases algae has been quantified according to size and phylum, *e.g.* “centric diatoms <10µm”. Also counts of algae have sometimes been done on cells per ml of water and sometimes as number of colonies present. As a consequence, analysis of this data has been done on algae of the following divisions:

Chlorophyta

Cyanobacteria

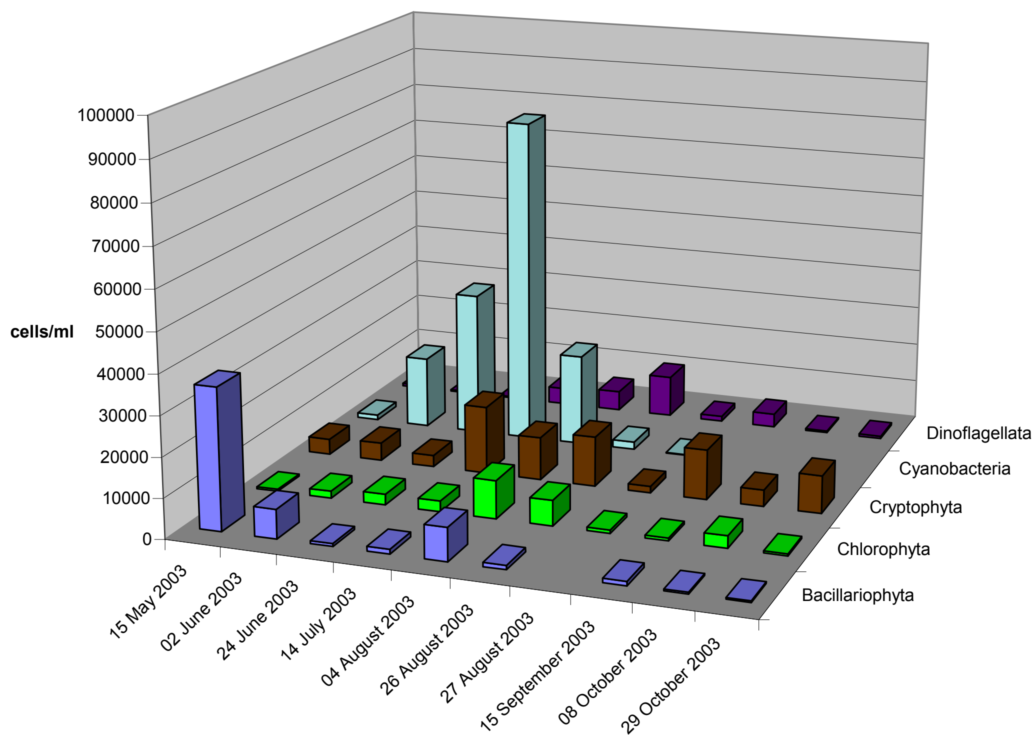
Bacillariophyta

Cryptophyta

Dinoflagellata

Also there were considerable gaps in the records, most notably in 2004. Unfortunately it is the data from 2004 compared to 2003 that would prove most useful as the mean *E.sieboldi* mean intensities of infection were high in 2003 and fell in 2004 (see Chapter 3). The comparative data on algal sampling is therefore concentrated on 2003 and 2005 as the latter year was similar to 2004 in terms of mean infection of trout with *E.sieboldi*. Figures 5.4 and 5.5 show the monthly cell counts from May to October for each of the algal divisions listed in 2003 and 2005

Figure 5.4 Algal cell counts for each of the divisions from May to October 2003.

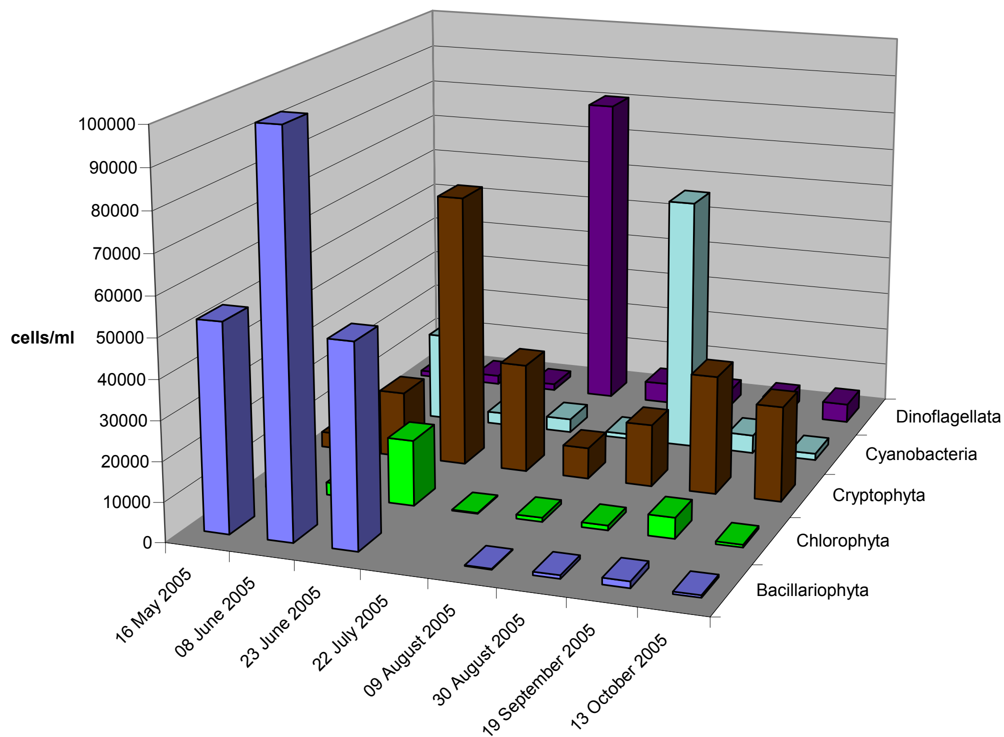


In 2003 the dominant algal type was the Cyanobacteria (blue green algae), which mainly consisted of *Anabaena* sp.

The Bacillariophyta (diatoms) were present in large number during the spring but these reduced to low levels by mid summer. This is the classic pattern for diatoms in freshwater lakes which depend on silicon for construction of cellular frustules. As silicon becomes limited, diatom numbers reduce. As a consequence, it is unlikely that diatoms perform a significant role in the nutrition of *E.sieboldi* nauplii. Cryptophyta (brown algae) were present at consistent levels of 5000-20000 cells per ml for most of the period when *E.sieboldi* nauplii would be in the reservoir.

The Chlorophyta (green algae) and Dinoflagellata were present in all samples at low levels (<5000ml^{-1}).

Figure 5.5 Algal cell counts for each of the divisions from May to October 2005.



In 2005, there were larger amounts of alga present in all monthly samples and Cyanobacteria were not dominant. It was not until late August that significant quantities appeared.

Bacillariophyta displayed a similar pattern to 2003, with large numbers of cells in the spring and low levels from mid summer.

Cryptophyta was present in higher numbers in 2005, and consisted of *Rhodomonas* and *Cryptomonas* species. At high numbers (>30000ml^{-1}), these were present as colonies of drifting mats.

Chlorophyta was present in low numbers for most of the period in 2005 similar to 2003. Dinoflagellata, however were the dominant algal type in August 2005 before falling again to low levels one month later.

In summary, it could be said that in the year of high *E.sieboldi* mean intensity of infection on the fish of Rutland Water, algal amounts were lower and there were fewer months where a single algal type dominated the sample. In 2005, when *E.sieboldi* infection levels were lower on the fish sampled, there were higher levels of algae and each month a different type of algae dominated as in a system in a state of flux.

5.4 Discussion

5.4.1 Food selection in *E.sieboldi* nauplii

The results of this study suggest that *E.sieboldi* nauplii are able to ingest a wide range of algal types and sizes when provided with a light source. Monoculture experiments recorded nauplii feeding on single celled algae with a size range from 5µm *Chlorella* sp. to 20µm *Haematococcus* sp. Also the shape of the algae did not inhibit feeding noticeably as some species used were spherical and others elongated (*Euglena* sp.). *Chlorella* is non motile, but the other species used were all flagellated motile algae. In the mixed cultures of filtered loch water, many different algae types existed (see 5.3.2.1). However it was not possible to identify any of the algae ingested as they degraded too quickly in the gut. It is probable that they were mainly small single cellular algae such as *Chlorella*, but the olive pigmentation may have come from small brown algae species or rotifers which were also present. No animal remains were detected in the gut or faecal matter of any nauplii examined in the study, but identifying such remains is problematic due to rapid degradation of delicate forms.

The results suggest that the brown algae *Cryptomonas* spp, was the most valuable to the nauplii nutritionally. In monocultures of this alga, the nauplii were sustained longer than in other monocultures, and the only development seen in controlled polycultures, were in cultures that included this genus. It was noted, however, that colonies of this alga were detrimental to the nauplii as they were easily rendered immotile when caught up in colony rafts. This may have a bearing on the success of *E.sieboldi* in periods of algal bloom. This was an unexpected result as it was assumed that increased food supply would increase the population but that has been shown to be not necessarily the case.

Despite the ability to feed on a wide variety of different types of algae, it was only in polycultures that development was observed and this suggests that a mixed diet may be necessary to provide the nutrients required for moulting to a new stage.

In Alston's (1994) attempts to feed ergasilid nauplii specific algae cells in a monoculture, he observed algal matter in the gut of a single nauplii of a culture of more than 300 examined, that were maintained in with *Chlamydomonas* spp. Attempts by Ben Hassine at culture (1983, cited in Alston 1994), similarly failed. The reasons for the failure of their monocultures are unknown but Alston (1994) suggested that it may have been due to the toxicity of the algal growth medium used which was transferred with the algae to the naupliar culture vessel. In this study, the algal cultures developed very rapidly to dense concentrations and therefore a minimum amount of growth media was transferred to the 1 l culture beakers in order to achieve the desired cell concentration. If algal densities had been lower, centrifuging of the algal cultures would have provided a means of collecting algae with minimum media. In future studies where there are difficulties in obtaining dense cultures, methods such as this would be preferred to transferring algal growth media to the naupliar culture.

Experimentation with algal monocultures is more widely reported in the development of free living copepods. As adult stages of the life cycle require nutrition from planktotrophy, nutritional supplies from different food types can be evaluated in terms of population growth, individual growth, fecundity, and egg hatching rate (Ceballos & Alvarez-Marques 2006;Fern+índez 1979;Price & Paffenhofer 1986). Cultures of many free living copepods are more advanced in technique and permit evaluation of

nutritional success in a range of environmental conditions. Fecundity is a common parameter in measuring maternal nutrition, as it is easily measured in laboratory conditions and effects can be reversed in a population by the substitute of dietary components (Parish, Wilson, & . 1978).

5.4.2 Differences in nauplii from P and F1 generation adult *E.sieboldi*

The feeding and size of the nauplii cultured from overwintered P generation adults in the spring differed substantially from those cultured from F1 generation adults in the summer. Nauplii from the P generation were larger and had lipid reserves immediately post-hatch. These were not evident in nauplii from the F1 generation. P generation nauplii developed and hatched at lower temperatures than those from F1, and development times in the egg were longer (see Chapter 4). The increased development time may account for larger nauplii as they have longer to use energy reserves in the egg. However it was surprising to see that they also maintained a lipid reserve post-hatch when F1 hatched nauplii did not. This may be a strategy for population survival for two reasons. Firstly, hatching earlier in the year in colder temperatures may be sub-optimal in terms of food availability. At this time the algae population is dominated by silicon rich diatoms which probably do not play a large part in the nutrition of the nauplii. No diatoms were found in the examination of nauplii cultured in filtered water, and the remains of frustules should have been easy to detect if they were there. A lipid reserve at this time may increase the survival rate of nauplii in a nutritionally poor environment. Secondly, there are fewer nauplii hatching at this point compared to the exponential increase seen later in the summer (see Chapter 4). Therefore it is vital for the population of *E.sieboldi* that as many nauplii hatch and develop at this time,

compared to late summer when the huge numbers of nauplii may be more expendable in population terms.

A change in strategy in terms of egg production, and subsequent juvenile feeding, at different times during the year is not unusual in copepods. In some free living copepods in marine and freshwater environments in response to sub-optimal environmental conditions for nauplii, diapausing eggs are produced (Grice & Marcus 1981;Wiggins, Macky, & Smith 1980). It has also been observed in parasitic crustaceans such as *Argulus* sp., when at certain times eggs are deposited on the substrate to form a seed bank for more optimal conditions (Hakalahti, Hakkinen, & Valtonen 2004). Although, less dramatic in terms of change in strategy, this study has identified possible differences in the form and performance of nauplii produced by *E.sieboldi* at different times of the year.

Wilson (1911) described ergasilids in the first three stages of nauplii as lecithotrophic. He observed that they did not possess a functional anus and feeding was not seen. In *E.sieboldi*, a fully functional anus and mouth parts were identified by (Abdelhalim, Lewis, & Boxshall 1991) in the NI stage nauplii, and is supported by observations in this study. The presence of a lipid reserve in some of the nauplii examined indicates that some maintenance functions and possibly development could be achieved without feeding in some nauplii but this is unlikely as all nauplii in starved cultures died within 3 days without any development. The lipid reserve may simply be remnants of intra-egg sac development. In contrast, in *Lernea*, and possibly all lernaeids, and also in the salmon louse (*Lepeophtheirus salmonis*) the nauplii do not feed and development is fuelled by an internal lipid store. As a result of this mechanism, the nauplii exhibit

neither mouth parts or armature that would allow food to be captured (Fryer 1978). This obligate lecithotrophy has been thought to have evolved from planktotrophy in many invertebrates in both marine and freshwater environments (Strathman 1978) . The change in strategy has consequences for maternal activity and juvenile success. Generally, lecithotrophic eggs are larger and larvae develop more rapidly as a result of inherent lipid reserves. In energetic terms, development of eggs therefore increases maternal energy demands, or at least reduces the clutch size of eggs produced (Hoegh-Guldberg & Emlet 1997). The possibility of closely related species such as those found in the ergasilids, some of which may be lecithotrophic (Ben Hassine & Raibaut 1981;Wilson 1911), and others that are planktotrophic as found in this study, allows for an evaluation of the different strategies from an evolutionary and energetic stand point.

The performance of the nauplii from P generation and F1 generation *E.sieboldi* in terms of development in cultures also differed. Although greater development in terms of stage reached was achieved by nauplii from the F1 generation in most cultures, the results suggested that nauplii from P generation performed better than those from F1 generation at the lower culture temperature of 10 ºC. This suggests a degree of conditioning to a certain temperature range either while the egg sac is attached to the adult, or possibly at a genetic level. At 15 ºC the F1 generation performed well, developing to NV in 13 days, compared to the P generation that did not develop past NI and only lasted for 6 days. At 20 ºC, both groups of nauplii performed less well, but still the F1 generation achieved development. This may suggest that all *E.sieboldi* nauplii perform less well during the warmest time of the year at Rutland Water, and may also contribute to the dearth in new infections seen in late August (see Chapter 3).

5.4.3 Analysis of historic data

The historic data from Anglian Water on the monthly algal counts was not as good quality as expected. Firstly, as the data set was incomplete and confused in terms of recording of algal types, there was some question as to the confidence that could be placed in it. Secondly, the results of the culturing experiment showed that *E.sieboldi* was able to select and ingest diverse types of algal cell, presumably to gain some nutritive benefit from them, as all the nauplii in these algal cultures performed better than starved nauplii. This was unexpected considering the failures of previous studies of this nature (see 5.1.2), to observe feeding on a single algal type. It was hoped that a certain type would be far superior in nutrient composition and consequently achieve better culturing results, which might account for a difference in the success of the *E.sieboldi* population during the study years. Although the results indicate that *E.sieboldi* is able to utilise different food types, it is inevitable that some types of phytoplankton, and particularly combinations of phytoplankton would be more beneficial. However as over 300 species of algae are identified in Rutland Water every year (Anglian Water historic records), it is highly unlikely to identify the beneficial feeds through data analysis and it was overly ambitious to expect to find a correlation between this data and observed development in laboratory cultures of just four types of algae.

Rutland Water is a fairly new water body at less than 40 years old. During this period, anthropogenic changes to the water quality have had large effects on the dominant algal species and the biodiversity of species in a system that could be described in a state of flux (Daldorph, Spraggs, Lees, Wheater, & Chapra 2001;Ferguson & Harper 1982;Kneale & Howard 1997). During the 1990s ferric dosing of the inlet water into

Rutland was carried out to control the algal populations that have a detrimental effect on water quality and cause problems in water treatment works (Randall, Harper, & Brierley 1999). The effects of the changes to phytoplankton during this period were probably still being felt at the time of this study. As a consequence of this unstable environment, higher organisms on the trophic scale such as *E.sieboldi* are also likely to be unstable in population terms. It may be that this study is observing changes in a parasite population that is caused by an environment in flux.

Without further development of techniques to culture ergasilid free living stages, evaluation of suitable nutritional food sources and its effects on a population are severely hampered. In this study some progress has been made in identifying different palatable algae for *E.sieboldi*, some combinations of which have permitted development through naupliar stages. With further progress in this area, it may become possible to estimate the success of the free living ergasilid in the light of specific field observations of quantities and qualities of different phytoplankton.

The population changes in *E.sieboldi* identified in Chapter 3, although possibly affected by the feeding and consequent success of the free living stages, has not been shown to correlate with any particular occurrence of an algae in the field observations. Further experimental studies on the nutritional requirements of all stages of the parasite are necessary to understand the interaction with population dynamics. The changes in population dynamics over the study years will be examined in Chapter 6 with the analysis of fishery management practices during the course of the study.

Chapter 6: Management of the Rutland Water trout fishery

6.1 Introduction

The wider objective of this study, as outlined in Chapter 1, was to identify potential methods for the control of *E.sieboldi* that could be implemented by fishery managers in a large potable water supply reservoir such as Rutland Water. Long term data on the many interactions of the ecology of the parasite and the operation of the fishery is necessary in order to understand the effects of environmental and anthropogenic changes. This chapter will examine some of the effects on parasite population level associated with fishery management in order to add to the wider knowledge of parasite ecology.

In Chapter 3, the pattern of new infections throughout the year was described, and it was shown that the mean infection levels of *E.sieboldi* on rainbow trout rose exponentially during the summer and autumn of 2003 to reach levels similar to the spring of that year. In 2004, the mean intensity of infection after the winter was still high, but with the stocking of new trout from April onwards, the mean intensity of infection fell and did not recover to those seen at the start of the year. In 2005, infection levels remained low, as in 2004. These findings presented an opportunity to look at aspects of the management of the fishery and the ecology of the parasite between years of high infections and low infections with a view to understanding the possible factors contributing to increased population levels.

The success of the *E.sieboldi* population may be due to one or more of three possible factors:

1) The environmental conditions for success of the parasite population were more optimal in 2003 than 2004 or 2005. For example, these conditions may be due to biotic or abiotic factors such as the quantity and nutritional value of food available to the free living stages, or a different temperature profile which affected reproduction.
2) Although the mean intensity of infection was approximately the same at the start of 2004 as the start of 2003, there may have been fewer fish in the reservoir at the start of 2004.
3) The density of rainbow trout hosts to the infective stage of the parasite may have changed during the study years. If there were fewer trout in the reservoir in the summer of 2004, *E.sieboldi* may have had more difficulty in finding a host.

Environmental factors, such as the reproductive activity of the parasite related to water temperature, and the possible success of the free living stages were considered in Chapters 4 and 5. The study showed that *E.sieboldi* had greater fecundity than previously thought, with a modelled prediction of up to 19 clutches of eggs being produced by post-overwinter P generation parasites. A dearth of new infections in late August was thought to be due to a new parasite cohort which behaved differently from parasites earlier in the year in terms of egg production, as they prepared to overwinter. Differences in the size and performance of nauplii of these 2 cohorts were also described in Chapter 5, those produced at the start of the year being larger and perhaps better conditioned for development at the lower temperatures at that time. The nauplii produced later in the year were smaller and performed best in terms of development at the higher temperatures seen in summer.

Although temperature records were not as detailed for 2003 and 2004 as they were for 2005, it was demonstrated that significant changes in water temperature would have some effect on the number of eggs produced overall, but possibly not sufficient to be responsible for the changes indicated. Similarly, the evidence for naupliar feeding and plankton analysis failed to associate the successful development of the parasite with any observed changes in phytoplankton. Thus, there was no conclusive evidence that the performance of the *E.sieboldi* population between 2003 and 2005 could be explained by any single observation of reproduction or free living stages related to the environmental conditions observed.

A further change to the environment that has not been considered previously is the effects of water level. Rutland Water, as a functional potable water supply, is operated to the demands of water customers. The water level and volume of the reservoir constantly change as water is pumped to meet the demand. This may have an effect on both the fish and *E.sieboldi* and will be considered in this study

Factors 2 and 3 require estimates of the fish population in Rutland Water in order to understand the population size of the parasite present. In a trout fishery where a large portion of the stock could potentially be changed for uninfected fish in a very short period of time, it is perhaps more useful to estimate parasite populations through fish stock assessment than through the traditional survey of parasite mean intensity and prevalence of infection.

This section describes changes in the fish population by examining stocking and catch records before and during the study years. By estimating the size of the overwintered

rainbow trout population at the start of the fishing season, and combining this estimate with mean intensity of infection recorded in Chapter 3, an estimate for the size of the *E.sieboldi* foundation population can be achieved. Similarly, estimating the size of the rainbow trout population when new infections occur in June, will allow a consideration of effects that management of the fish stock levels during the study years had on parasite success. This information may also inform future management choices for Rutland Water, in operating a trout fishery with infections of *E.sieboldi*, and possible management suggestions will be considered.

6.2 Materials and Methods

6.2.1 Estimate of rainbow trout population

Anglers who have purchased a day ticket are requested to inform the fishery managers of the number of fish they have caught. These returns are totalled each week and entered on to a database to allow estimates of stock and the need for replacements.

Figures for weekly catch and any restocking that took place were provided by Anglian Water and are contained in Appendices 1-5. During the course of the study, fish were stocked from 7 different trout farms. No attempt at differentiating these stocks due to origin was made. Only stockings of rainbow trout are considered. Stockings of brown trout constituted less than 5% of the stocking overall and are not considered here. Rainbow trout stocked were of a minimum weight of 650g per fish. The mean weight of rainbow trout stocked into Rutland Water during the study years was 750g (Anglian Water *pers. comm.*).

A model to estimate the standing stock of rainbow trout at any given time was based upon the angler catch of rainbow trout, the number of fish stocked and estimated losses from the reservoir. The purpose of estimating the changing fish stock during the year was to estimate the number of overwintered fish present in the system at the start of the year and therefore the number of P generation *E.sieboldi* that are the foundation for further generations later in the season. The purpose of estimating the stock of rainbow trout in mid June is to identify the number of rainbow trout hosts available to become infected with *E.sieboldi* when the F1 generation of parasites first infect fish.

Terms used in the analysis of this data and are explained below.

Returns:	A card detailing number of fish caught submitted by an angler after a days fishing.
Catch:	Number of fish reported as caught and removed from the reservoir in a day.
Catch rate:	Catch / returns (reported as catch rate per day).
Standing stock:	The estimated number of rainbow trout in the reservoir at a given time.

6.2.2 Reservoir water level

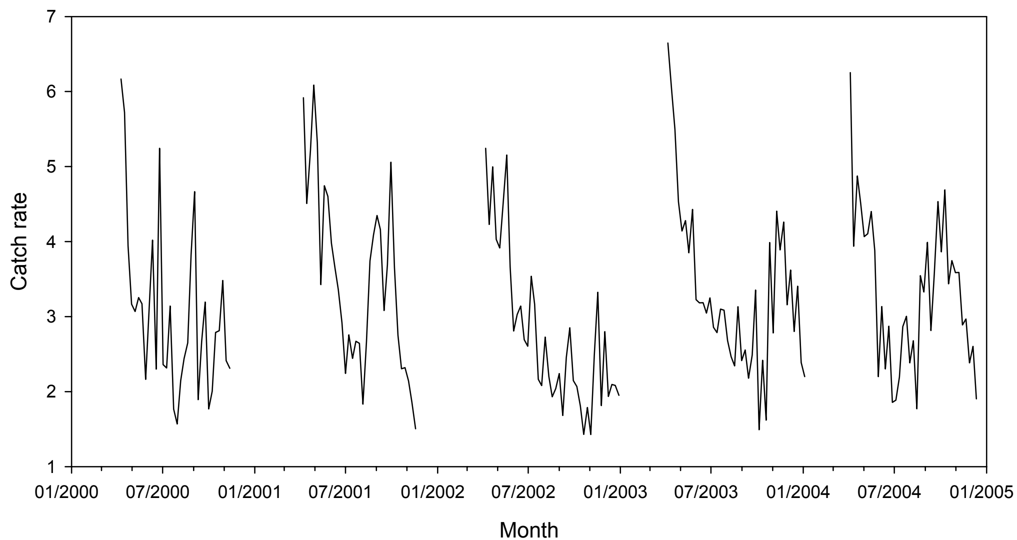
Reservoir water level was measured by Anglian Water at the same time every night using a float switch recording device located on the Lim tower reservoir monitoring station in the central basin of Rutland Water. Telemetry from this station was provided to Anglian Water who kindly supplied the author with the records. All water level measurements are expressed in metres above ordinance datum (mod), a global reference for comparison of heights in different parts of the world.

6.3 Results

6.3.1 Assumptions in modelling of stocking figures

The pattern of mean weekly catch rate for 2000 to 2004 is illustrated in Figure 6.1. The catch rate reported was assumed to be proportional to the standing stock as demonstrated in rainbow trout fisheries by (Pawson 1982). The annual catch rate (number of fish caught per angler) was consistent in pattern over the 5 years, *i.e.* there was no gradual increase or decrease in catch rate over time. For this reason it is assumed that the number of fish available to be caught per angler was also consistent between these years.

Figure 6.1 Mean weekly catch rate between January 2000 and January 2005.



The pattern of catch rate in all years shows that catch rate is initially high at the start of the season when water temperatures were low and the largest single stocking of 15-20000 trout took place. Catch rates fell during the summer and improved again in the autumn as the water temperature cooled. The summary statistics for total catch and stocking in each year from 2000 to 2004 are shown in Table 6.1

Table 6.1 Total rainbow trout stocked and reported as caught in each year from 2000 to 2004.

Year	Total Stocked	Reported Caught	% Caught	Total Returns	Caught / Returns
2000	97369	48434	49.7	16057	3.02
2001	101713	54325	53.4	14587	3.72
2002	95138	47145	49.6	14730	3.20
2003	88412	44837	50.7	12633	3.55
2004	88458	41332	46.7	11969	3.45

Between 2000 and 2004, mean percentage of rainbow trout reported as caught each year was approximately 50% of the total fish stocked in that year. As there is little possibility for trout to escape from the reservoir, the remaining 50% were either caught by anglers who did not post a return, were lost from the system due to mortality (including predation), or remained uncaught to overwinter to the next year.

The standing stock of rainbow trout at any given time in the angling season is a sum of how many fish have overwintered from the previous the year, plus how many have been stocked into the reservoir in that year minus what has been caught. It must also take account of losses from the system due to natural mortalities and fish that have been caught but not recorded. To put this into equation form, at the end of the first week of the angling season, the standing stock would equal:

$$SS_1 = (OW + ST_1) \times (1-L) - FC$$

Where SS_1 is standing stock at the end of week 1 of the angling season, OW is overwintered fish, ST_1 is fish in week 1, L is losses from the system that includes mortality rate and unreported catches and FC is fish caught.

In the second week of the angling season, the standing stock would equal

$SS_2 = ((SS_1 + ST_2) \times (1-L)) - FC$

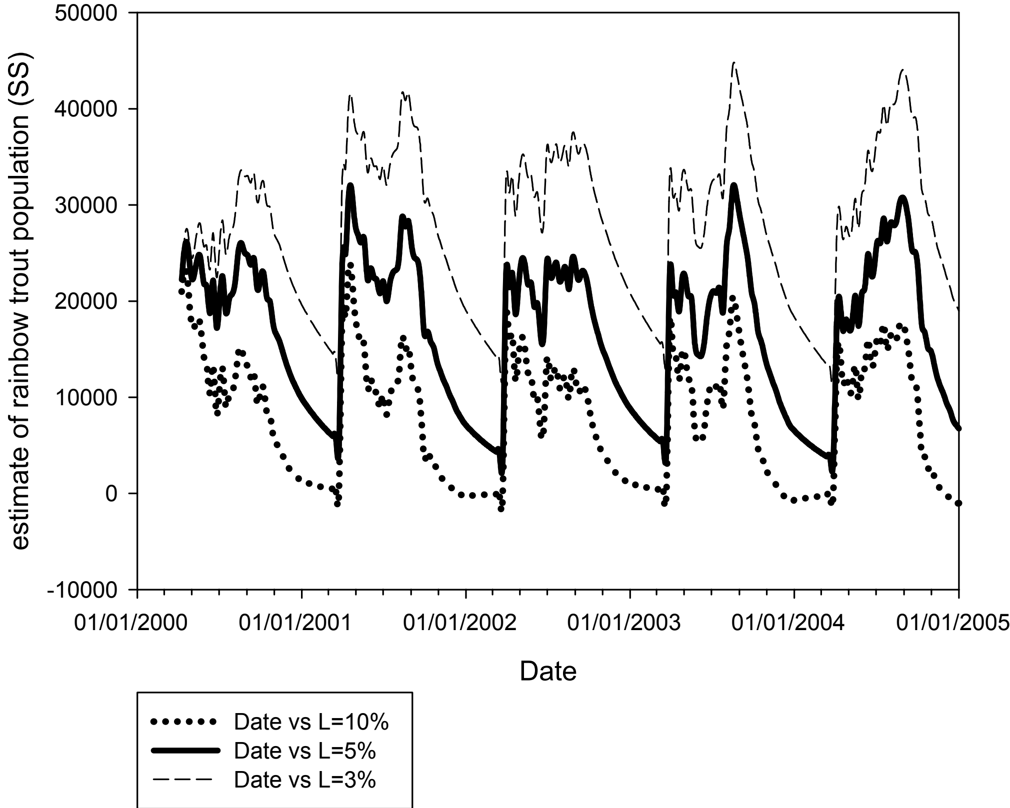
and this would be repeated in the generic form

$SS_n = ((SS_{n-1} + ST_n) \times (1-L)) - FC$

ST and *FC* are both provided in the fisheries data on a weekly basis. *L* is the figure that represents a loss from the system of 50% of the fish stocked each year due to mortality and unreported catch. L is assumed to be a constant and equally applicable to overwintered fish as to newly stocked fish.

In terms of weekly loss of standing stock, *L* is likely to be within the range of 1 and 10% to account for the 50% loss in total when new stock is increased throughout the year. To test this, *SS* was calculated on fishery data for 2000 to 2004 using the value for *L* as either 3, 5 or 10%. The correct figure for *L* should result in an estimated population throughout the year that maintains a large enough number of fish to be caught, while providing a suitable number of overwintered fish for the following season. Figure 6.2 illustrates the estimated population when *L* is 3, 5 or 10%.

Figure 6.2 Estimates of rainbow trout population in Rutland Water between 2000 and 2005 where the L statistic (% loss of stock per week due to mortality and unrecorded angler catches) is 3, 5 or 10%.



When L = 10% loss per week the population estimate is too low. In some weeks it shows a negative population.

At 3%, the population estimate is too high. If this figure was correct it would indicate that in April of each year when the angling season began, there were approximately 15000 overwintered fish to which 20000 new fish were additionally stocked. It is extremely unlikely that 1 in 2 or 1 in 3 fish at the start of the year were overwintered – there are simply too few overwintered fish caught for this to be the case (Anglian Water *pers. comm.*).

At *L* = 5 % population loss each week, the population figure is the more likely approximate. This estimates that 3-5000 fish overwinter (estimates using standing stock formula every week between April 2000 and April 2005) and are present at the start of the angling year. When 15-20000 new fish were stocked this would give an initial ratio of 1 in 5 fish as overwintered. This is a satisfactory figure to explain the catch ration seen at the fishery at the start of the angling season as reported by the anglers and wardens of Rutland Water.

6.3.2 Estimate of overwintered rainbow trout numbers and *E.sieboldi* population size at the start of each angling season.

Using the functions described in 6.2.2, where *L* = 0.95 (5% loss of stock per week), the number of overwintered rainbow trout at the start of each angling season (April 1st 2001 to 2005) were estimated and are shown in Table. 6.2. The mean intensity of infection of sampled overwintered rainbow trout at the start of the angling seasons (2001-2005) are also shown in Table 6.2, as is an estimate of the *E.sieboldi* population size present on the rainbow trout of Rutland Water calculated by multiplying the two figures (based on 100% prevalence of infection of overwintered rainbow trout in April 2003, 2004 and 2005 – see Chapter 3).

The combination of overwintered stock estimate and mean intensity of infection (see Chapter 3) suggests that in April 2003, there were in the region of 12 million *E.sieboldi* infecting 4876 rainbow trout. In April 2004, the number of *E.sieboldi* infecting rainbow trout was 31% less at an estimate 8.3 million infecting 3451 fish, although the mean intensity of fish sampled was approximately the same.

Table 6.2 Estimates of overwintered rainbow trout population and the population of *E.sieboldi* infecting these fish immediately before the start of the angling seasons of 2001 – 2005.

Date	Overwintered rainbow trout estimate derived from SS equation	Mean intensity of infection of overwintered rainbow trout*	Estimate of *E.sieboldi* population size infecting overwintered fish.
April 2001	5368	unknown	unknown
April 2002	3893	unknown	unknown
April 2003	4876	2468	12.03 million
April 2004	3451	2406	8.30 million
April 2005	3802	322	1.22 million

*based on mean intensity of infection of all overwintered fish caught in the spring before new infections commence.

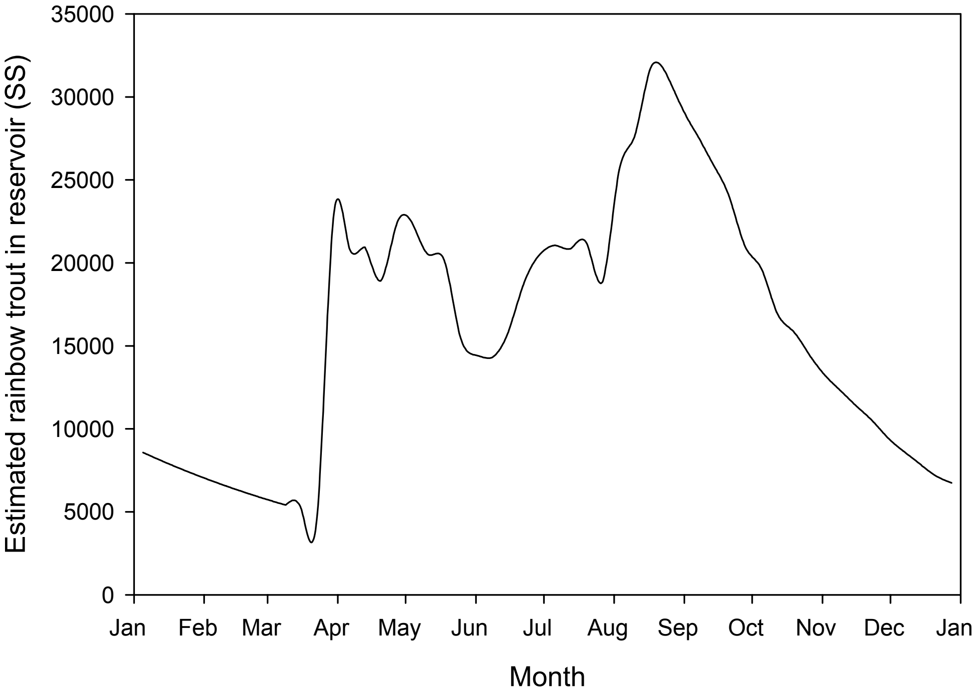
6.3.3 Host availability and *E.sieboldi* population size infecting rainbow trout during 2003

Figure 6.3 shows the estimate number of rainbow trout in the reservoir in 2003 using the formula for standing stock.

The angling season in 2003 opened in the first week of April. Prior to the season opening, there were 19000 rainbow trout stocked over 2 weeks from mid March, with weekly stocking taking place until the first week of September. The standing stock on the 1st of April was therefore 19000 + fish overwintered fish from 2002 (estimated as 4876 fish). The population of *E.sieboldi* on rainbow trout was estimated at approximately 12 million. The first progeny of these parasites infected fish in mid June. Prevalence of infection was 100% and mean intensity of infection of stock and early stock fish (combined) was 126 parasites per fish. An estimation of the standing stock at this time using the standing stock formula suggests that there were a total of 15730

rainbow trout in the reservoir. Assuming the mean intensity of infection and prevalence of fish examined in the sample at this time were representative of the whole population, the data suggests infection with 1.98 million F1 parasites by mid June.

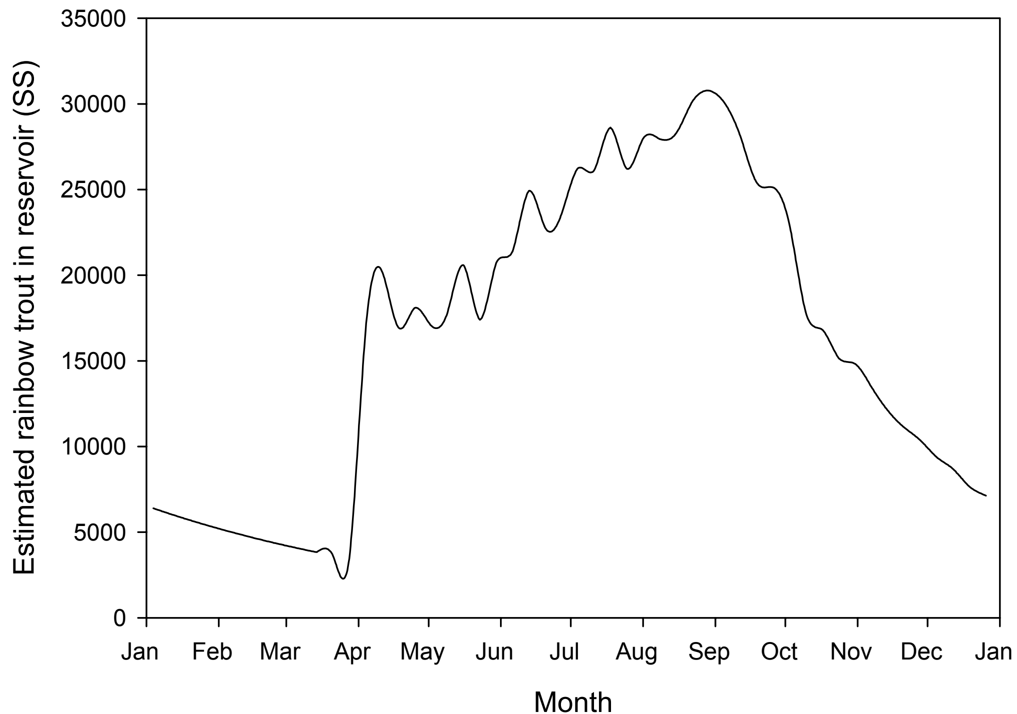
Figure 6.3 Total estimated number of rainbow trout in Rutland Water during 2003.



6.3.4 Host availability and *E.sieboldi* population size infecting rainbow trout during 2004

Figure 6.4 shows the estimate number of rainbow trout in the reservoir in 2004 using the formula for standing stock.

Figure 6.4 Total estimated number of rainbow trout in Rutland Water during 2004.



The angling season opened in April 2004 and stocking of rainbow trout at the start of the season was with a slightly smaller number of 15000 fish. Weekly stocking took place until the end of October, later in the year than in 2003. A smaller number of parasites were present at the start of 2004 (estimated 31% fewer) as there were fewer overwintered fish. The F1 progeny of these parasites infected rainbow trout in mid June and the sample suggested that 100% prevalence of infection was again apparent. The mean intensity of infection however was lower at 6 *E.sieboldi* per fish (see Chapter 3). The data suggests that in mid June, there were 24916 rainbow trout that had become infected with 0.15 million F1 *E.sieboldi*. This was a 92% reduction in the number of parasites compared with the same point 1 year earlier.

One aim of this study was to determine if there was a lower density of rainbow trout hosts available for infection by *E.sieboldi* in 2004 compared with 2003 which may explain why the *E.sieboldi* population was lower. The results of the standing stock analysis suggest that there were more rainbow trout in the reservoir in June 2004 when the F1 generation of *E.sieboldi* infected fish. Host density in 2004 was therefore greater than in 2003 and it would be expected to see lower mean intensities of infection. However, the success of *E.sieboldi* in 2004 compared with 2003 based on the estimated numbers of P parasites present at the start of both years to the number of F1 parasites infecting fish in the summer, suggests that even fewer overwintered fish were present between April and June 2004 than the formula estimated or that a further factor had reduced the success of the P generation to produce F1 infections in 2004.

6.3.5 Reservoir water level

Figure 6.5 shows the measured water level of Rutland water between January 2000 and January 2005. The water level fluctuates each year according to operational demand and climate. The mean water level during this period was 82.3 mod (metres above ordinance datum). The reservoir water level during the period when new *E.sieboldi* infections took place in 2003 and 2004 is shown in Figure 6.6.

Figure 6.5 Rutland Water level measured at the Lim tower in the central basin at midnight every day between 1st January 2002 and 1st January 2005.

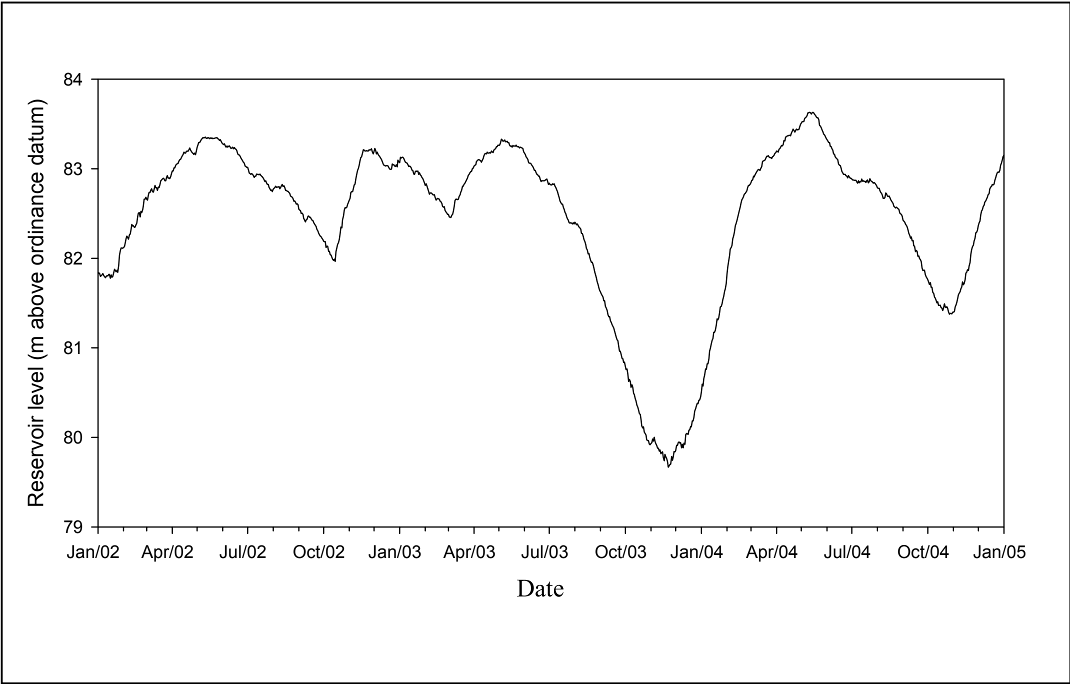
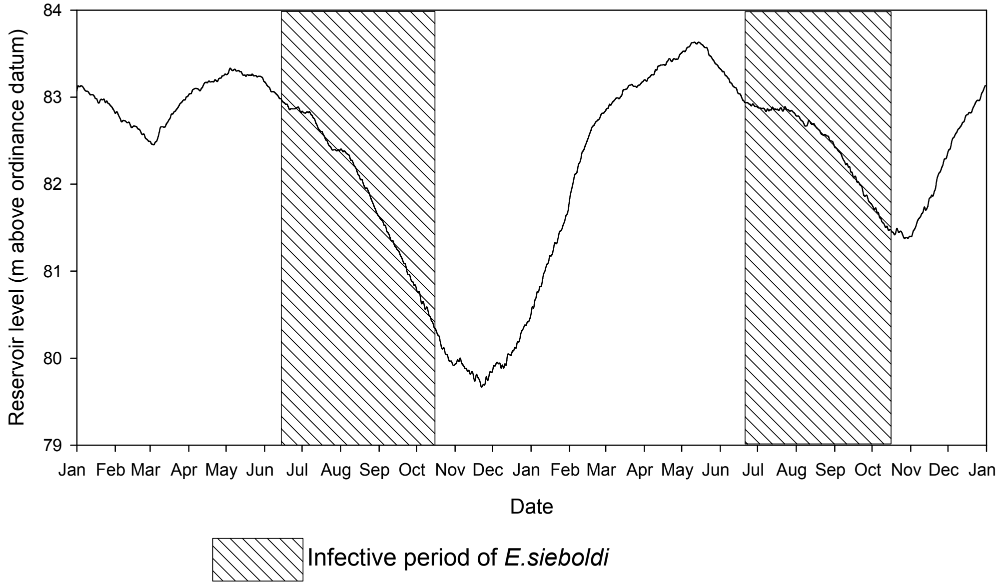


Figure 6.6 Rutland Water level measured at the Lim tower in the central basin at midnight every day between 1st January 2003 and 1st January 2005. Shaded area indicates the period when new infections of *E.sieboldi* were recorded on the fish of Rutland Water.



The reservoir water level during the *E.sieboldi* infective period in 2003 was similar to that in 2004 until August, when the water level began to fall at a greater rate than was seen in 2004. The mean reservoir level between 1st August and October 15th in 2003 was 81.4 mod. In the same period in 2004, the water level was 82.3 mod. Also of interest is that during the winter before the 2003 infective period, the water level was at a normal operating level. During the winter prior to the 2004 infective period, the water level was much lower, dropping in November 2003 to its lowest level recorded during this period at 79.7 mod.

6.4 Discussion

6.4.5 The modelling of rainbow trout stock levels

Traditional stock assessment methods include tag and recapture, hydro acoustic surveys, or netting surveys and are performed for a variety of reasons (Gullard 1971). Hydro acoustic methods of assessment were considered but it was felt that analysis of the stock of rainbow trout would not be able to be separated from other species and that the littoral zone would not be able to be surveyed due to disturbance causing the fish to avoid the area close to the boat (Malégorzata & Marek 2006). Tagging and recapture methods were also considered. Unfortunately, the numbers of fish required to generate a statistically useful return of fish made this option untenable for this study. Physical stock assessment should be performed in future work however, as it allows a more accurate assessment of fish stocks, with fewer assumptions. It can also accurately measure the infection levels of fish that were introduced into the reservoir at different times. However, fish tagging and recapture methods do require significant resources and assistance from anglers to be successful, and data generated only gives stock assessment in the past not the current levels which a model that is based on catch effort

analysis does estimate (Hilborn & Walters 1992). Tag loss and increased mortality rates may also be detrimental to the study (Vehanen 1997). Net capture techniques were not desirable due to the possible damage caused to the economically important stock caused by gill netting, and the problems with topography encountered during netting for coarse fish (see Chapter 3).

For this study, a model based on catch effort was used because it made use of readily available fishery data, provided an estimate of historical and current stock levels, and required no further resources to achieve a result.

There were a number of assumptions made in the assessment of rainbow trout stock. The number of fish that were caught each week and not reported by anglers was unknown. As the system for anglers to submit a return did not change during the study, it was assumed that the mean percentage of anglers failing to submit a return remained approximately the same in each year. In future studies, an accurate measure of this could be made by comparing returns to till receipts. During this the study, till receipt information was not available. However, data of this sort would not assist in monitoring the performance of season ticket anglers who purchased a year permit at the start of the season and are encouraged to make a detailed return at the end of the year. It cannot also be assumed that anglers failing to submit a return were equally as successful as anglers who did submit a return. It is likely that an angler who has caught no fish is more likely to neglect to submit a return.

Losses from the system due to mortality were likely to vary slightly between years, due to factors such as food availability or predation by changing populations of cormorants

(*Phalacrocorax carbo*). However, since 2000, the trout stocked have been larger, having increased from a mean weight of 500g to 750g. This was a measure aimed at reducing losses to a large wintering population of cormorants at the reservoir (Anglian Water Wardens *pers. comm.*). It has been suggested from studies on cormorant gut content analysis at large lake fisheries that most of the diet of cormorants is comprised of smaller coarse fish species rather than larger commercial species (Engstrom 2000). As no other causes for mortalities in large numbers are known to have occurred during the study period, and in the absence of more informative data on this subject, it was assumed that losses from the system due to mortality remained constant between study years. We do not know how the mortality rate of fish varied throughout a single year. It may be that more fish died during the winter due to cold temperatures, lack of feeding and increased predation. Alternatively, mortality might be higher in the summer when oxygen levels are lower in the warmer temperatures and fish are stressed after being stocked. Given the lack of data on this variation, for the purpose of analysis, it was assumed that the rainbow trout population mortality rate was constant throughout the year.

Numbers of overwintering fish are likely to vary from year to year depending on the success of anglers and environmental conditions, especially in the latter months of the year when no new stockings were taking place. The increase in the mean rainbow trout stock size from 500g to 750g in 2000, may also increase the overwintering success of the population, as body mass has been linked to overwintering survival in other salmonids such as brown trout and brook trout (Hutchings 1993).

A study by Pawson (1982) using catch per unit effort and catch succession techniques, to monitor stock levels in a trout fishery, suggested that catchability was implicit with stock determination. In the case of a fishery with an infection problem, this may not be the case where high infection levels reduce the ability of anglers to catch a standing stock.

The incorporation of the catch data in the stock assessment in this study assumed that the residence time of a rainbow trout in the reservoir had no bias on the likelihood of its being caught. This is substantiated by (Pawson 1986), who found no indication that rainbow trout newly stocked into a fishery were more vulnerable to fly fishing than longer-term residents. However, Pawson's study was conducted on a much smaller water body than Rutland Water, where the behaviour of resident fish may be very different. Future studies should try to incorporate the potential for residence induced performance against angling. This could be accomplished by using tagging and recapture methods in parallel with examination of all angler caught fish. This would also provide details of stock turnover at different times of the year, a measure that has been associated with reducing the numbers of infected stock in a fishery as a potential control against an infection with *Argulus* (Taylor, Sommerville, & Wootten 2006). It would also allow different cohorts of trout stocked into the reservoir to be monitored in terms of numbers remaining and the levels of infection of these different stocks.

6.4.6 The number of overwintering parasites and host availability

The analysis of angler catch and stocking records provided an estimate of the overwintering stock present at the end and beginning of the angling season. This enabled an estimation of the size of the *E.sieboldi* population infecting the rainbow trout

in the reservoir. The overwintered population of fish is the host for the foundation population of *E.sieboldi* that is responsible for producing generations of infections in the forthcoming year. These fish host the largest infections of *E.sieboldi* of any rainbow trout during the year, and as they are relatively few in number, they provide a good target for management strategies that seek to reduce the population of the parasite.

The estimation that there were 31% fewer parasites present at the start of the 2004 angling season compared with the start of the 2003 season, was certain to have a "knock on" effect on the success of *E.sieboldi* later in 2004. Due to the subsequent generations being dependent on the successful reproduction of the overwintered P generation, a small reduction in their numbers would have a magnified effect amongst subsequent generations.

However, the 92% reduction in the numbers of the initial F1 infections in 2004 were surprisingly high. It may be that initial sampling of the fish in 2004 was immediately after infections had begun and sampling of the fish in 2003 was 1 to 2 weeks after infections had begun. To some extent this is substantiated by the 2003 June sample which contained 'early stock' rainbow trout that had slightly higher infection levels than 'stock' rainbow trout. Even taking this into account, the initial drop in the infection levels cannot be attributed to a 31% decline in numbers of the *E.sieboldi* P generation alone. A further examination of the stocking figures for 2003 and 2004 may go some way to explain this. The pre-stock of rainbow trout in 2003 was 19000 fish. In 2004 this figure was 15000 fish. This had the effect of diluting the overwintered fish more in 2003 than in 2004, in essence giving more protection to these fish from anglers in 2003 than in 2004. For this reason, more fish are likely to have remained uncaught for a

longer period in 2003 and consequently the *E.sieboldi* P generation would have been sustained for longer, at a larger size, and therefore produced more progeny.

A potential reason for a reduction in the *E.sieboldi* population during 2004, was that if there had been a lower host density, this may have had detrimental effects on the success of parasite transmission, leading to a lower *E.sieboldi* population. With fewer fish, it may have been more difficult for the infective stage of the parasite to find a host. This was shown to be not the case as the model of estimated rainbow trout standing stock, suggested that when new infections were first observed in June 2004, there were more trout available for infection than at the same time in 2003.

6.4.7 The effects of water level

There were reduced water levels in the reservoir recorded during the winter of 2003-2004. The effects of this on the parasite population are largely unknown, but as this occurred at a pivotal point between a year with larger numbers of *E.sieboldi* (2003) and a year with fewer *E.sieboldi* (2004), it can not be ignored and is considered here.

The normal pattern water level in Rutland Water is high water in the winter, reducing during the summer and filling up again during the following autumn and winter. The below average precipitation rate during the winter of 2003 to 2004 (UK meteorological office web data), may have been in part responsible for water levels 3m below average at this time. This exposed much of the littoral fringe to desiccation with a consequent loss of vegetation, especially in the shallower areas of the reservoir at the western extremes of the north and south arms. A previous study by (Warlow & Oldham 1982) on the feeding of brown and rainbow trout in Rutland Water examined the gut contents

of fish at different times of the year. During the winter when water levels would normally increase, gut content analysis showed that most trout became dependent on terrestrial food such as earthworms, as aquatic insect and zooplankton levels in the reservoir levels were low. Loss of this food source in the winter between 2003 and 2004, may have reduced the survival of overwintering fish and therefore the P generation *E.sieboldi* beyond the estimates of the standing stock model.

From August to October 2004, the water levels were higher than those seen in the corresponding period of 2003. This was the period when the largest numbers of new infections takes place and also when free living stages of *E.sieboldi* are expected to be most abundant. Higher water levels at this time may reduce the chances of infective stages of the parasite finding a host, although this study did not identify how and where this occurs. Perhaps more importantly, higher water levels would reduce water temperature and phytoplankton numbers and therefore numbers of planktotrophic zooplankton, including the naupliar stages of *E.sieboldi* (see Chapter 5). Studies on the fish ectoparasite *Argulus* have identified a reduction of water level with increased temperatures during the summer to correspond with increased infection rates of fish (Gault, Kilpatrick, & Stewart 2002;Shafir & Van As 1986). However, Taylor *et al.* (2006) found that a drop in water level was a preventative measure for infections of *Argulus* in UK trout fisheries, although the authors considered that this might be a chance occurrence in their epidemiological model. It seems likely that a drop in water level would have different environmental implications for both parasite and host depending on the time of year that it occurred. The occurrence of an unusual water level reduction at a critical point of change in infection parameters in this study suggests

that it is worthy of further investigation and should be considered in future epidemiological studies.

6.5 Elements of *E.sieboldi* population biology that may be useful to fishery management

The findings of this study have given information on the activities of the *E.sieboldi* population that may be useful in the management of Rutland Water trout fishery. The information presented may be of use in a wider geographical area and in the context of both natural and managed fisheries.

The study showed that rainbow trout are susceptible to infection with high numbers of the gill parasite *E.sieboldi*. Through field observations, cage trials, tank trials, and laboratory investigations it has been demonstrated that the overwintering P generation of adult parasites started producing egg sacs when water temperature was approximately 7 ºC, but this may vary according to the profile of water temperature during the winter and the degree days accrued.

As multiple clutches of eggs are produced by P generation parasites (see Chapter 4), the progeny of the P generation, the F1 generation develop through 12 free living stages in the plankton before infecting fish in June, July and August, but probably at a declining rate as previously overwintered fish were increasingly removed from the reservoir. During July and August the progeny of F1 parasites develop and hatch within 2 weeks of infecting fish. The dearth of new infections seen in late August was possibly a period where the rate of new F1 infections had decreased due to reduction or death of the P

generation. It may also be that at high water temperatures, the successful development of the free living stages of the parasite was reduced.

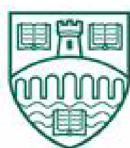
The progeny of the F1 parasites, the more populous F2 generation, began to infect fish in late August / early September and continued until October. This accounted for the rapid increase of infection levels seen during August and September (see 3.3.1.2). It is possible that a F3 generation was produced from the F2 parasites and infected fish in late September and October but as the pre-clutch interval was longer in F2 parasites as they prepared to overwinter (see Chapter 4), the size of an F3 generation would have been smaller.

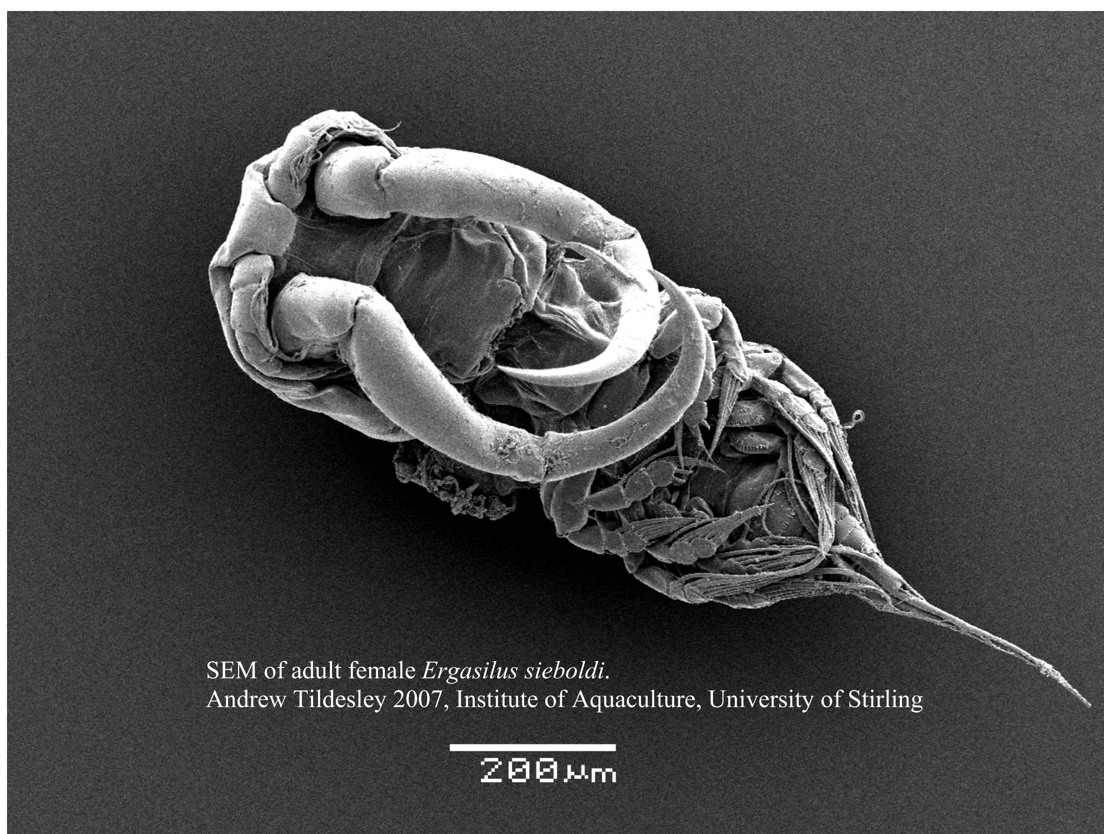
Parasites infecting fish in October overwinter very successfully before they in turn are classed as the P generation in the following year. During this period egg development still continues within the ovary of the parasite but no egg sacs are produced until the water temperature rises to spring temperatures. As the population of rainbow trout in the reservoir at this time is small compared with the high numbers of fish during the angling season, removing as many overwintered fish from the reservoir before the parasites begin egg production would have a larger effect on reducing the overall *E.sieboldi* population. This could be accomplished by extending the angling season into the winter months or reducing the size of the initial stocking of naïve fish in the spring so that overwintered fish are form a larger proportion of the angler's catch at the start of the fishing season. The use of alternative stocks may also be beneficial in reducing *E.sieboldi* numbers. The stocking of diploid rainbow trout or brown trout as an alternative to triploid rainbows may help this situation in 2 ways. Firstly this study has shown that both diploid rainbow and brown trout are less susceptible to infection than

triploid rainbows and secondly, some anecdotal evidence has suggested that diploid rainbow trout may have a reduced survival ability overwinter compared to triploid rainbow trout (Solomon, Environment Agency web publication, accessed 2008), due to the weakened state of fish following gonadal development. However, studies of winter survival in US streams by Dillon *et al.* (2000) report no statistical difference in survival between triploid and diploid rainbow trout. Loss of overwintered fish, a valuable resource to a fishery, could be countered by stocking the better winter performing triploid fish after the infective period of the parasite had ended in November.

Careful monitoring of fish stock levels using standing stock analysis is desirable during the angling season. Maintaining stock turn over at as high a rate as possible will reduce the number of parasites and therefore the number of later infections occurring. The use of alternative stocks to triploid fish was discussed in Chapter 3 and is to be encouraged as these have been shown to be less susceptible to infection with *E.sieboldi*.

In 2007, a report of management suggestions was presented to the study sponsors Anglian Water for consideration and is presented in the following pages. Fishery management is a complicated process and operation of a successful fishery is dependant on encountering and finding solutions to many problems. The report of management suggestions is intended to inform the sponsor on potential methods to reduce the infection levels of *E.sieboldi* on the trout of Rutland Water, but it is recognised that some recommendations would have detrimental consequences in other areas of fishery management and it is ultimately the choices made that determine the future success of the fishery.





SEM of adult female *Ergasilus sieboldi*. Andrew Tildesley 2007

Management suggestions for Rutland Water to control the copepod parasite *Ergasilus sieboldi*

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Introduction

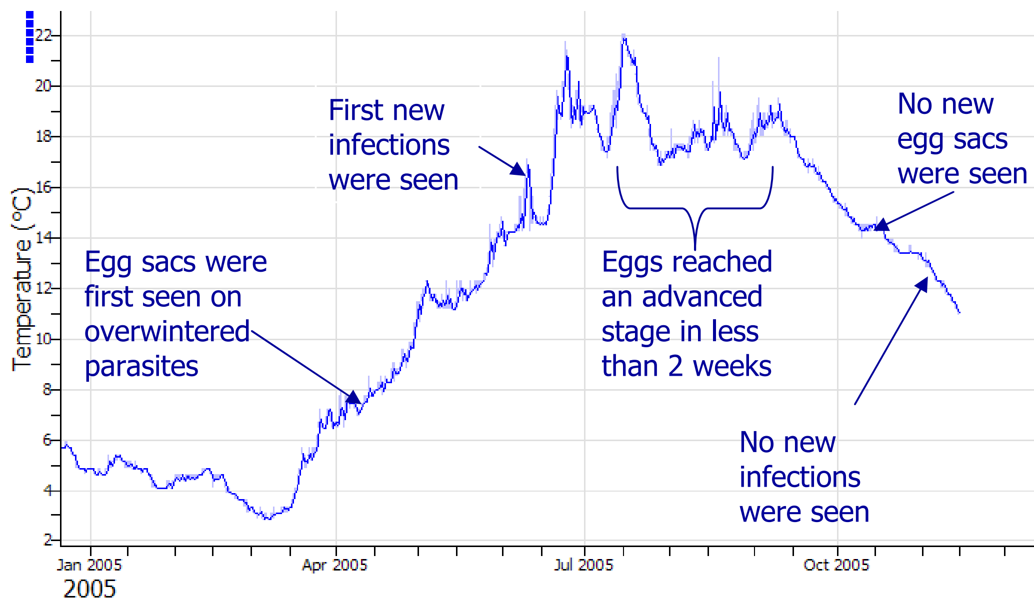
The copepod parasite *Ergasilus sieboldi* was first recorded on trout in Rutland Water in 2002. The intensity of infection and the reproductive status of the parasite were monitored in a programme conducted by the University of Stirling and Anglian Water from 2003 to 2006. *E. sieboldi* is a parasite known to be present throughout England on a wide range of fish host species. Only the adult female is parasitic, the nauplius, copepodid and adult male stages are all free living planktonts. During the course of the study, *E. sieboldi* was found attached to the gills of 7 of the 9 species of fish examined. These include the two species that are stocked in Rutland, rainbow trout and brown trout. The large numbers of the parasite that were present on the fish in 2003 were considered by the fishery to have caused a reduction in angler success and a loss of condition to the fish. It has been suggested in previous studies that poorly conditioned fish are susceptible to secondary infections and increased predation. There is also an adverse impact on the fishery if anglers catch fish they perceive as “diseased”.

E. sieboldi usually attaches to the primary gill filaments of the host fish and causes damage by its feeding and movement on the gill surface. During the summer months when temperatures are highest, damage to the respiratory surface of the host has been reported by others to cause a range of signs from lethargy, loss of appetite and condition, to emaciation and death in extreme cases. During the course of this study, a wide range of levels of infection were seen, including individual fish with more than 6000 parasites.

This document suggests some management strategies that might be employed or altered to reduce the population size of the parasite within the reservoir. There is not a single, simple solution to control parasites, especially in large water bodies and control and management of the parasite can only be effective when using a number of approaches. It is therefore recommended that as many of the suggestions are implemented as possible simultaneously. Some of the suggestions will not be practical in all circumstances as many considerations need to be taken into account in operating a trout fishery. This document provides the principles of management for the reduction of the parasite population, the scientific evidence from the study and also some practical suggestions that are examples of methods that may be useful in Rutland Water.

Principle findings of the study

The graph below summarises the observations made in 2005 of *E sieboldi* infections on trout in Rutland water. It illustrates the parasite population changes found in relation to temperature and typifies the annual cycle of events.



- The parasite successfully overwintered on trout each year. The numbers of parasites infecting fish before winter were similar to the following spring, indicating minimal losses during this period.
- During the overwintering period the parasite did not extrude egg sacs.
- The reproductive activity of the parasite was temperature dependant.
- The overwintering parasites began producing eggs in April when temperatures rose above 7°C.
- Eggs hatched from late April onwards depending on temperature and within 48 hours were replaced with new egg sacs. At peak temperatures, the parasite can extrude and hatch a new batch of eggs every 7 days or less.
- The free living stages developed in the reservoir and were adapted to feed on a range of algae types/species. They first reached the infective adult female stage in early June, mated with free living males, and attached to a fish host. They then started to produce multiple batches of egg sacs until the temperature fell below approximately 14-15°C. and then entered the overwintering phase.
- No new infections occurred after October.
- Coarse fish that were examined all had low levels of infection. Triploid rainbow trout were the most susceptible hosts examined, with diploid rainbow trout and brown trout infected at lower levels.
- Parasite numbers were highest in 2003, with 2004-2006 showing similar, lower levels of infection.

Principle 1 – reduce the size of parasite population in the reservoir before it becomes reproductively active.

The numbers of parasites in any season are determined by the size of the overwintering generation. It is this generation that produces the first new parasites of the season which themselves reproduce to provide further generations in the same year. By reducing the overwintering population, it limits the number of parasites in all generations in a given year.

Individual overwintering fish may carry thousands of parasites. As no suitable method has been found to remove the parasites from the fish, it would be advantageous to remove these fish from the reservoir.

This can be achieved in a number of ways. Currently fish are stocked between March and September. Fishing generally improves in terms of catch per unit effort after September so fewer fish need to be stocked. It is recommended to stop stocking at the end of August, thus reducing the population size of trout in the reservoir in later months whilst maintaining an acceptable catch rate for anglers.

In addition, it would be beneficial to extend the period of angling activity to the winter months. Currently no fishing takes place from January to April, and therefore relatively large numbers of infected fish remain in the system. If it is impossible to open the reservoir during this period, anglers might be allowed to target these fish before the initial stocking takes place in March. 1 to 2 weeks of angling activity at this time could make a significant reduction in the overall parasite population. This could be given as an opportunity for free fishing to season ticket holders from the previous year.

It is known that overwintering fish are a valuable resource to Rutland Water as anglers target them for their generally larger size and fighting capabilities.

To provide anglers with similar but uninfected fish in place of the infected overwintering fish which should be removed, stocking could recommence in November after the infective period of the parasite had ended, or simply stock a representative population of larger fish in the first stocking in March.

Gill from a rainbow trout in March showing *E. sieboldi* in a non-ovigerous state.
Andrew Tildesley 2007, Institute of Aquaculture, University of Stirling

Gill from an overwintered rainbow trout in April showing *E. sieboldi* in ovigerous state.
Andrew Tildesley 2007, Institute of Aquaculture, University of Stirling

Principle 2 – Increase stock turnover.
The study suggests that the largest factor for determining the number of parasites on a single fish is the amount of time that the fish is present in the reservoir during the infective period from June to October. Currently the stock turnover is estimated at 3 to 4 weeks. At times of peak infection, this figure is too high to keep the parasite numbers at a low level.
To reduce the turnover time, it is recommended to reduce the size of the initial stocking in March, currently at 18-20000 fish and to trickle stock these fish over a longer period of time. The current stocking of a large number of fish in March produces high figures for catch vs. angler effort at a time when water temperatures are low and fishing is generally easier than summer months. In addition to the initial stocking, weekly stocking takes place until September, to replace those fish taken from the reservoir. It is recommended to increase the frequency of stocking and to reduce the numbers of fish per stocking in order to increase turnover rate. This is especially true of the peak infection months of July-September. Currently the weekly stocking levels vary considerably, with some weeks receiving several thousand trout in a single delivery. Stock turnover could be increased by stocking in smaller batches several times per week as necessary. The use of the existing trout cages to hold fish prior to release is an option to achieve the trickle stock. Although fish in the cages will still become infected, it appears that the net sides reduce infection rates considerably.
Additionally it is recommended to ban catch and release of fish. Anglers who catch a fish and return it to the reservoir are increasing stock turnover time. It could also be argued that a fish that is returned to the reservoir, if it survives, could be in a weakened and stressed condition which may increase it's susceptibility to infection. Rutland Water is an international competition venue for highly skilled anglers. Regular competition events could be timed to provide a tool to increase stock

turnover. Fish become infected from June to October with infection rates highest from July to September. Competitions and practice days taking place during this period can increase the stock turnover and thus decrease the mean intensity of infection. Competitions also take place in two formats. The first allows anglers to catch up to a maximum of 6 fish and they record the total weight of the fish. If they finish earlier than other competitors they are given a time bonus. The second format does not include any time bonuses. For this reason, the first format often involves anglers trying to catch fish as fast as they can and targeting newly stocked fish. The second format with no time bonuses will see anglers trying to target the larger fish that have been resident in the reservoir for longer. The latter approach will tend to remove the fish from the system that have a higher parasite burden and for this reason the second type of competition should be encouraged.

Principle 3 – The use of alternative fish stocks

Research has shown that diploid rainbows and brown trout are less susceptible to infection than triploid rainbows. It is recommended to stock a greater proportion of these fish during times of high parasite transmission rates (July – September). However, it should be noted that most diploid rainbow trout produced are intensively farmed for the table market. They are consequently not of the same size and quality as fish produced for re-stocking into trout fisheries. If good quality diploid trout can be sourced from farm production they should be used.

Most trout stocked into the reservoir are in the range of 700-900g. In the study, no significant differences in susceptibility have been identified in trout of different sizes within this range. Larger, grown on fish of 3kg or more have been shown to be in a generally better condition than smaller fish. The parasite burdens are often lower and a fish of this size is probably more robust in fighting an infection of this parasite, though there may be behavioural or even morphological factors involved in their resistance to infection.

It would clearly be un-economical to stock rainbow trout of this size in sufficient numbers into a reservoir as large as Rutland, but it may be possible to supplement the existing stock with some fish of this size as a replacement for fewer overwintering trout.

Principle 4 – General

It is important to assume some general "best practice" principles in order to operate a management strategy with the presence of a known parasite and the possibility of the introduction of new pathogens. The following are recommendations for the operation of Rutland trout fishery based on these principles.

- Ensure anglers' equipment is as clean as possible to prevent the transfer of pathogens to or from the reservoir. It is recommended to use compulsory disinfectant baths for fishing nets and fish holding bags/boxes or to provide anglers with nets and bags to use from clean stock. Adult female parasites have been shown to survive for more than 5 days off the host if kept in water at low temperatures. Eggs can survive even longer and are viable at a wide range of ambient temperatures.
- As a general principle, monitor health status of stocked fish before introduction.
- Reservoir water levels should be kept as high as possible during the infective period. Any planned maintenance to the reservoir that involves the reduction of water level should be performed outside of the infective period. The

parasite reproduction has been shown to be temperature dependant. Decreased water levels increase the mean water temperature and increases the rate of parasite reproduction. The hosts are also concentrated into a smaller volume, potentially bringing them into more contact with the infective stage of the parasite. Other studies of *E. sieboldi* have suggested that the parasite thrives in waters where there is little or no aquatic vegetation to slow the dispersion of the parasite. Reducing the water level below the littoral fringe would therefore increase dispersion.

- Suspension of catch and release policy. Catch and release slows stock turnover and stresses fish. The practice should be avoided throughout the season, but especially during the peak infective period of July to September.
- Regular monitoring of parasite levels should take place by Anglian Water. An increased burden in the early part of a year could be an indication of the potential for significant problems in the mid to late summer.
- Incoming water should be filtered to remove incoming fish and free living parasites wherever possible.
- The removal of higher predators such as pike and cormorants may not be beneficial in control of the parasite as these predators will remove fish from the system and therefore remove parasites. Unhealthy fish are likely to have large parasite burdens and are likely to be more susceptible to predation.
- It is recommended to investigate the use of trout that have been fed on medicated feeds prior to stocking. The use of emamectin benzoate to control sea lice and argulus infections has been documented. Fish fed on medicated diets are now commercially available for restocking and the efficacy against *E. sieboldi* should be investigated. It may be possible to stock these fish at times of greatest parasite transmission in order to obtain a level of protection.

Summary

The copepod parasite *E. sieboldi* has been associated with a reduction in angler success at Rutland Water. A study has elucidated many aspects of the life cycle and dynamics of the parasite population in this reservoir. The size and nature of Rutland Water restricts the management solutions available to deal with a parasite problem. A number of key findings have suggested management changes to reduce the size of the parasite problem. A number of approaches should be taken to affect an impact on the parasite population. In recent years, the parasite population has been smaller than 2002-2003 but parasite populations may naturally fluctuate from year to year and the risk of an increase in parasite numbers is always present without careful management.

Plates

Plate 1. SEM of adult female *E.sieboldi*. Ventral view.

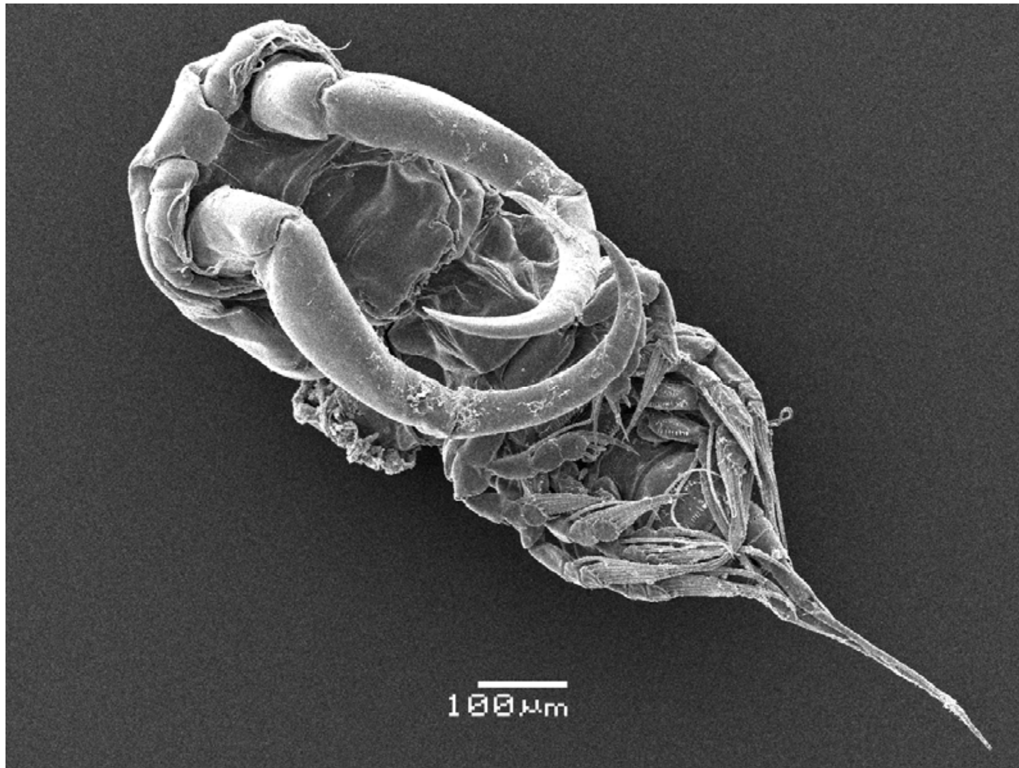


Plate 2. SEM of antennae of adult female *E.sieboldi.* M is mouth cone, A is antenna.

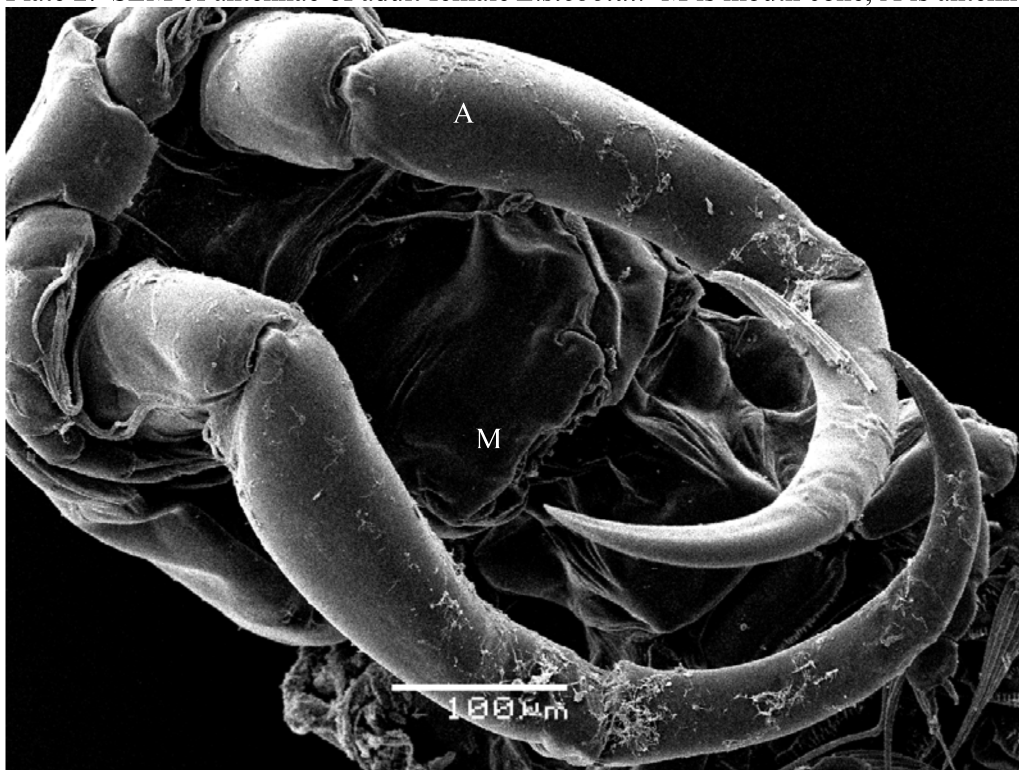


Plate 3. Mouth parts of adult female *E.sieboldi*. Ventral view. Mn = mandible, Mx = maxilla.

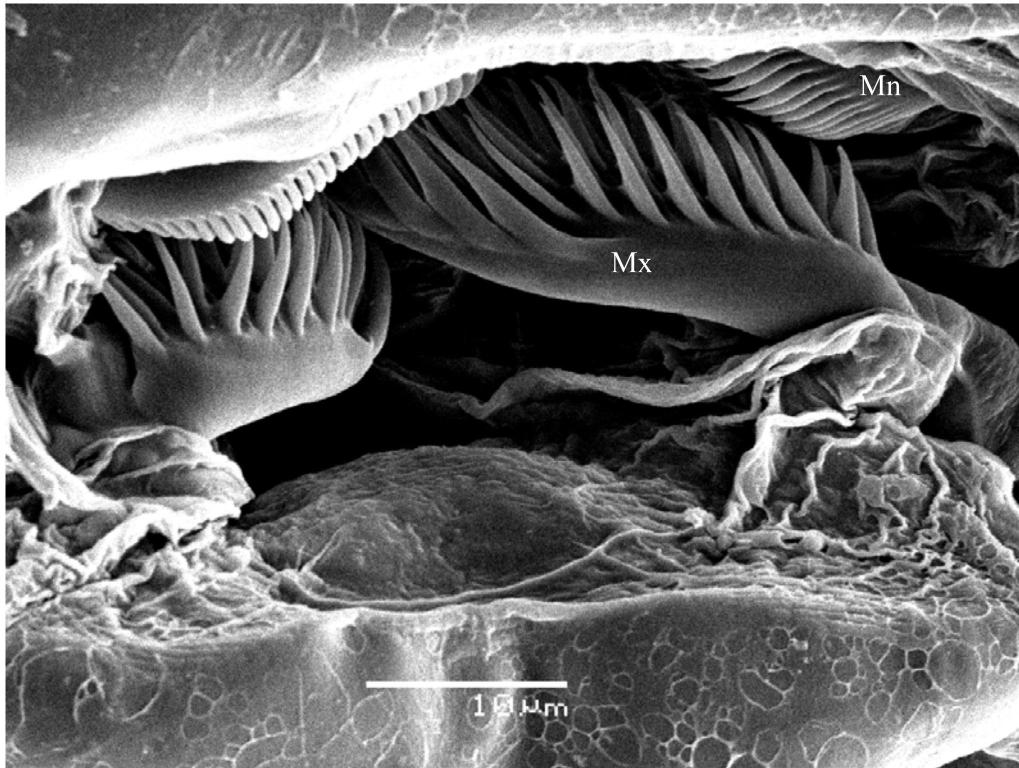


Plate 4. Extensive serrations on the lateral margins of swimming legs. Ventro-lateral view.

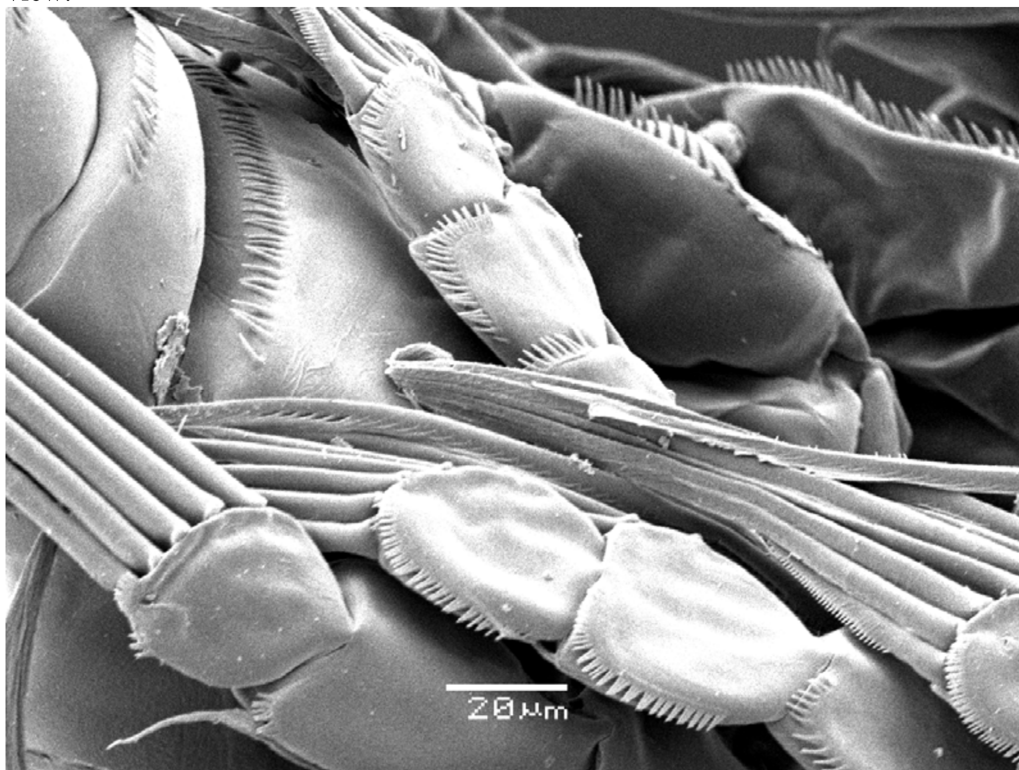


Plate 5. Serrations on the ventral margins of the abdominal somites. Ventro-lateral view.

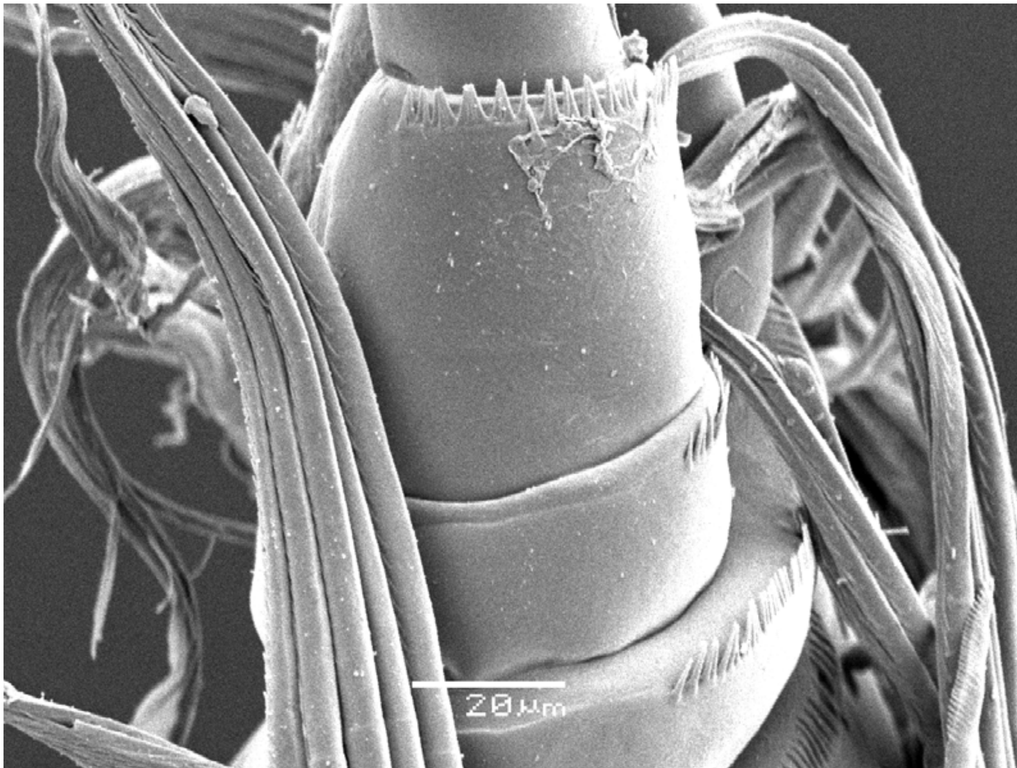


Plate 6. Ariel view of Rutland Water.

Reproduced from Google Earth

Plate 7. Photograph of experimental cages. Photographer was facing north-west.

Plate 8. A typical overwintered rainbow trout (above) and a recently stocked rainbow trout (below). Both caught by angling in April 2004.

Plate 9. Ovigerous *E.sieboldi* with white egg sacs.

Plate 10. Ovigerous *E.sieboldi* with advanced egg sacs.

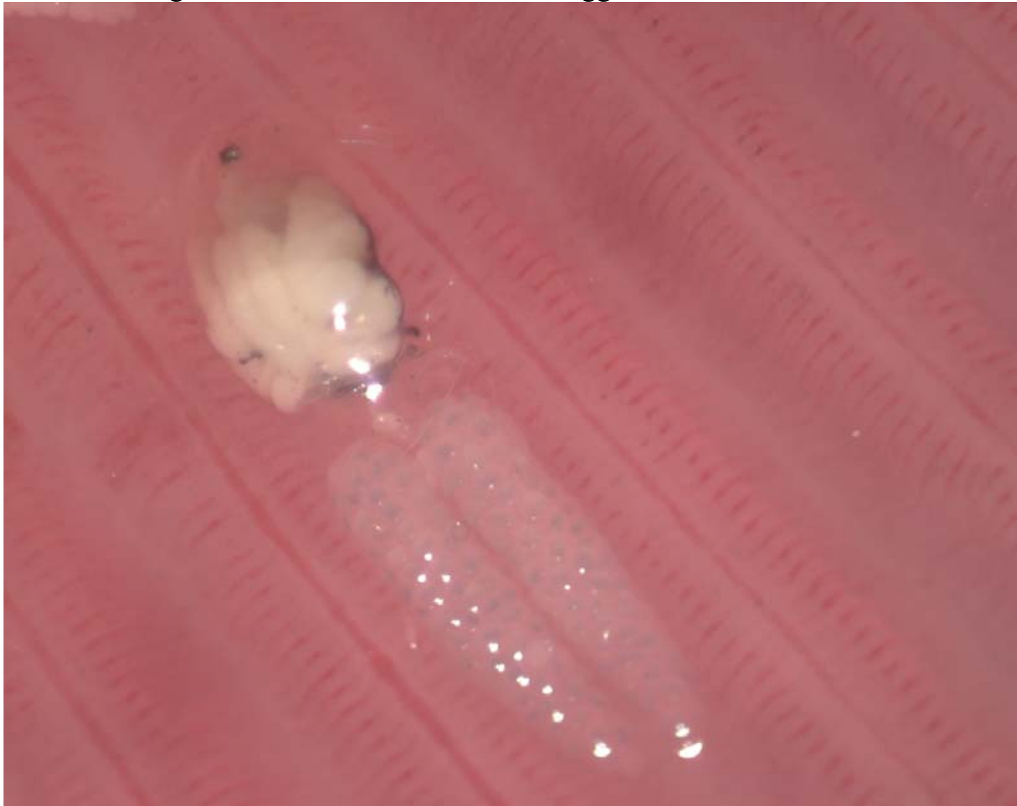


Plate 11. Gill from an angler caught rainbow trout in March 2004. *E.sieboldi* are in a non-ovigerous state. Some mucus production is evident on the gill.

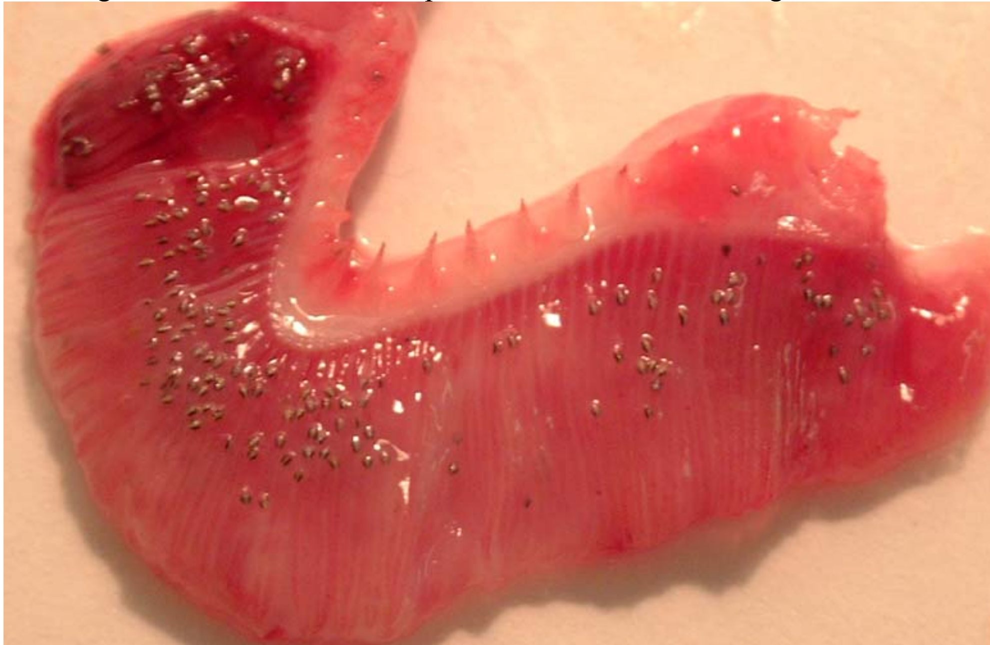


Plate 12. Gill from an angler caught rainbow trout in July 2004. *E.sieboldi* are in an ovigerous state. Extensive mucus production is evident on the gill.

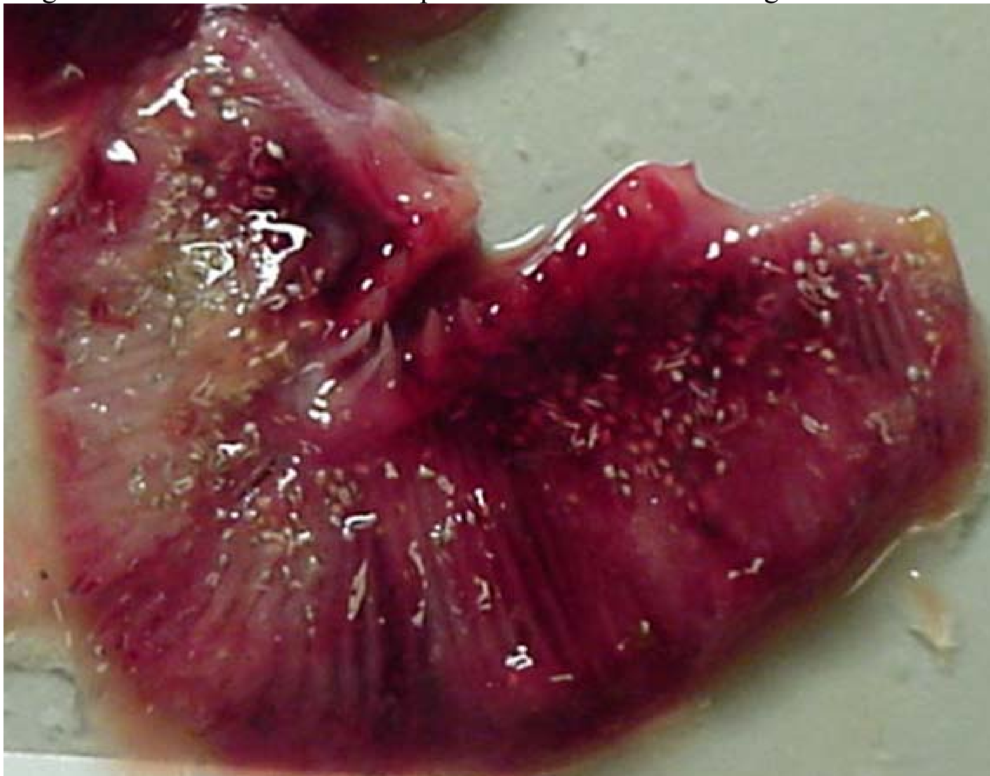


Plate 13. Digital image of *E.sieboldi* in non-ovigerous, ovigerous and ovigerous advanced states.

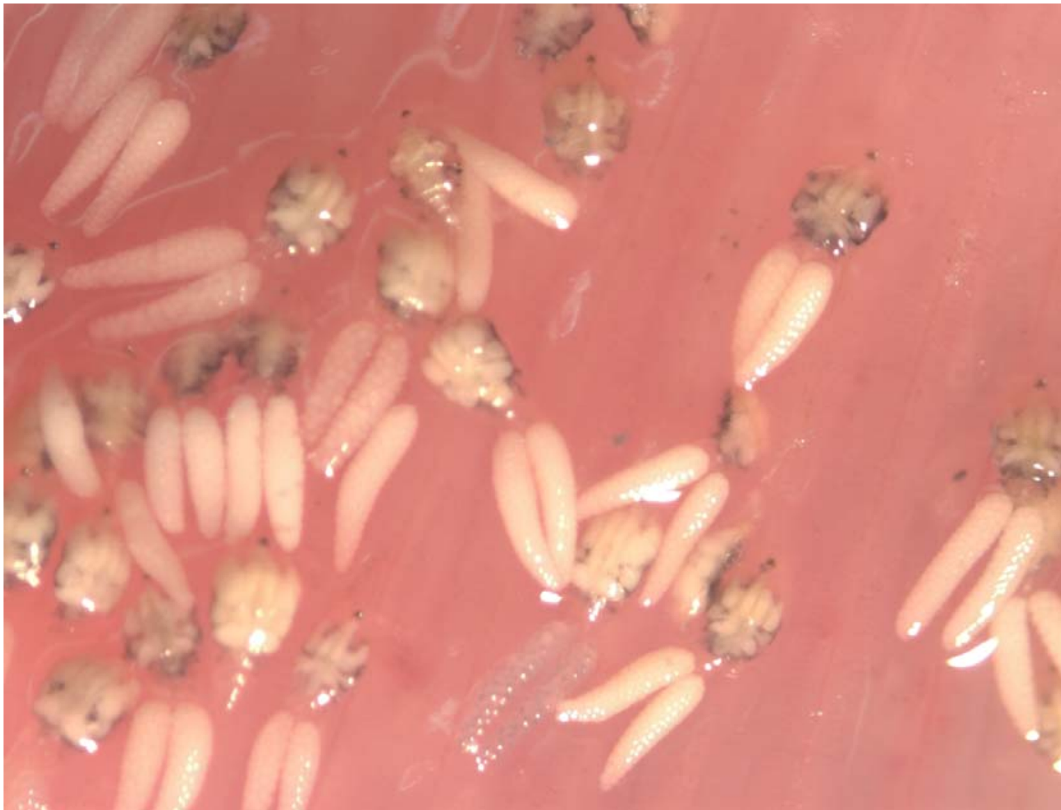


Plate 14. Digital image of the third gill arch on the right hemibranch from *O. mykiss* individual RT4 showing a moderate infection of *E. sieboldi.*

Plate 15. A higher magnification of the ergasilid infection shown in Plate 14. By using the digital zoom the development stage of each individual parasite can be determined.

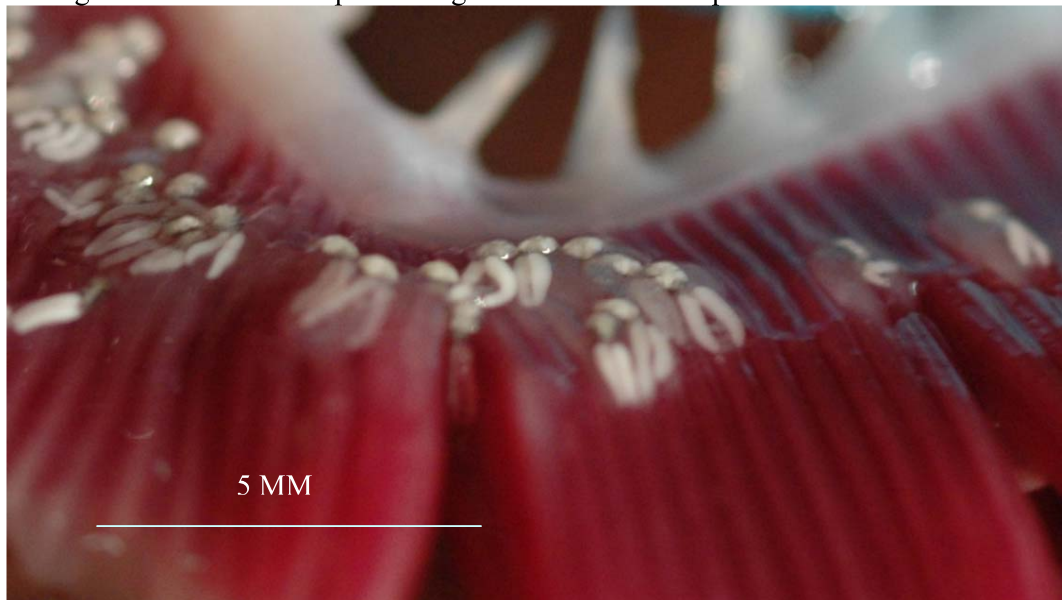


Plate 16. A negative image of that shown in Plate 15. By examining a negative image an indication of the reproductive status of each *E. sieboldi* can also be obtained. Although this was not the method applied here to determine reproductive status, it does permit subtle changes in grey scale captured in photographs to be used to show differences between mature and immature egg sacs.

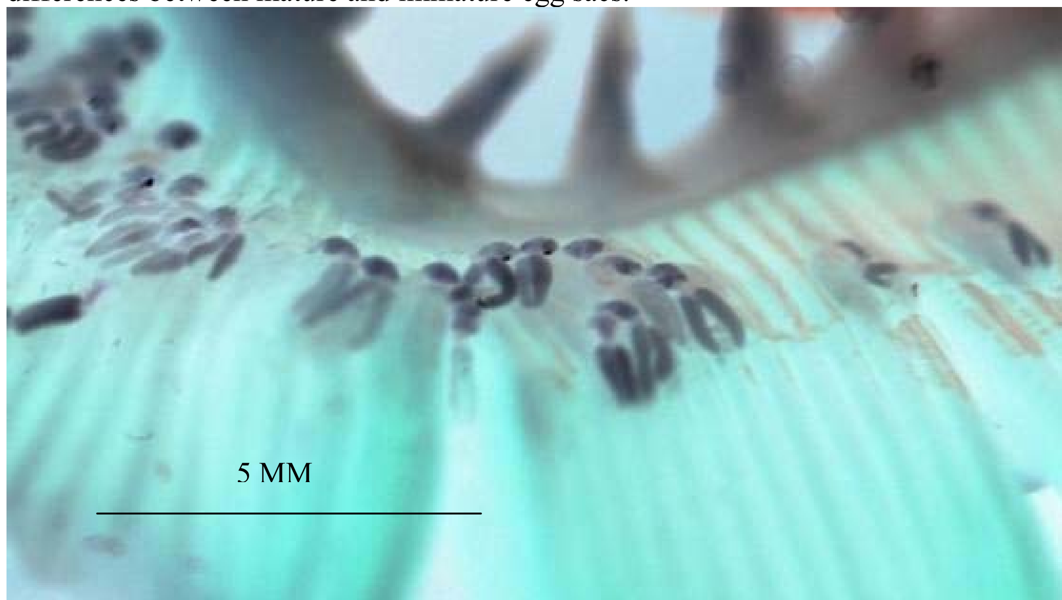


Plate 17. Light micrograph of the cephalosome indicating general ovary shape of *E.sieboldi* (dorsal view) showing paired ovary with 8 diverticula.

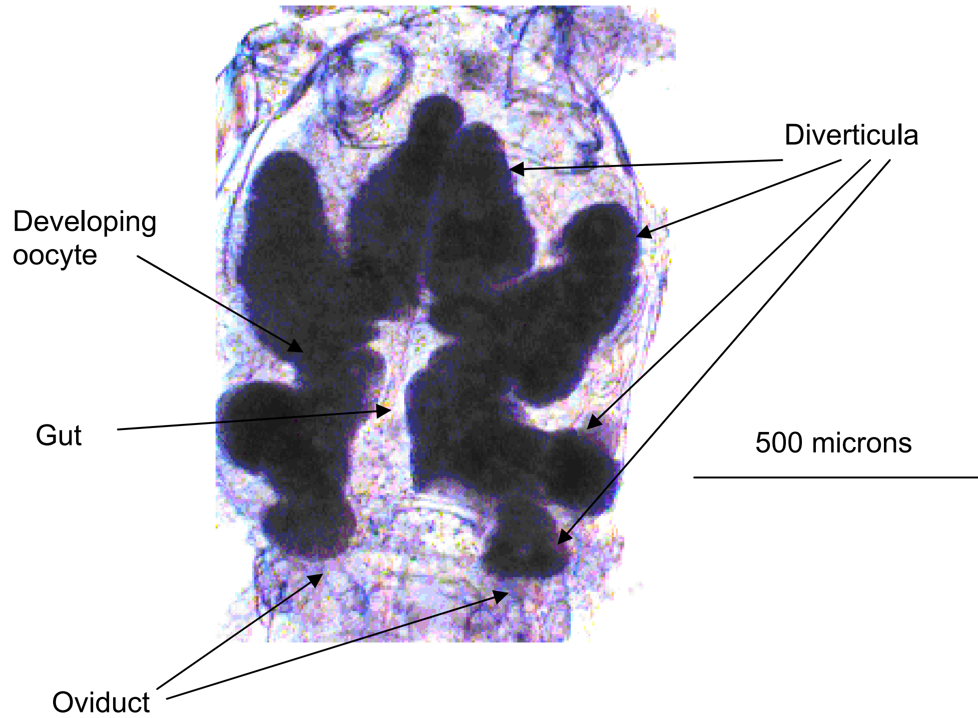


Plate 18. Light micrograph of *E.sieboldi* during oviposition. Oocytes are extruded from the posterior ovarian diverticula and are carried along paired oviducts which converge immediately anterior to the genital complex. Ventral view.

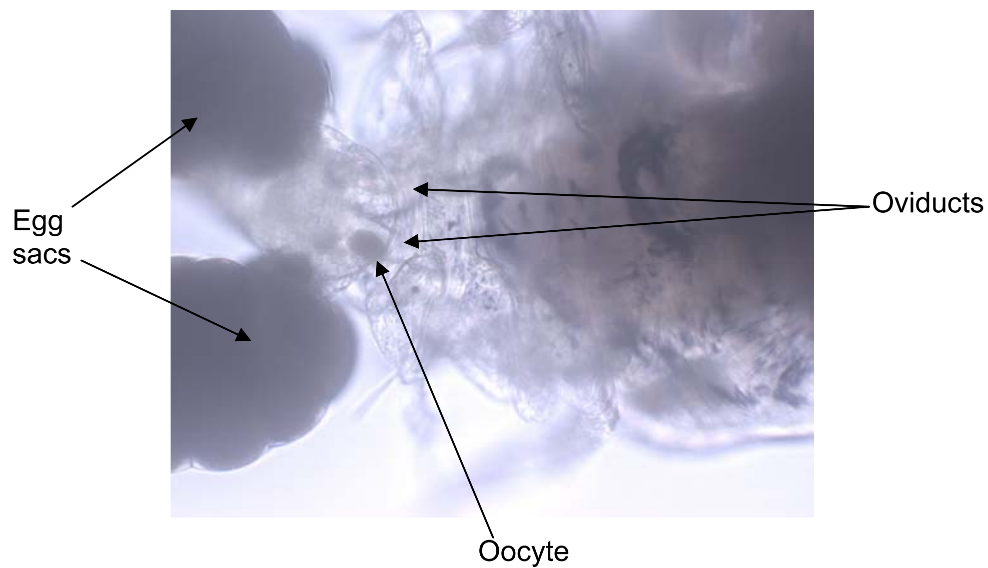


Plate 19. Phase contrast light micrograph of *E.sieboldi* during oviposition.

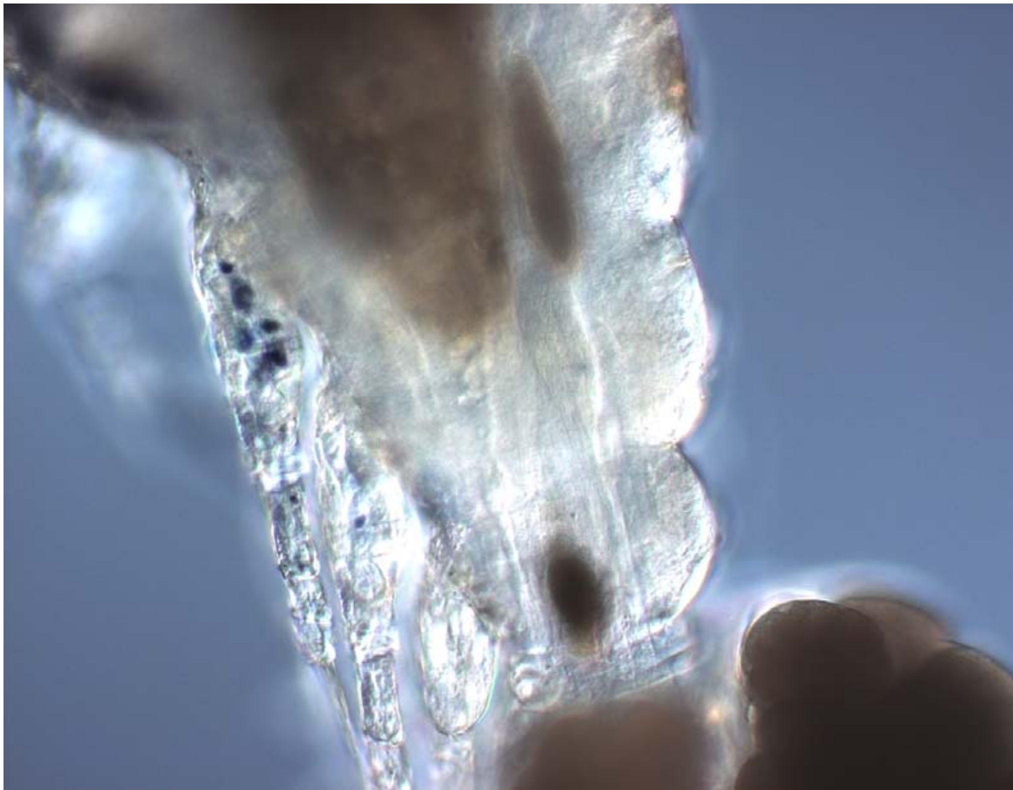


Plate 20. SEM of *E.sieboldi* cephalosome. Dorsal view. The cephalosome is expanded due the increased mass of developing ovarian diverticula within..

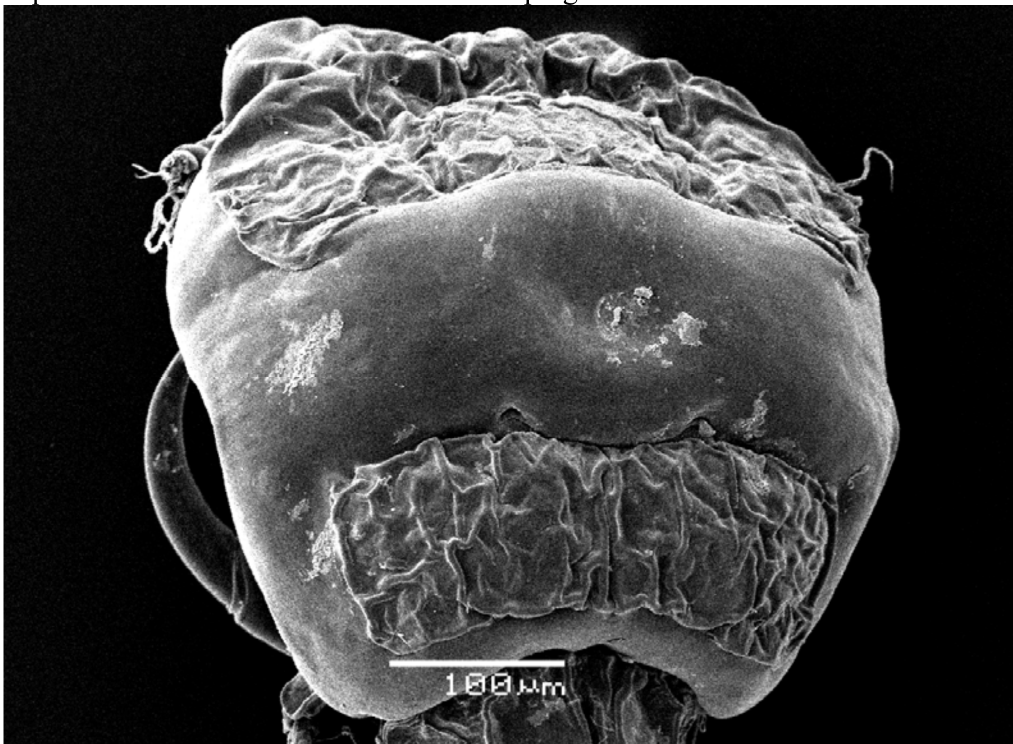


Plate 21. Lateral view of *E.sieboldi* cephalosome to illustrate the 3 dimensional aspects of ovary shape. A. Phase contrast light micrograph. B. Negative phase contrast. C. Diagram of ovary shape.

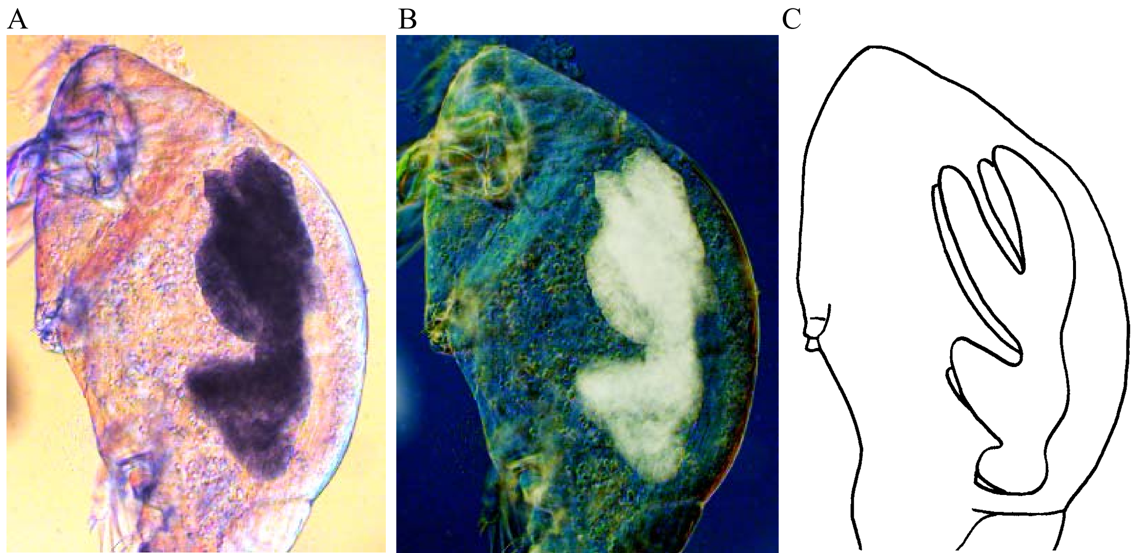


Plate 22. Negative light micrographs illustrating the variation of ovary development in *E.sieboldi.*

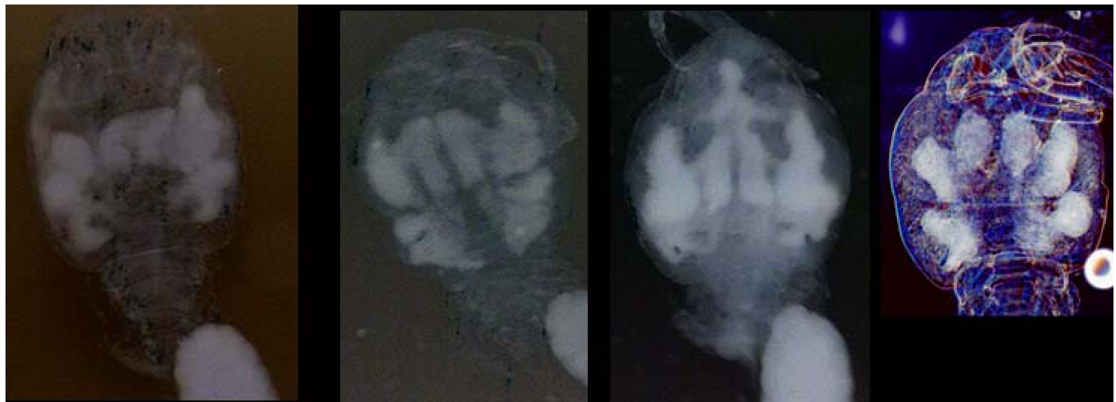


Plate 23. Light micrograph of *E.sieboldi* nauplii hatching from advanced stage egg sac. Dark eggs with no developing nauplii visible are assumed to be non-viable.

Plate 24. Light micrograph of *E.sieboldi* nauplius in process of hatching I. The egg has become separated from the egg sac. Note the extension of the caudal knob to the surface of the egg capsule. Rostrum of nauplius has emerged from the egg capsule.

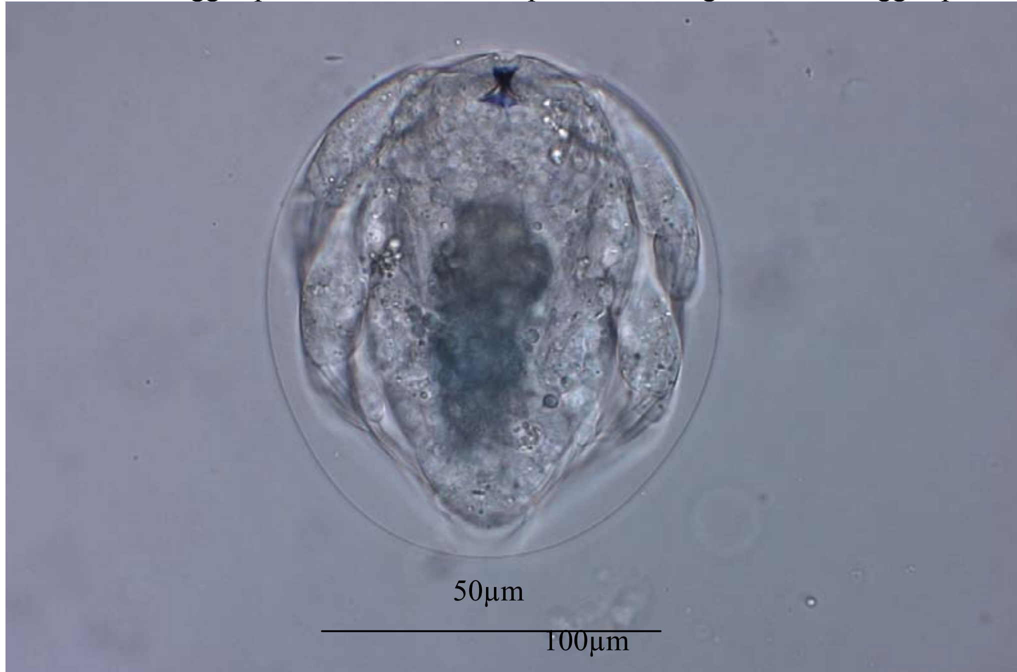


Plate 25. Light micrograph of *E.sieboldi* nauplius in process of hatching II. Antenna and antennules of nauplius have moved to the surface of the egg capsule causing it to expand its width while pulling further the breach in the egg capsule further over the rostrum.

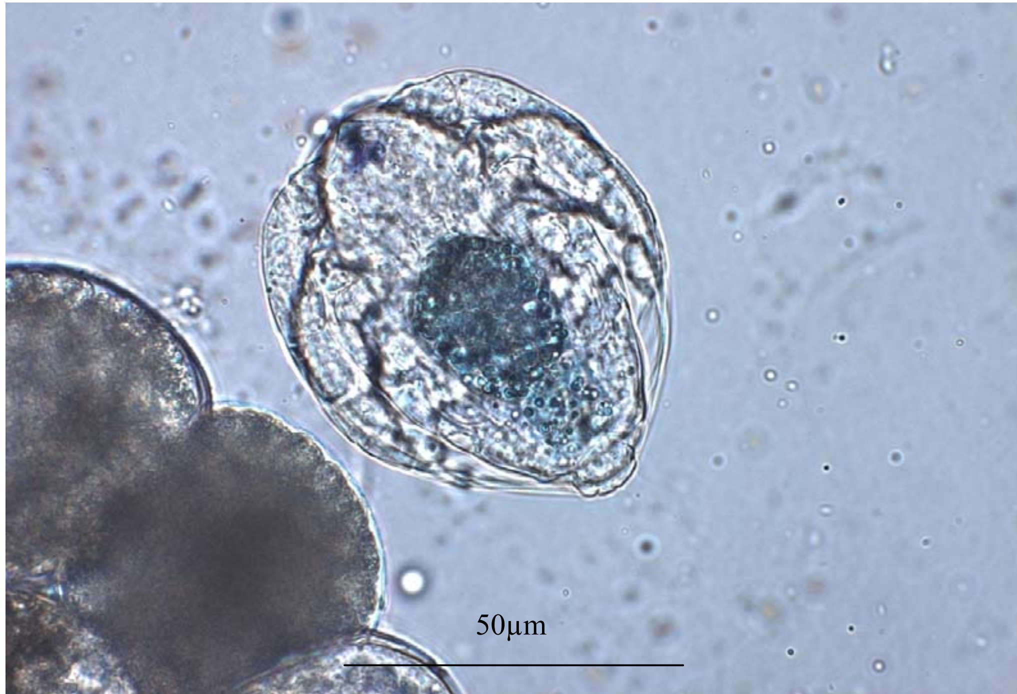


Plate 26. Light micrograph of *E.sieboldi* nauplius in process of hatching III. The breach in egg capsule has increased in size and moved to the posterior half of the nauplius. The antennules have now become free of the egg while the antennae move to free the nauplius from the envelope entirely.

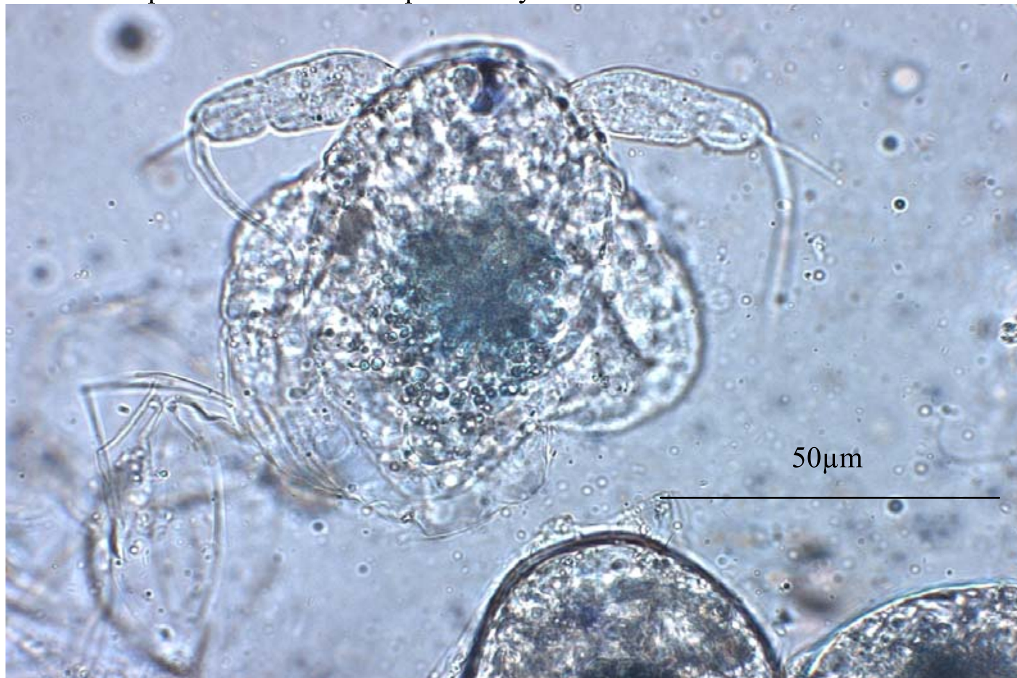


Plate 27 and Plate 28. Light micrographs of *E.sieboldi* nauplius in process of hatching IV. The nauplius is now free of the capsule and begins to raise antennules and antennae into the swimming position.

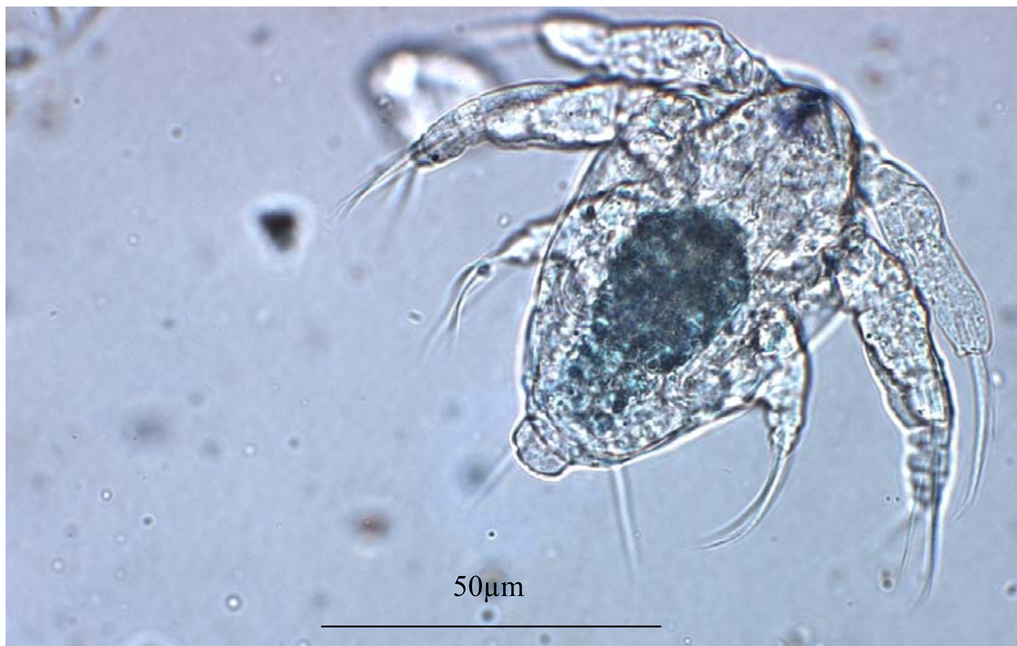


Plate 29. Light micrograph of *E.sieboldi* nauplius in process of hatching VI. The hatched nauplius stage NI with fully extended antennae and antennules.

Plate 30. Light micrograph of *E.sieboldi* nauplius in process of hatching V. The egg capsule remains as the nauplius swims away.

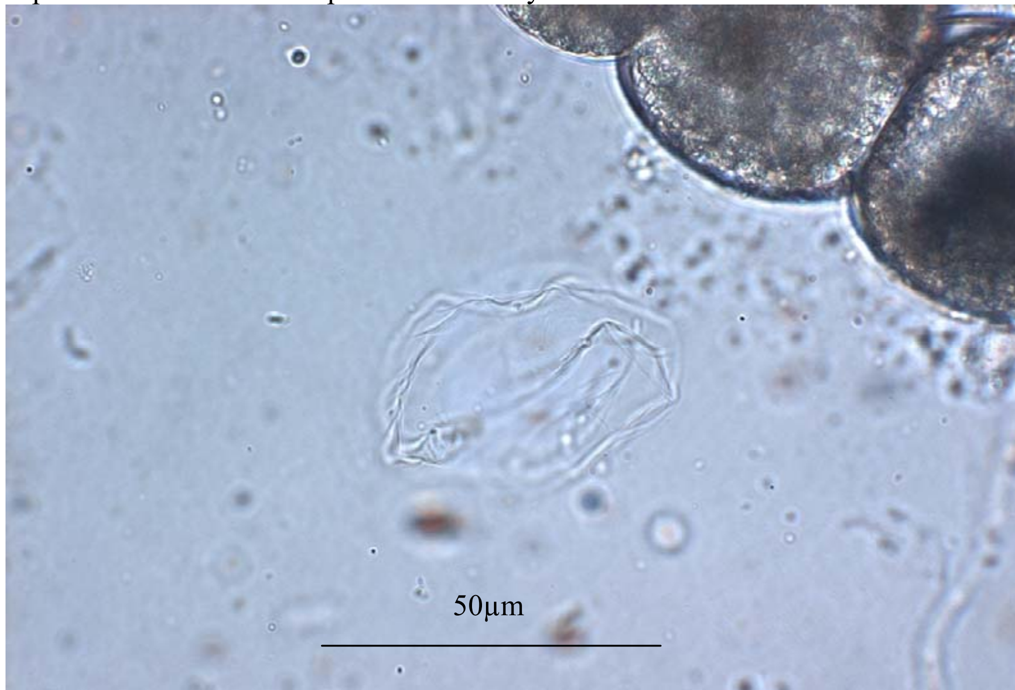


Plate 31. Light micrograph of *E.sieboldi* stage NI nauplius in filtered loch water. Ventral view. Hind gut contains a bolus of dark olive matter.

Plate 32. Light micrograph of *E.sieboldi* stage NII nauplius in filtered loch water. Dorsal view. Hind gut contains a bolus of light brown matter. Fore gut contains areas of green that may reflect recent feeding on green alga.

Plate 33. Light micrograph of *Cryptomonas* sp. algae.

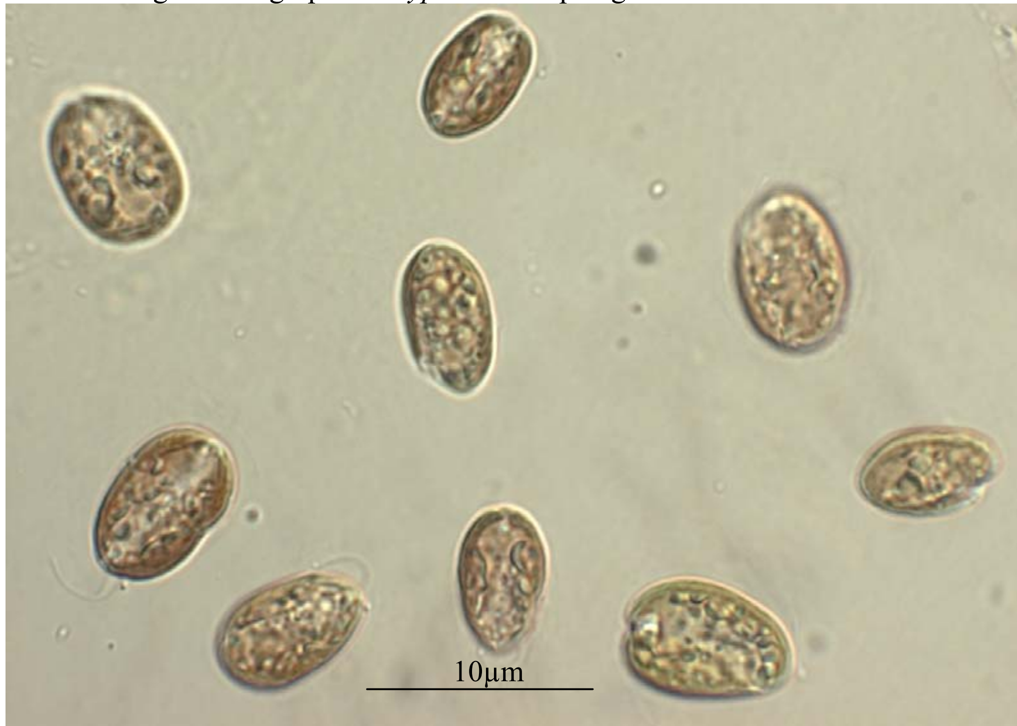


Plate 34. Light micrograph. *E.sieboldi* stage NI nauplius in culture of *Cryptomonas* sp. algae. Nauplius has just been added to the culture and has not yet fed.

Plate 35. Light micrograph. Dorsal view of *E.sieboldi* stage NI nauplius. Note bolus in hind gut of *Cryptomonas* sp. algal cell.

Plate 36. Light micrograph. *E.sieboldi* stage NI nauplius in culture of *Chlorella* sp. algae. Hind gut contains condensed algal cells and fore gut contains numerous algal cells in the process of digestion.

Plate 37. Light micrograph of *Haematacoccus* sp. algae.

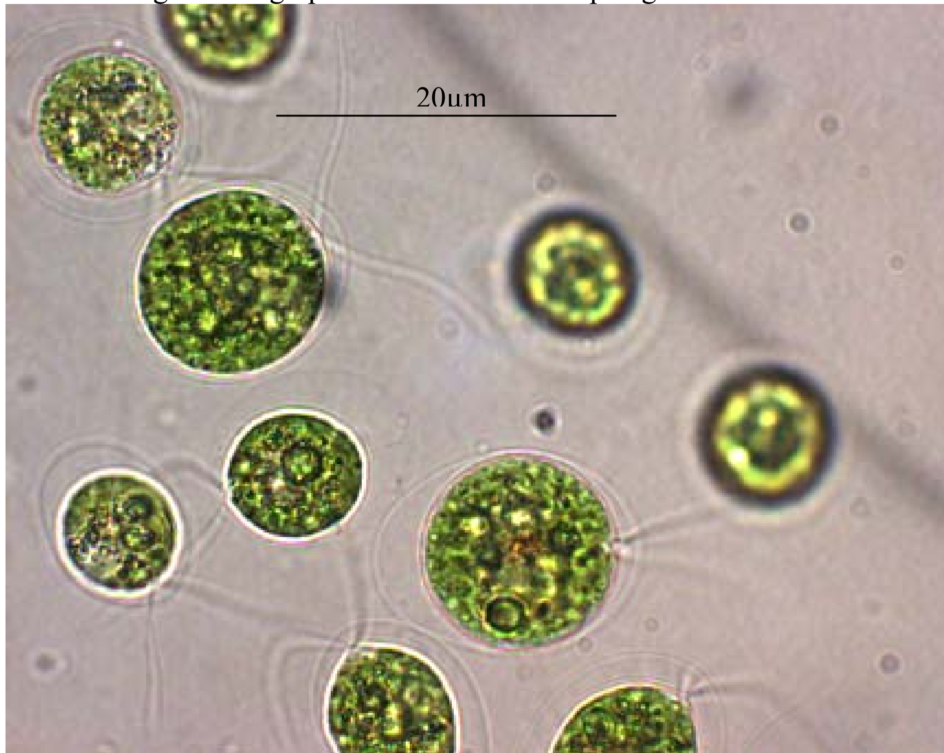


Plate 38. Light micrograph. *E.sieboldi* stage NI nauplius in culture of *Haematococcus* sp. algae. Hind gut contains an entire algal cell and peristaltic movement of gut aids in the breakdown of another algal cell.

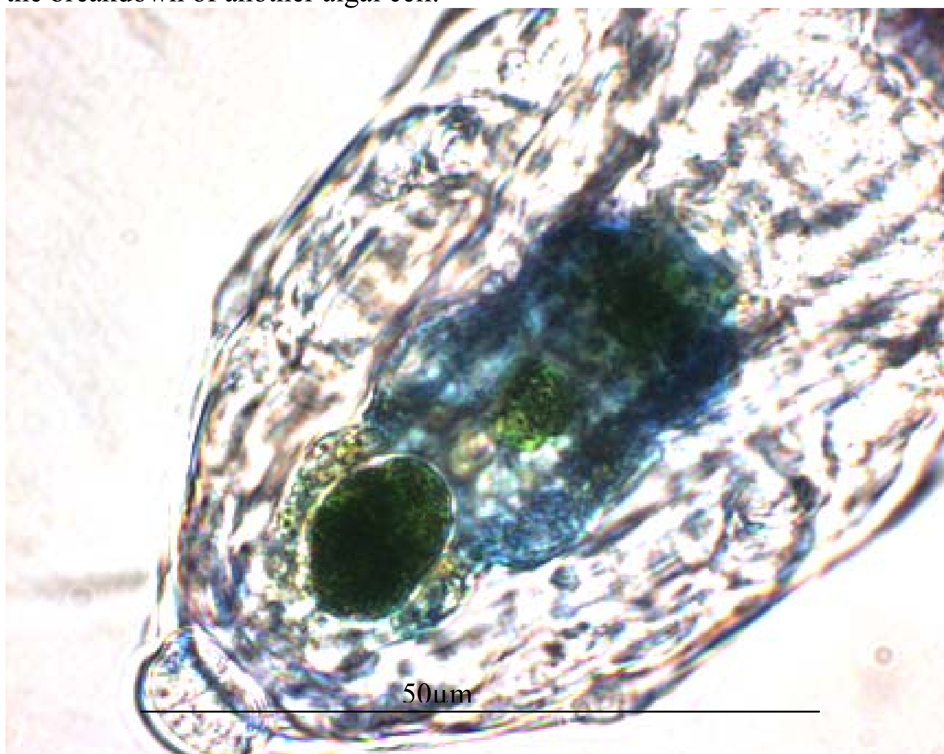


Plate 39. Light micrograph. *E.sieboldi* stage NII nauplius from polyculture. Hind gut contains brown and green algae cells.

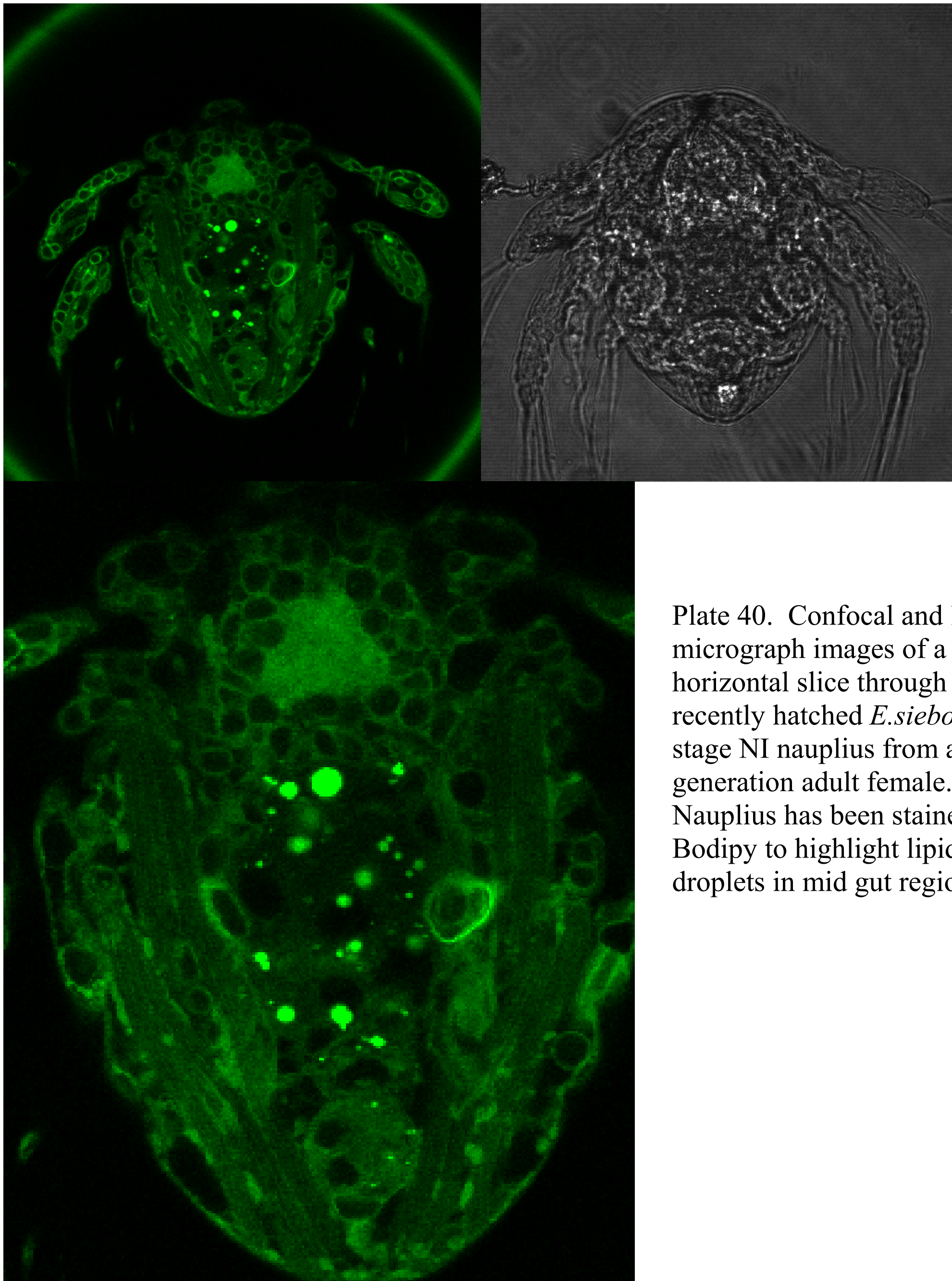


Plate 40. Confocal and light micrograph images of a horizontal slice through a recently hatched *E.sieboldi* stage NI nauplius from a P generation adult female. Nauplius has been stained with Bodipy to highlight lipid droplets in mid gut region.

Plate 41. Lateral view of *E.sieboldi* NI stage nauplius. Note pigmented cells lining gut. *A* = anus, *Ck* = caudal knob, *G* = gut, *Hg* = hind gut, *M* = mouth.

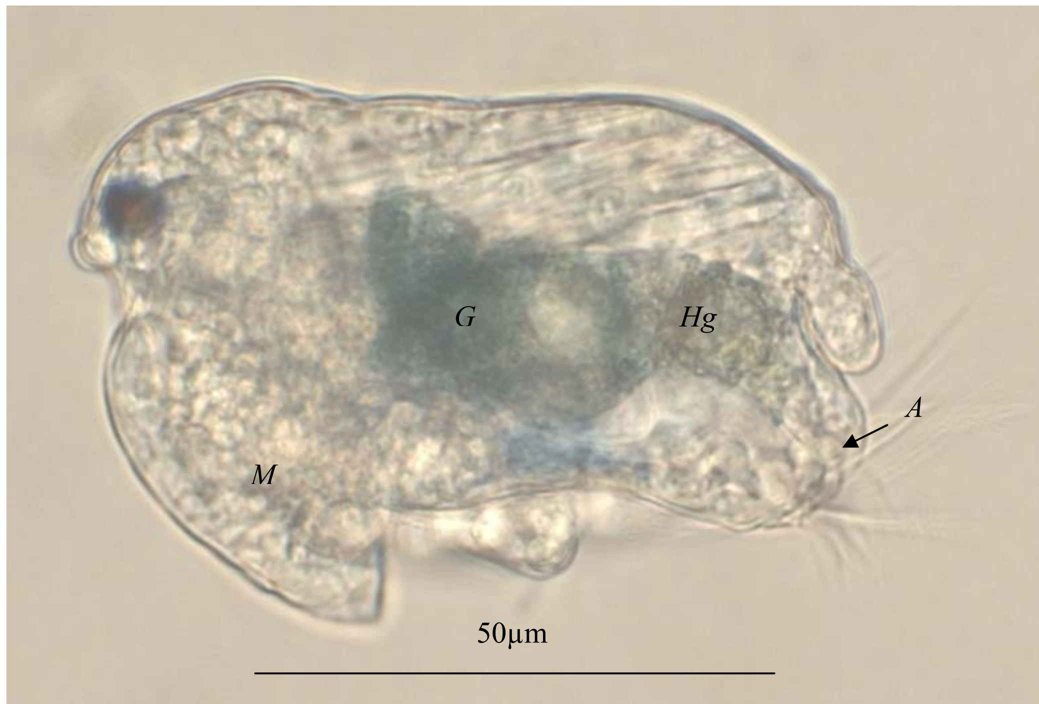


Plate 42. Lateral view of *E.sieboldi* NII stage nauplius. Note increased number of pigment cells and dorsal migration of caudal knob.

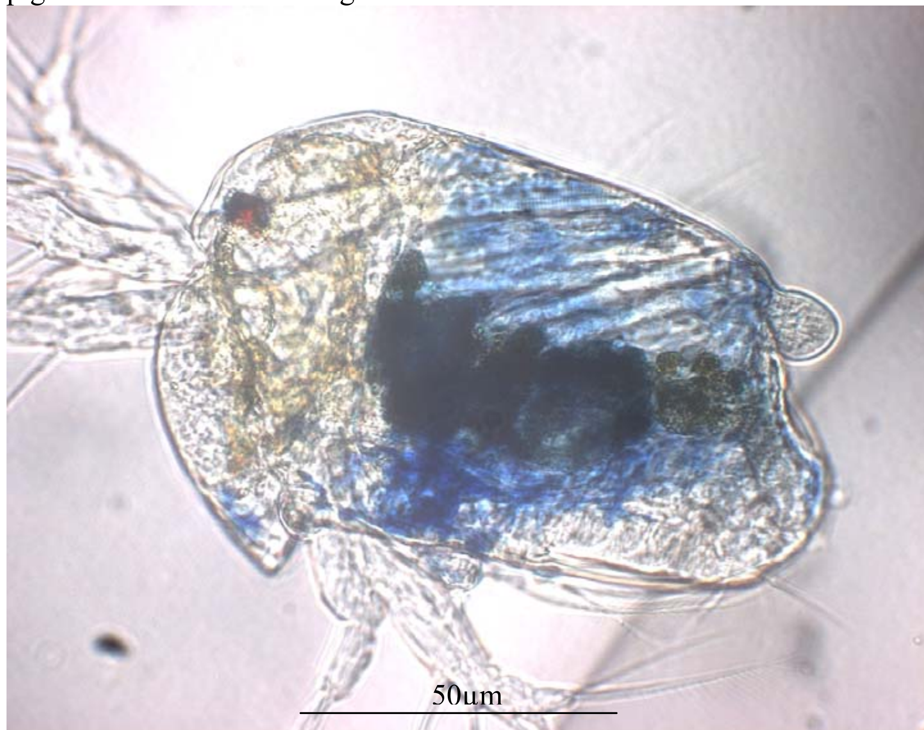


Plate 43. Lateral view of gut of *E.sieboldi* NII stage nauplius. Note bolus of matter formed into a faecal pellet in the hind gut.

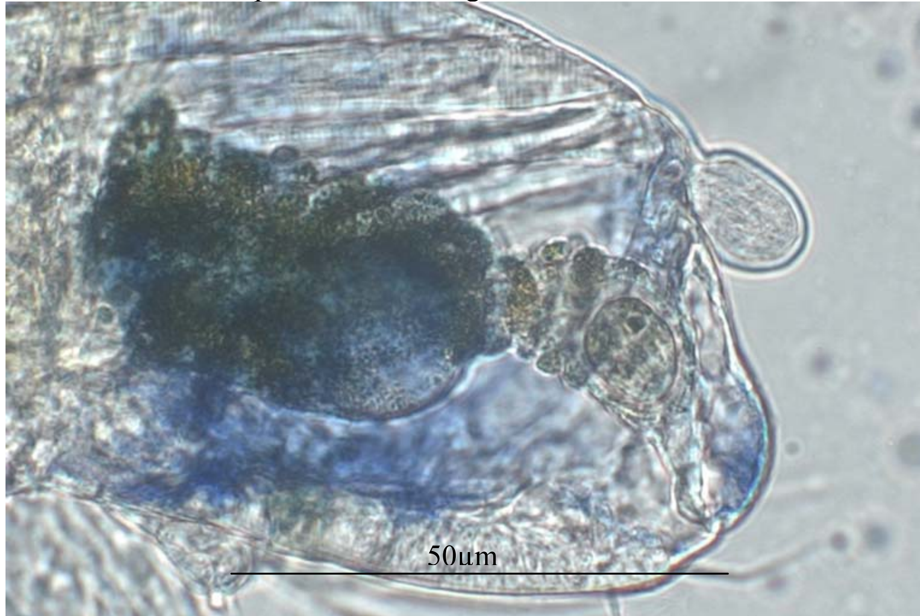


Plate 44. Light micrograph. *E.sieboldi* stage NI nauplius having just extruded a faecal pellet consisting of *Cryptomonas* sp. algae.

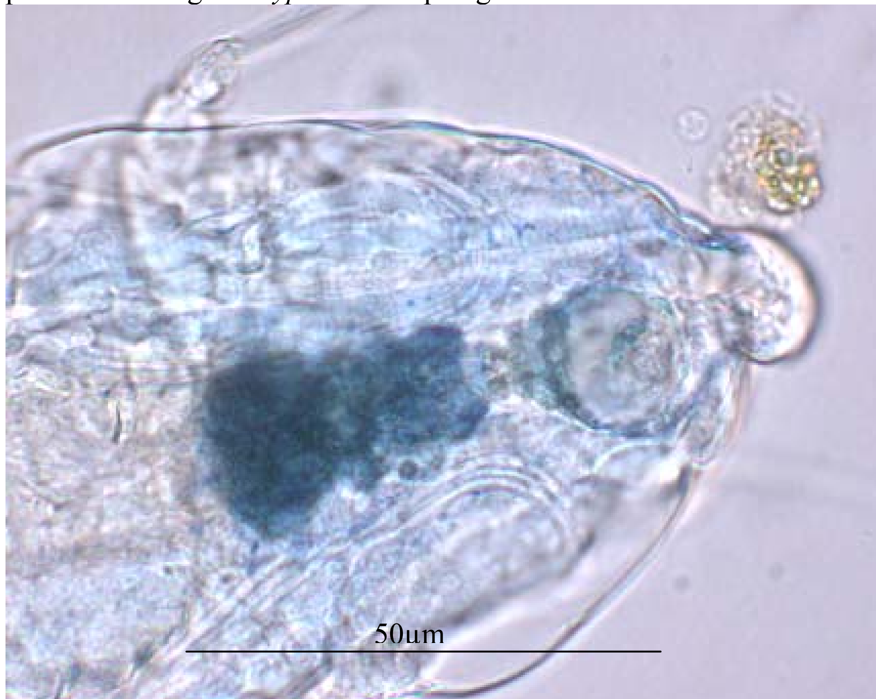
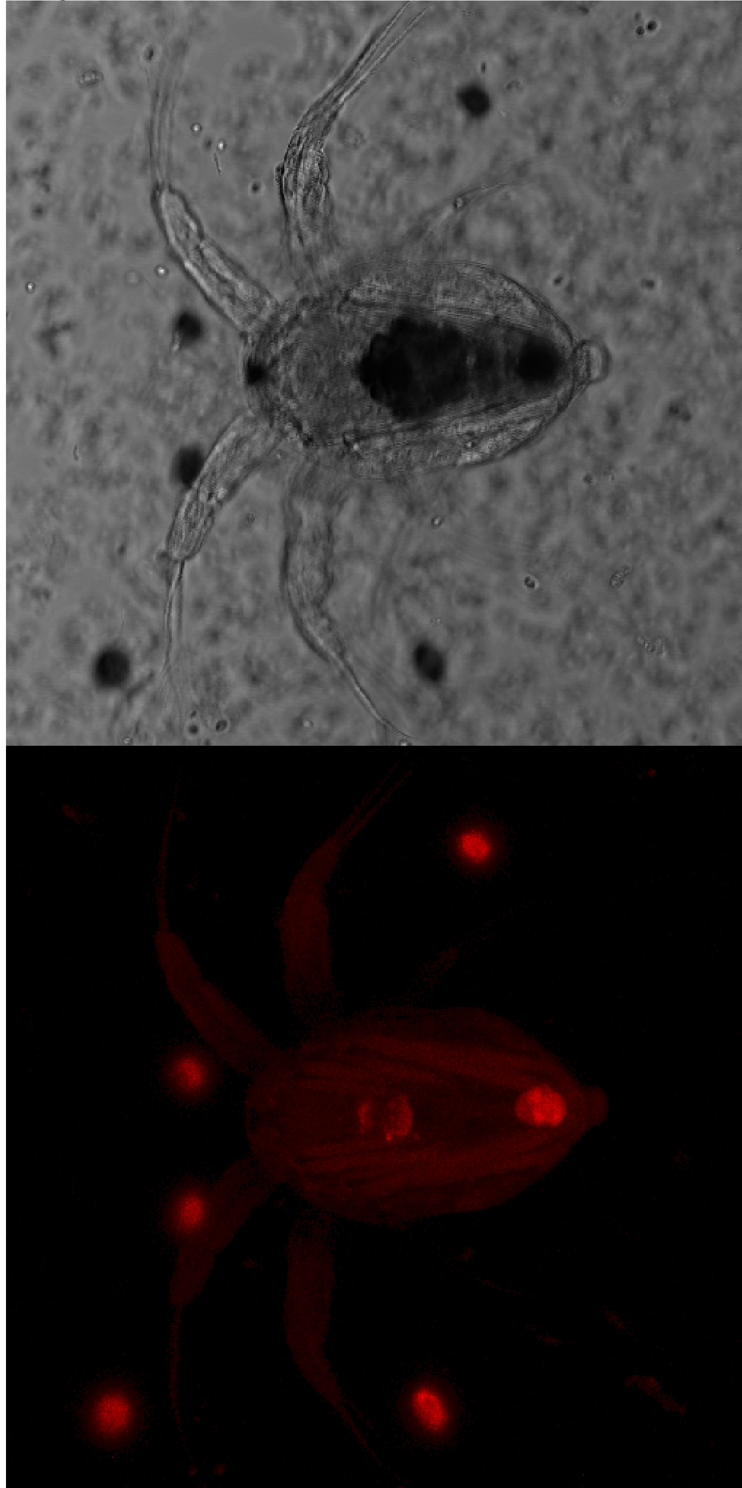


Plate 45. Light micrograph and confocal image of *E.sieboldi* stage NI nauplius in mono culture of *Haematococcus* sp. A confocal laser scanning micrograph of algal cells shows both an entire algal cell in the hind gut and another algal cell being broken down in the fore gut.



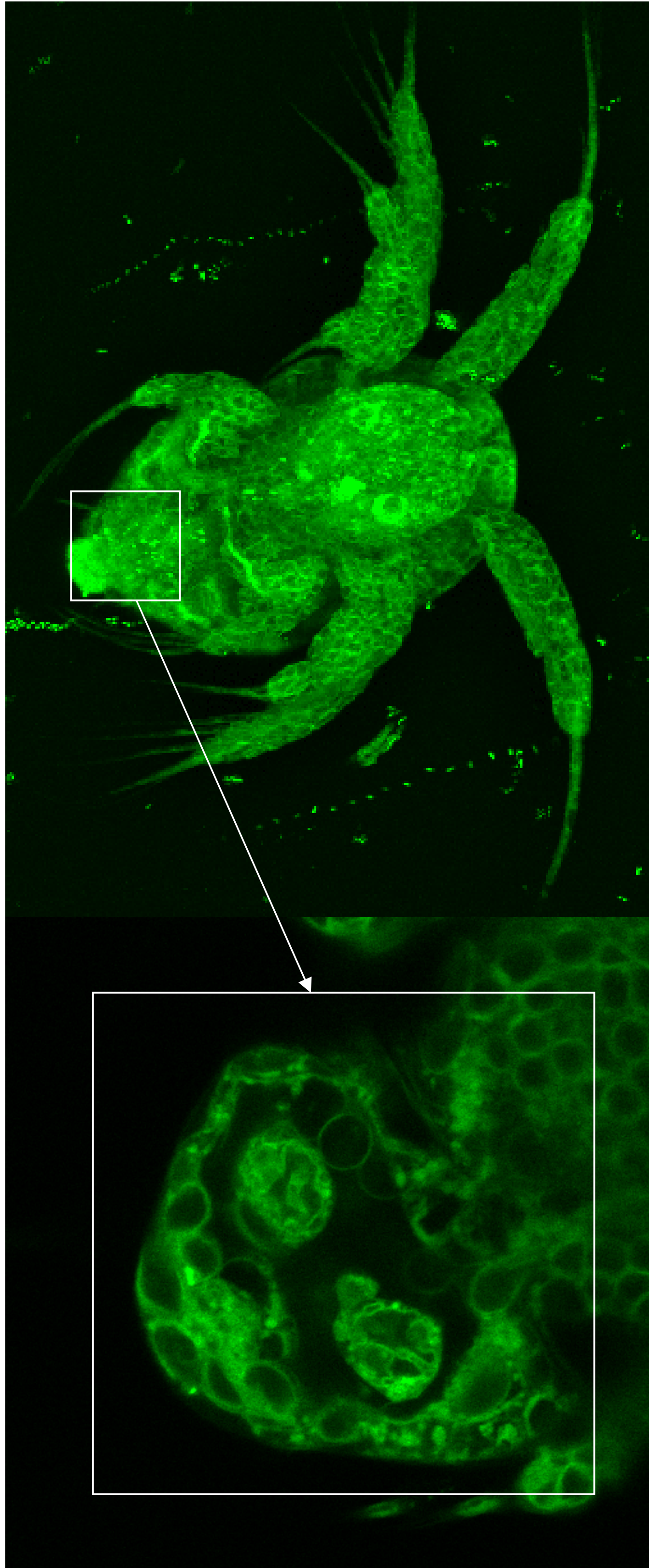


Plate 46. Laser scanning confocal image of the ventral surface of *E.sieboldi* stage NI nauplius. Inset is a horizontal slice image generated by the confocal microscope of two organelles in the caudal region of the nauplius located on either side of the hind gut which may have a digestive glandular function.

Appendix 1 Rutland Water rainbow trout catch and stocking data - 2000

Date	Returns	Cumulative returns	Catch	Cumulative catch	Stock	Cumulative stock	Catch rate
Pre Season					20000	20000	
09/04/2000	222	222	1369	1369	0	20000	6.2
16/04/2000	236	458	1350	2719	6275	26275	5.7
23/04/2000	532	990	2098	4817	3375	29650	3.9
30/04/2000	532	1522	1684	6501	0	29650	3.2
07/05/2000	612	2134	1878	8379	3375	33025	3.1
14/05/2000	564	2698	1835	10214	4875	37900	3.3
21/05/2000	560	3258	1776	11990	3150	41050	3.2
28/05/2000	635	3893	1375	13365	0	41050	2.2
04/06/2000	732	4625	2281	15646	3125	44175	3.1
11/06/2000	689	5314	2769	18415	1200	45375	4.0
18/06/2000	705	6019	1622	20037	6300	51675	2.3
25/06/2000	705	6724	3697	23734	0	51675	5.2
02/07/2000	363	7087	857	24591	4500	56175	2.4
09/07/2000	478	7565	1107	25698	5000	61175	2.3
16/07/2000	832	8397	2613	28311	0	61175	3.1
23/07/2000	620	9017	1096	29407	3500	64675	1.8
30/07/2000	421	9438	661	30068	2400	67075	1.6
06/08/2000	600	10038	1289	31357	3868	70943	2.1
13/08/2000	344	10382	839	32196	5324	76267	2.4
20/08/2000	358	10740	949	33145	3200	79467	2.7
27/08/2000	445	11185	1703	34848	2125	81592	3.8
03/09/2000	564	11749	2632	37480	3777	85369	4.7
10/09/2000	358	12107	678	38158	1000	86369	1.9
17/09/2000	316	12423	843	39001	2800	89169	2.7
24/09/2000	601	13024	1919	40920	0	89169	3.2
01/10/2000	273	13297	483	41403	2800	91969	1.8
08/10/2000	315	13612	632	42035	2400	94369	2.0
15/10/2000	478	14090	1333	43368	0	94369	2.8
22/10/2000	272	14362	765	44133	1400	95769	2.8
29/10/2000	362	14724	1260	45393	0	95769	3.5
05/11/2000	429	15153	1035	46428	800	96569	2.4
12/11/2000	264	15417	610	47038	800	97369	2.3
19/11/2000	124	15541	279	47317	0	97369	2.3
26/11/2000	168	15709	321	47638	0	97369	1.9
03/12/2000	112	15821	300	47938	0	97369	2.7
10/12/2000	64	15885	180	48118	0	97369	2.8
17/12/2000	95	15980	135	48253	0	97369	1.4
24/12/2000	35	16015	95	48348	0	97369	2.7
31/12/2000	42	16057	86	48434	0	97369	2.0

Appendix 2 Rutland Water rainbow trout catch and stocking data - 2001

Date	Returns	Cumulative returns	Catch	Cumulative catch	Stock	Cumulative stock	Catch rate
Pre Season					19800		
08/04/2001	217	217	1284	1284	3600	23360	5.9
15/04/2001	431	648	1942	3226	9800	33160	4.5
22/04/2001	481	1129	2509	5735	4800	37960	5.2
29/04/2001	568	1697	3457	9192	1800	39760	6.1
06/05/2001	595	2292	3163	12355	3500	43260	5.3
13/05/2001	541	2833	1854	14209	2500	45760	3.4
20/05/2001	760	3593	3605	17814	5500	51260	4.7
27/05/2001	619	4212	2848	20662	0	51260	4.6
03/06/2001	618	4830	2459	23121	5000	56260	4.0
10/06/2001	758	5588	2769	25890	3125	59385	3.7
17/06/2001	753	6341	2530	28420	3550	62935	3.4
24/06/2001	581	6922	1694	30114	1500	64435	2.9
01/07/2001	443	7365	993	31107	3500	67935	2.2
08/07/2001	358	7723	987	32094	0	67935	2.8
15/07/2001	373	8096	911	33005	3700	71635	2.4
22/07/2001	330	8426	882	33887	3434	75069	2.7
29/07/2001	320	8746	846	34733	2444	77513	2.6
05/08/2001	264	9010	484	35217	3070	80583	1.8
12/08/2001	321	9331	854	36071	6650	87233	2.7
19/08/2001	396	9727	1482	37553	2040	89273	3.7
26/08/2001	467	10194	1909	39462	4100	93373	4.1
02/09/2001	253	10447	1100	40562	0	93373	4.3
09/09/2001	488	10935	2030	42592	2100	95473	4.2
16/09/2001	376	11311	1158	43750	2100	97573	3.1
23/09/2001	368	11679	1358	45108	0	97573	3.7
30/09/2001	760	12439	3843	48951	0	97573	5.1
07/10/2001	282	12721	1030	49981	2200	99773	3.7
14/10/2001	442	13163	1215	51196	1100	100873	2.7
21/10/2001	259	13422	597	51793	800	101673	2.3
28/10/2001	276	13698	640	52433	0	101673	2.3
04/11/2001	148	13846	317	52750	0	101673	2.1
11/11/2001	140	13986	260	53010	0	101673	1.9
18/11/2001	89	14075	134	53144	0	101673	1.5
25/11/2001	135	14210	285	53429	0	101673	2.1
02/12/2001	92	14302	186	53615	0	101673	2.0
09/12/2001	110	14412	300	53915	0	101673	2.7
16/12/2001	55	14467	180	54095	0	101673	3.3
23/12/2001	55	14522	135	54230	0	101673	2.5
30/12/2001	65	14587	95	54325	0	101673	1.5

Appendix 3 Rutland Water rainbow trout catch and stocking data - 2002

Date	Returns	Cumulative returns	Catch	Cumulative catch	Released	Stock	Cumulative stock	Catch rate
Pre Season						19800		
07/04/2002	578	578	3031	3031	513	3250	23050	5.2
14/04/2002	691	1269	2922	5953	292	5800	28850	4.2
21/04/2002	633	1902	3162	9115	466	0	28850	5.0
28/04/2002	529	2431	2132	11247	459	6350	35200	4.0
05/05/2002	520	2951	2036	13283	422	6100	41300	3.9
12/05/2002	757	3708	3406	16689	654	4400	45700	4.5
19/05/2002	734	4442	3783	20472	734	3000	48700	5.2
26/05/2002	702	5144	2573	23045	398	4000	52700	3.7
02/06/2002	493	5637	1384	24429	191	0	52700	2.8
09/06/2002	823	6460	2491	26920	500	4000	56700	3.0
16/06/2002	731	7191	2295	29215	345	0	56700	3.1
23/06/2002	609	7800	1640	30855	215	2800	59500	2.7
30/06/2002	365	8165	951	31806	70	9700	69200	2.6
07/07/2002	433	8598	1531	33337	486	1500	70700	3.5
14/07/2002	643	9241	2036	35373	146	3500	74200	3.2
21/07/2002	497	9738	1076	36449	136	3500	77700	2.2
28/07/2002	348	10086	724	37173	85	0	77700	2.1
04/08/2002	388	10474	1058	38231	31	3000	80700	2.7
11/08/2002	331	10805	729	38960	228	2600	83300	2.2
18/08/2002	513	11318	991	39951	289	0	83300	1.9
25/08/2002	230	11548	469	40420	64	4852	88152	2.0
01/09/2002	237	11785	531	40951	19	1320	89472	2.2
08/09/2002	248	12033	417	41368	36	0	89472	1.7
15/09/2002	438	12471	1075	42443	309	3166	92638	2.5
22/09/2002	307	12778	875	43318	279	2000	94638	2.9
29/09/2002	275	13053	590	43908	17	500	95138	2.1
06/10/2002	432	13485	893	44801	23	0	95138	2.1
13/10/2002	339	13824	613	45414	23	0	95138	1.8
20/10/2002	203	14027	290	45704	7	0	95138	1.4
27/10/2002	202	14229	361	46065	4	0	95138	1.8
03/11/2002	110	14339	157	46222	0	0	95138	1.4
10/11/2002	31	14370	77	46299	0	0	95138	2.7
17/11/2002	65	14435	216	46515	0	0	95138	3.3
24/11/2002	91	14526	262	46777	0	0	95138	2.9
01/12/2002	65	14591	182	46862	0	0	95138	2.8
08/12/2002	31	14622	60	46922	0	0	95138	1.9
15/12/2002	52	14674	109	47031	0	0	95138	2.1
22/12/2002	36	14710	75	47106	0	0	95138	2.1
29/12/2002	20	14730	39	47145	0	0	95138	2.0

Appendix 4 Rutland Water rainbow trout catch and stocking data - 2003

Date	Returns	Cumulative returns	Catch	Cumulative catch	Released	Stock	Cumulative stock	Catch rate
Pre Season	0	0	0	0	0	19000	19000	
06/04/2003	240	240	1595	1595	0	1000	20000	6.6
13/04/2003	546	786	3292	4887	0	4600	24600	6.0
20/04/2003	644	1430	3539	8426	11	2700	27300	5.5
27/04/2003	416	1846	1885	10311	30	6600	33900	4.5
04/05/2003	429	2275	1776	12087	50	3050	36950	4.1
11/05/2003	486	2761	2080	14167	16	1400	38350	4.3
18/05/2003	658	3419	2533	16700	36	3400	41750	3.8
25/05/2003	810	4229	3588	20288	25	0	41750	4.4
01/06/2003	639	4868	2061	22349	23	1800	43550	3.2
08/06/2003	658	5526	2095	24444	34	2800	46350	3.2
15/06/2003	633	6159	2016	26460	25	4400	50750	3.2
22/06/2003	325	6484	990	27450	19	5000	55750	3.0
29/06/2003	336	6820	1092	28542	275	4000	59750	3.3
06/07/2003	323	7143	923	29465	5	2662	62412	2.9
13/07/2003	409	7552	1140	30605	9	2100	64512	2.8
20/07/2003	212	7764	657	31262	1	2100	66612	3.1
27/07/2003	396	8160	1222	32484	6	0	66612	3.1
03/08/2003	213	8373	571	33055	1	8500	75112	2.7
10/08/2003	152	8525	375	33430	2	4000	79112	2.5
17/08/2003	204	8729	478	33908	0	6300	85412	2.3
24/08/2003	204	8933	639	34547	4	2000	87412	3.1
31/08/2003	196	9129	473	35020	4	0	87412	2.4
07/09/2003	469	9598	1198	36218	0	1000	88412	2.6
14/09/2003	179	9777	390	36608	0	0	88412	2.2
21/09/2003	261	10038	649	37257	0	0	88412	2.5
28/09/2003	534	10572	1790	39047	0	0	88412	3.4
05/10/2003	154	10726	230	39277	0	0	88412	1.5
12/10/2003	711	11437	1718	40995	5	0	88412	2.4
19/10/2003	134	11571	217	41212	16	0	88412	1.6
26/10/2003	151	11722	602	41814	1	0	88412	4.0
02/11/2003	192	11914	534	42348	9	0	88412	2.8
09/11/2003	64	11978	282	42630	8	0	88412	4.4
16/11/2003	81	12059	315	42945	7	0	88412	3.9
23/11/2003	73	12132	311	43256	0	0	88412	4.3
30/11/2003	165	12297	521	43777	0	0	88412	3.2
07/12/2003	95	12392	344	44121	0	0	88412	3.6
14/12/2003	111	12503	311	44432	0	0	88412	2.8
21/12/2003	94	12597	320	44752	0	0	88412	3.4
28/12/2003	31	12628	74	44826	0	0	88412	2.4
04/01/2004	5	12633	11	44837	0	0	88412	2.2

Appendix 5 Rutland Water rainbow trout catch and stocking data - 2004

Date	Returns	Cumulative returns	Catch	Cumulative catch	Released	Stock	Cumulative stock	Catch rate
Pre Season								
04/04/2004	119	119	744	744	40	15000	15000	6.3
11/04/2004	407	526	1603	2347	110	6200	21200	3.9
18/04/2004	464	990	2262	4609	218	0	21200	4.9
25/04/2004	405	1395	1825	6434	265	4000	25200	4.5
02/05/2004	495	1890	2013	8447	121	2000	27200	4.1
09/05/2004	505	2395	2072	10519	172	3800	31000	4.1
16/05/2004	494	2889	2174	12693	268	6200	37200	4.4
23/05/2004	556	3445	2157	14850	280	0	37200	3.9
30/05/2004	601	4046	1322	16172	127	5800	43000	2.2
06/06/2004	295	4341	925	17097	85	2760	45760	3.1
13/06/2004	391	4732	900	17997	56	5800	51560	2.3
20/06/2004	482	5214	1384	19381	182	500	52060	2.9
27/06/2004	187	5401	347	19728	34	2400	54460	1.9
04/07/2004	399	5800	753	20481	32	4850	59310	1.9
11/07/2004	238	6038	522	21003	25	1800	61110	2.2
18/07/2004	379	6417	1086	22089	65	5150	66260	2.9
25/07/2004	326	6743	979	23068	49	0	66260	3.0
01/08/2004	219	6962	522	23590	20	3800	70060	2.4
08/08/2004	198	7160	530	24120	38	2000	72060	2.7
15/08/2004	220	7380	390	24510	47	2200	74260	1.8
22/08/2004	150	7530	532	25042	21	4000	78260	3.5
29/08/2004	146	7676	486	25528	22	2800	81060	3.3
05/09/2004	180	7856	718	26246	48	1600	82660	4.0
12/09/2004	189	8045	532	26778	5	0	82660	2.8
19/09/2004	352	8397	1257	28035	58	0	82660	3.6
26/09/2004	508	8905	2302	30337	476	3500	86160	4.5
03/10/2004	271	9176	1047	31384	111	0	86160	3.9
10/10/2004	842	10018	3948	35332	1306	0	86160	4.7
17/10/2004	339	10357	1165	36497	134	1200	87360	3.4
24/10/2004	221	10578	828	37325	118	0	87360	3.7
31/10/2004	169	10747	606	37931	40	1098	88458	3.6
07/11/2004	173	10920	621	38552	78	0	88458	3.6
14/11/2004	240	11160	693	39245	100	0	88458	2.9
21/11/2004	128	11288	380	39625	54	0	88458	3.0
28/11/2004	86	11374	205	39830	27	0	88458	2.4
05/12/2004	179	11553	466	40296	87	0	88458	2.6
12/12/2004	114	11667	217	40513	21	0	88458	1.9
19/12/2004	196	11863	595	41108	0	0	88458	3.0
26/12/2004	65	11928	138	41246	0	0	88458	2.1
02/01/2005	41	11969	86	41332	0	0	88458	2.1

References

Abdelhalim, A. I. (1990) *Morphology and Epidemiology of some Parasitic Copepods (Poecilostomatoida: Ergasilidae) from British Freshwater Fish*. PhD thesis. Royal Holloway and Bedford New College, University of London. 279pp.

Abdelhalim, A. I., Lewis, J. W. & Boxshall, G.A. (1991) The life-cycle of *Ergasilus sieboldi* Nordmann (Copepoda: Poecilostomatoida), parasitic on British freshwater fish. *Journal of Natural History*, **25**, 559-582.

Alanara, A. & Brannas, E. (1996) Dominance in demand-feeding in Arctic charr and rainbow trout: the effect of stocking density. *Journal of Fish Biology*, **48**, 242-254.

Allendorf, F.W. Leary, R.L. (2005) Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout *Conservation Biology* **2,** 170-184

Alston, S. (1994) *The Taxonomy, Epidemiology and Behaviour of* Ergasilus briani *Markewitsch 1933 (Copepoda Poecilostomatoida)*. PhD thesis. Royal Holloway and Bedford New College, University of London. 264pp.

Alston, S., Boxshall, G. & Lewis, J. (1996) The life-cycle of *Ergasilus briani* Markewitsch, 1993 (Copepoda: Poecilostomatoida). *Systematic Parasitology*, **35**, 79-110.

Anon (2004) Trout production 2003: A survey of trout production in England and Wales. *Trout News* **38**, 5-8

Balling, T. E. & Pfeiffer, W. (1997) Location-dependent infection of fish parasites in Lake Constance. *Journal of Fish Biology*, **51**, 1025-1032.

Bandilla, M., Hakalahti, T., Hudson, P. J. & Valtonen, E. T. (2005) Aggregation of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout (*Oncorhynchus mykiss*): a consequence of host susceptibility or exposure? *Parasitology*, **130**, 169-176.

Barker, D.E. & Cone, D.K. (2000) Occurrence of *Ergasilus celestis* (Copepoda) and *Pseudodactylogryrus anguillae* (Monogenea) among wild eels (*Anguilla rostrata*) in relation to stream flow, pH and temperature and recommendations for controlling their transmission among captive eels. *Aquaculture* **187**, 261-274.

Baud, A., Cuoc, C., Grey, J., Chappaz, R. & Alekseev, V. (2004) Seasonal variability in the gut ultrastructure of the parasitic copepod *Neoergasilus japonicus* (Copepoda, Poecilostomatoida). *Canadian Journal of Zoology*, **82**, 1655-1666.

Ben Hassine, O. K. & Raibaut, A. (1981) Réalisation expérimentale du cycle évolutif de *Ergasilus lizae* Kroyer, 1863, Copépode parasite de Poissons Muglidés. Premiers résultats de l'infestation. *Archives de l`Instut Pasteur de Tunis*, **58**, 423-430.

Ben Hassine, O. K. (1983) *Les Copepodes Parasites de Poissons Mugilidae en Mediterranee Accidentale (Cotes Francaises et Tunisiennes): Morphologie, Bio-*

ecologie, Cycles Evolutifs. Universite de Sciences et Techniques du Langedoc. 471pp (In French).

Bennion, H., Hilton, J., Hughes, M., Clark, J., Hornby, D., Fozzard, I., Phillips, G. & Reynolds, C. (2005) The use of GIS based inventory to provide a national assessment of waters at risk from eutrophication in Great Britain. *Science of the Total Environment*, **344**, 259-273.

Blanc, J. M.; Poisson, H., Quillet, E. (2006) A blue variant in the rainbow trout, *Oncorhynchus mykiss* Walbaum. *Journal of Heredity*, **97**, 89-93.

Bricker, K.S., Gannon, J.E., Roberts, L.S. & Torke, B.G. (1978) Observations on the ecology and distribution of free-living Ergasilidae (Copepoda, Cyclopoida). *Crustaceana*, **35**, 313-317.

Bricknell, I. R., Bron, J. E., & Bowden, T. 2006, Diseases of gadoid fish in cultivation: a review, *ICES Journal of Marine Science,* **63**, 253-266.

Budino, B., Cal, R. M., Piazzon, M. C. & Lamas, J. (2006) The activity of several components of the innate immune system in diploid and triploid turbot. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology*, **145**, 108-113.

Campbell, A., Frazer, B. D., Gilbert, N., Gutierrez, A. P. & Mackauer, M. (1974) Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology*, **11**, 431-438.

Ceballos, S. & Alvarez-Marques, F. (2006) Seasonal dynamics of reproductive parameters of the calanoid copepods *Calanus helgolandicus* and *Calanoides carinatus* in the Cantabrian Sea. *Progress in Oceanography*, **70**, 1-26.

Cloutman, D. G. & Becker, D. A. (1977) Some ecological aspects of *Ergasilus centrarchidarum* Wright (Crustacea-Copepoda) on largemouth and spotted bass in Lake Fort-Smith, Arkansas. *Journal of Parasitology*, **63**, 372-376.

Czeczuga, B. (1980) Carotenoids in fish. XI. Carotenoid level in tench, *Tinca tinca* (L.), affected by the presence of parasitizing *Ergasilus sieboldi* Nordm. (Crustacea, Copepoda). *Acta Parasitologica Polonica*, **27**, 101-107.

Daldorph, P.W.G., Spraggs, G. E., Lees, M. J., Wheater, H. S. & Chapra, S. C. (2001) Integrated lake and catchment phosphorus model - A eutrophication management tool. II: Application to Rutland Water. *Journal of the Chartered Institution of Water and Environmental Management*, **15**, 182-189.

Dezfuli, B. S., Giari, L., Konecny, R., Jaeger, P. & Manera, M. (2003) Immunohistochemistry, ultrastructure and pathology of gills of *Abramis brama* from Lake Mondsee, Austria, infected with *Ergasilus sieboldi* (Copepoda). *Diseases of Aquatic Organisms*, **53**, 257-262.

Dillon, J.C., Schill, D.J. & Teuscher, D.M. (2000) Relative return to creel of triploid and diploid rainbow trout stocked into eighteen Idaho streams. *North American Journal of Fisheries Management*, **20**, 1-9.

Dorovskich, G. N., Ekimova, I.V. & Rocheva, S. A. (1985) Dependence of length of antennae of *Ergasilus sieboldi* (Copepoda, Ergasilidae) on the hosts age. *Parazitologiya*, **19**, 483-484. (In Russian with English abstract).

Einszporn, T. (1965) Nutrition of *Ergasilus sieboldi* Nordmann. II. The uptake of food and the food material. *Acta Parasitologica Polonica*, **13**, 373-382.

Einszporn-Orecka, T. (1970) Quantitative changes in the circulating blood of tenches (*Tinca* L.) infested by *Ergasilus sieboldi* Nordmann. *Polish Archives of Hydrobiology*, **17**, 463-481.

Einszporn-Orecka, T. (1973a) Changes in the picture of peripheral blood of tench, *Tinca* (L.) under the influence of *Ergasilus sieboldi* Nordmann, I. Composition of morphotic blood constituents of uninfected fish in annual cycle. *Acta Parasitologica Polonica,* **21**, 393-411.

Einszporn-Orecka, T. (1973b) Changes in the picture of peripheral blood of tench, *Tinca* (L.) under the influence of *Ergasilus sieboldi* Nordmann, II. Changes in the leuckocytic system. *Acta Parasitologica Polonica,* **21**, 485-499.

El-Rashidy, H. & Boxshall, G. (1999) Ergasilid copepods (Poecilostomatoida) from the gills of primitive Mugilidae (grey mullets). *Systematic Parasitology*, **42**, 161-186.

Engstrom, H. (2000) Long term effects of cormorant predation on fish communities and fishery in a freshwater lake. *Ecography*, **24**, 127-138

Fernández, F. (1979) Nutrition studies in the nauplius larva of *Calanus pacificus* (Copepoda: Calanoida). *Marine Biology*, **53**, 131-147.

Fenton, A., Hakalahti, T., Bandilla, M. & Valtonen, E.T. (2006) The impact of variable hatching rates on parasite control: a model of an aquatic ectoparasite in a Finnish fish farm. *Journal of Applied Ecology*, **43**, 660-668.

Ferguson, A.J.D. & Harper, D.M. (1982) Rutland Water phytoplankton: the development of an asset or a nuisance? *Hydrobiologia*, **88**, 117-133.

Frost, W.E. (1974) *A Survey of Rainbow Trout in Britain and Ireland*. Salmon and Trout Association. 36 pp.

Fryer, G. (1969) The parasitic copepod *Ergasilus sieboldi* Nordmann, new to Britain. *Naturalist*, **909**, 49-51.

Fryer, G. (1970) Biological aspects of parasitism of freshwater fishes by crustaceans and molluscs. Symposium of the British Society for Parasitology, **8,** 103-118.

Fryer, G. (1978) Free-living stages of freshwater parasitic Copepoda. *Binnengewasser*, **26**, 344-367.

Fryer, G. (1982) *The Parasitic Copepoda and Branchiura of British Freshwater Fishes, a Handbook and Key.* Freshwater Biological Association Scientific Publication, Ambleside. 87pp.

Fryer, G. & Andrews, C. (1983) The parasitic copepod *Ergasilus briani* Markewitsch in Yorkshire: an addition to the British fauna. *Naturalist*, **108**, 7-10.

García de Leániz, C., Fleming, I.A., Einum, S., Verspoor, E., Consuegra, S., Jordan, W.C., Aubin-Horth, N., Lajus, D.L., Villanueva, B., Ferguson, A., Youngson, A.F. &. Quinn, T.P. (2007) Local Adaptation, Chapter 7, In: *The Atlantic Salmon: Genetics, Conservation and Management.* Verspoor, E., Stradmeyer, L. and Nielson, J.L. (eds.),.Blackwell Publishing Ltd,Oxford. 195-235

Gaterell, M.R., Morse, G.K. & Lester, J.N. (1995) A valuation of Rutland water using environmental economics. *Environmental Technology*, **16**, 1073-1082.

Gault, N.F.S., Kilpatrick, D.J. & Stewart, M.T. (2002) Biological control of the fish louse in a rainbow trout fishery. *Journal of Fish Biology*, **60**, 226-237.

Gnadeberg, W. (1948) Beitrage zur biology und entwicklung des *Ergasilus sieboldi* v. Nordmann (Copepoda Parasitica). *Zeitschrift fur Parasitenkunde*, **14**, 103-180. (In German).

Grabda-Kazubska, B., Baturo-Warszawska, B. & Pojmanska, T. (1987) Dynamics of parasite infestation of fish in Lakes Dgal Wielki and Warniak Poland in connection with introduction of phytophagous species. *Acta Parasitologica Polonica*, **32**, 1-28.

Grice, G.D. & Marcus, N.H. (1981) Dormant eggs of marine copepods. *Oceanography Marine Biology Annual Review*, **19**, 125-140.

Gullard, J. A. (1971) Manual of methods for fish stock assessment. Part I. Fish population analysis. *The Journal of Applied Ecology*, **8**, 616-617.

Hahn, D. A. & Denlinger, D. L. (2007) Meeting the energetic demands of insect diapause: nutrient storage and utilization. *Journal of Insect Physiology*, **53**, 760-773.

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

Halisch, W. (1939) Anatomie und biologie von *Ergasilus minor* Halisch. *Zeitschrift fur Parasitenkunde*, **11**, 284-330. (In German).

Hawkins, D. (2001) *Morphology and Epidemiology of the Ergasilid (Copepoda: Poicilostomatoida) Parasites of British Freshwater Fish.* PhD thesis, Royal Holloway College, University of London. 265pp.

Hedrick, R.P., McDowell, T.S., Gay, M., Marty, G.D., Georgiadis, M.P. & MacConnell, E. (1999) Comparative susceptibility of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* to *Myxobolus cerebralis*, the cause of salmonid whirling disease. *Diseases of Aquatic Organisms*, **37**, 173-183.

Hedrick, R. P., McDowell, T. S., Gay, M., Marty, G. D., Fosgate, G. T., Mukkatira, K., Myklebust, K. & El-Matbouli, M. (2003) Susceptibility of two strains of rainbow trout (one with suspected resistance to whirling disease) to *Myxobolus cerebralis* infection. *Diseases of Aquatic Organisms*, **55**, 37-44.

Hilborn, R. & Walters, C. J. (1992) *Quantitative Fisheries Stock Assessment.* Chapman & Hall. 579pp.

Hutchings, J. A. (1993) Behavioural implications of intraspecific life history variation. In: *Behavioural Ecology of Fishes*, vol. 11 F. Huntingford & P. Toricelli, eds., Harwood Acedemic, Erice, 187-205.

Hoegh-Guldberg, O. & Emlet, R. B. (1997) Energy use during the development of a lecithotrophic and a Planktotrophic Echinoid. *The Biological Bulletin*, **192**, 27-40.

Hudson, P. L., Bowen, C. A. & Stedman, R. M. (1994) New records of *Ergasilus* (Copepoda, Ergasilidae) in the Laurentian Great-Lakes, including a lake wide review of records and host associations. *Canadian Journal of Zoology*, **72**, 1002-1009.

Hunt, P.C. & O'Hara, K. (1973) Overwinter feeding in rainbow trout. *Journal of Fish Biology*, **5**, 277-280.

Huys, R. & Boxshall, G. A. (1991) *Copepod Evolution*. The Ray Society,London. 468pp.

Jhingan, E., Devlin, R. H. & Iama, G. K. (2003) Disease resistance, stress response and effects of triploidy in growth hormone transgenic coho salmon. *Journal of Fish Biology*, **63**, 806-823.

Kabata, Z. (1970) *Diseases of Fish I. Crustacea as Enemies of Fish.* T.F.H. Publications, New Jersey. 171pp.

Kabata, Z. (1979) *Parasitic Copepoda of British Freshwater Fish.* The Ray Society, London. 667pp.

Kahan, D., Berman, Y. & Bar-el, T. (1988) Maternal inhibition of hatching at high population densities in *Tigriopus japonicus*. *Biological Bulletin*, **174**, 139-144.

Kashkovsky, V. V. & Kashkovskayasolomatova, V. P. (1985) A study of *Ergasilus sieboldi* population (Copepoda, Ergasilidae) in Lake Arakul. *Parazitologiya*, **19**, 195-205.

Kearn, G. C. (2004) Poecilostomatoid copepods. In: *Leeches, Lice and Lampreys.* Springer, Amsterdam, 214-236.

Kirk, R. S. (2000) *Checklist of Parasites from British Freshwater Fish*. National Fisheries Laboratory, Environment Agency, Bristol, 14pp.

Kneale, P. E. & Howard, A. (1997) Statistical analysis of algal and water quality data. *Hydrobiologia*, **349**, 59-63.

Kuang, P. R. & Liu, D. S. (1992) Comparative study on the thorax-plate of Ergasilidae (parasitic Copepoda) by scanning electron microscopy. *Acta Zoologica Sinica*, **38**, 352-354.

Kuperman, B. I. & Shulman, R. E. (1972) An experimental investigation of the effect of temperature on some parasites of the pike. *Vestnik - Leningrad University (Biol.),* **27**, 5-15. (In Russian with English abstract).

Kuperman, B. I. & Shulman, R. E. (1977) On the influence of some abiotic factors on the development of *Ergasilus sieboldi* (Crustacea, Copepoda). *Parazitologiya*, **11**, 117-121. (In Russian with English abstract).

Lincoln, R.F.& Scott, A.P. (1984) Sexual maturation in triploid rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology*, **25**, 385-392

Malégorzata, G. & Marek, J. (2006) Acoustical estimates of fish and zooplankton distribution in the Piaseczno reservoir, Southern Poland. *Aquatic Ecology*, **40**, 211-219.

Marcus, N.H. (1982) Photoperiodic and temperature regulation of diapause in *Labidocera aestiva* (Copepoda: Calanoida). *Biological Bulletin*, **162**, 45-52.

Margolis, L, Esch, G. W., Holmes, J. C., Kuris, A. M. & Schad, G. A. (1982) The use of ecological terms in parasitology. *The Journal of Parasitology*, **68**, 131-133.

Mirzoeva, L.M. (1972) The life cycle and biology of *Sinergasilus lieni* Yin, 1949 (Crustacea, Copepoda, Parasitica). *Parazitologiya*, **6**, 252-258.

Molnar, K. & Szekely, C. (1997) An unusual location for *Ergasilus sieboldi* Nordmann (Copepoda, Ergasilidae) on the operculum and base of pectoral fins of the pikeperch (*Stizostedion lucioperca* L.). *Acta Veterinaria Hungarica*, **45**, 165-175.

Moody, J. & Gaten, E. (1982) The population dynamics of eyeflukes *Diplostomum spathaceum* and *Tylodelphys clavata* (Digenea: Diplostomatidae) in rainbow and brown trout in Rutland Water: 1974-1978. *Hydrobiologia*, **88**, 207-209.

Moore, D. E. (1982) Establishing and maintaining the trout fishery at Rutland Water. *Hydrobiologia*, **88**, 179-189.

Mugridge, R. E. R., Stallybrass, H. G. & Holman, A. (1982) *Neoergasilus japonicus* (Crustacea: Ergasilidae). A parasitic copepod new to Britain. *Journal of Zoology*, **197**, 551-557.

Myers, J. M. & Hershberger, W. K. (1991) Early growth and survival of heat-shocked and tetraploid-derived triploid rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **96**, 97-107.

Naich, M., Bilqees, F. M. & Khan, A. (2000) The incidence of *Ergasilus* sp. infection on *Abramis brama* in Paulton's Park Lake, near Romsey, Hampshire, U.K. *Proceedings of Parasitology*, 51-59.

National Rivers Authority (1995) National Angling Survey. *Fisheries Technical Report*, Stationary Office Books, 35pp.

Nordmann, A. von (1832) Mikrographische beitrage zur naturgeshichte der wirbellosen thiere. *Quarto. G. Reimer*, Berlin **2,** 1-150. (In German)

Norrbin, M. F. (1994) Seasonal patterns in gonad maturation, sex ratio and size in some small, high-latitude copepods: implications for overwintering tactics. *Journal of Plankton Research*, **16**, 115-131.

Ojha, J. & Hughes, G.M. (2001) Effect of branchial parasites on the efficiency of the gills of a freshwater catfish, *Wallago attu. Journal of Zoology,* **255**, 125-129.

Oldewage, W. H. & Van As, J. G. (1987) Observations on the attachment of a piscine gill parasitic ergasilid. *South African Journal of Zoology*, **22**, 313-317.

Paperna, I. & Zwerner, D.E. (1976) Studies on *Ergasilus labracis* Kroyer (Cyclopidea-Ergasilidae) parasitic on striped bass, *Morone saxatilis*, from Lower Chesapeake Bay. 1. Distribution, life-cycle, and seasonal abundance. *Canadian Journal of Zoology*, **54**, 449-462.

Parish, K. K. & Wilson, D. F. (1978) Fecundity studies on *Arcatia tonsa* (Copepoda: Calanoida) in standardised culture. *Marine Biology*, **46**, 65-81.

Pawson, M. G. (1982) Recapture rates of trout in a 'put-and-take' fishery: Analysis and management. *Aquaculture Research*, **13**, 19-31.

Pawson, M. G. (1986) Performance of rainbow trout, *Salmo gairdneri* Richardson, in a pot-and-take fishery, and influence of anglers behaviour on catchability. *Aquaculture Research*, **17**, 59-73.

Piasecki, W., Goodwin, A. E., Eiras, J. C. & Nowak, B. F. (2004) Importance of Copepoda in freshwater aquaculture. *Zoological Studies*, **43**, 193-205.

Pojmanska, T. (1984) An analysis of seasonality of incidence and maturation of some fish parasites with regard to thermal factor 1. General methods *Ergasilus sieboldi*. *Acta Parasitologica Polonica*, **29**, 217-228.

Pojmanska, T. & Dzika, E. (1987) Parasites of bream *Abramis brama* L. from Lake Goslawskie Poland affected by long-term thermal pollution. *Acta Parasitologica Polonica*, **32**, 139-162.

Price, H. J. & Paffenhofer, G. A. (1986) Effects of concentration on the feeding of a marine copepod in algal monocultures and mixtures. *Journal of plankton research*, **8**, 119-128.

Randall, S., Harper, D. & Brierley, B. (1999) Ecological and ecophysiological impacts of ferric dosing in reservoirs. *Hydrobiologia*, **395**, 355-364.

Roubal, F.R. (1986) Comparative pathology of some monogenean and copepod ectoparasites on the gills of *Acanthopagrus australis* (family Sparidae). *Journal of Fish Biology* **34**, 503-514.

Rubio-Godoy, M. & Tinsley, R.C. (2004) Comparative susceptibility of brown trout and rainbow trout to *Discocotyle sagittata* (Monogenea). *Journal of Parasitology*, **90**, 900-901.

Ruotsalainen, M. (1984) On the occurrence of *Ergasilus sieboldi* in vendace (*Coregonus albula* L.) in Lake Kallavesi, Central Finland. In: *Proceedings of 4th Multicolloquium of Parasitology*, Special Volume.

Sarieyyupoglu, M. & Saglam, N. (1992) *Ergasilus sieboldi* and *Argulus foliaceus* in *Capoeta trutta* caught from polluted region of Keban Dam Lake. *Journal of Ege University Aquatic Products*, **8**, 31-42.

Schaperclaus, W., Kulow, H. & Schreckenbach, K. (1992) *Fish Diseases.* Translated by Chan, M.S.R..A.A. Balkerna, Brookfield, USA. 1398pp.

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

Solomon, D. (2001) *The Potential for Restocking using All-Female Triploid Brown Trout to avoid Genetic Impact upon Native Stocks.* Environment Agency, Bristol. 6pp.

Starovoytov, V.K. (1990) Interactions among several gill parasites of zander. *Hydrobiological Journal / Gidrobiologicheskiy Zhurnal*, **6**, 67-69.

Strathman, R. R. (1978) The evolution and loss of feeding larval stages of marine invertebrates. *Evolution*, **32**, 894-906.

Taylor, N.G.H., Sommerville, C. & Wootten, R. (2006) The epidemiology of *Argulus* spp. (Crustacea: Branchiura) infections in stillwater trout fisheries. *Journal of Fish Diseases*, **29**, 193-200.

Thatcher, V. E. & Boeger, W. A. (1983) Pathology of fishes of Brazilian Amazonia 3. Histologic changes in branchiae produced by *Ergasilus brasergasilus* and Acusicola Crustacea Cyclopoida Ergasilidae. *Acta Amazonica*, **13**, 441-452.

Torres, P., Villalobos, L., Woelfl, S. & Puga, S. (2004) Identification of the copepod intermediate host of the introduced broad fish tapeworm, *Diphyllobothrium latum*, in southern Chile. *Journal of Parasitology*, **90**, 1190-1193.

Tucker, C. S., Sommerville, C. & Wootten, R. (2000) An investigation into the larval energetics and settlement of the sea louse, *Lepeophtheirus salmonis*, an ectoparasitic copepod of Atlantic salmon, *Salmo salar*. *Fish Pathology*, **35**, 137-143.

Tuuha, H., Valtonen, E.T. & Taskinen, J. (1992) Ergasilid copepods as parasites of perch *Perca fluviatilis* and roach *Rutilus rutilus* in central Finland: Seasonality, maturity and environmental influence. *Journal of Zoology (London)*, **228**, 405-422.

Urawa, S., Muroga, K. & Kasahara, S. (1980a) Naupliar development of *Neoergasilus japonicus* (Copepoda: Ergasilidae). *Bulletin of the Japanese Society of Scientific Fisheriesi,* **46**, 941-947.

Urawa, S., Muroga, K., Kasahara, S., Dumont, H. J. & Green, J. (1980b) Studies on *Neoergasilus japonicus* (Copepoda: Ergasilidae), a parasite of freshwater fishes. II. Development in copepodid stage. Journal of the Faculty of applied Sciences, *Hiroshima University.* **19**, 21-38,

Urawa, S., Muroga, K. & Kasahara, S. (1991) Growth and fecundity of the parasitic copepod *Neoergasilus japonicus* (Ergasilidae). *Bulletin of the Plankton Society of Japan*, Special Volume, 619-625.

Vehanen, T. (1997) Fish and fisheries in a large regulated peaking-power river reservoirs in northern Finland, with special reference to the efficiency of brown trout and rainbow trout stocking. *Regulated Rivers Research and Management*, **13**, 1-11.

Warlow, A. D. & Oldham, R. S. (1982) Temporal variations in the diet of brown trout (*Salmo trutta*) and rainbow trout (*Salmo gairdneri*) in Rutland Water. *Hydrobiologia*, **88**, 199-206.

Wiggins, G. B., Macky, R. J. & Smith, I. M. (1980) Evolutionary and ecological strategies of animals in annual temporary ponds. *Archives of Hydrobiology*, **58**, 97-206.

Williams, C. F. (2007) *Impact Assessment of Non-native Parasites in Freshwater Fisheries in England and Wales.* PhD thesis. University of Stirling. 412pp.

Wilson, C. B. (1911) North American parasitic copepods belonging to the family Ergasilidae. *Proceedings of the United States National Museum*, **39**, 263-400.

Zharikova, T. I. (1993) Effect of anthropogenic water pollution on ectoparasites of bream (*Abramis brama*). *Zoologicheskij Zhurnal*, **72**, 73-83.

Zmerzlaya, E. I. (1972) *Ergasilus sieboldi* Nordmann 1832 its development, biology and epizootological significance. *Izvestiya Gosudarstvennogo Nauchno-Issledovatel'skogo. Instituta Ozernogo i Rechnogo Rybnogo Khozyaistva*, **591**, 132-177.