

Teija Hakalahti

Studies of the Life History of a Parasite
A Basis for Effective Population Management







ABSTRACT

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Studies of the life history of a parasite – a basis for effective population management

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Diss.

In this thesis, the life history traits of the ectoparasite *Argulus coregoni* were examined during its life cycle. Water temperature was a key determinant of temporal patterns of *A. coregoni* population abundance and dynamics; neither parasites attached to the host nor egg-hatching were recorded at temperatures below ~10°C. Parasite transmission to their hosts was characterized by peak recruitment in spring followed by extended, but slower recruitment. Egg-hatching was triggered by cooling treatments, the magnitude of which was dependent on the period of cooling. Each female parasite, however, produced eggs that hatched over many hatching opportunities. This delayed emergence pattern can be seen as an adaptation to unpredictable, risky environments, where such bet-hedging may allow some infection to occur even after periodic absences of hosts. Female *A. coregoni* preferred to lay their eggs on dark substrates in the deepest water in a 2 m-deep canal, with the egg population showing an aggregated distribution pattern. Non-random distribution of eggs coupled with variable hatching may result in differences in host exposure to parasites, which was shown to be a dominant causative factor behind the observed aggregated distribution of *A. coregoni* on their rainbow trout (*Oncorhynchus mykiss*) hosts. Rainbow trout did not seem to develop any resistance mechanisms to prevent parasite settlement after first encounters with *A. coregoni* nor any general trend in repeated susceptibility of fish to parasite attachment observed. Both these findings suggest that the fish were of equal genetic susceptibility. Nevertheless, the attachment of parasite infective stages, that carried a finite energy resource for host seeking, seemed to be opportunistic and non-selective. Although the infectivity of *A. coregoni* remained high up to death, delayed starts with respect to host searching time were reflected on growth in the altered growth rate of settled parasites. Over time, parasites that had spent more time searching for a host reached the length of parasites that attached to a host faster. *A. coregoni* thus appeared to compensate for delayed growth resulting from an extended period off-host by accelerated growth, although, this was shown to incur a cost through decreased life-expectancy. This study has shown that individual parasites are more flexible in their life history strategies than has hitherto been understood. The results from this thesis provide parameter estimations for a detailed host-macroparasite model that will capture both host and parasite population dynamics, and can be used for planning cost-effective management against ectoparasite infestations at fish farms.

Key words: Aggregated distribution; *Argulus coregoni*; bet-hedging; compensatory growth; ectoparasite; life history; population dynamics; transmission.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals (I-V).

- I Hakalahti, T. & Valtonen, E. T. 2003: Population structure and recruitment of the ectoparasite *Argulus coregoni* Thorell (Crustacea: Branchiura) on a fish farm. *Parasitology* **127**: 79-85.
- II Hakalahti, T., Häkkinen, H. & Valtonen, E. T. 2004: Ectoparasitic *Argulus coregoni* hedge their bets - studies on egg hatching dynamics. *Oikos* **107**: 295-302.
- III Hakalahti, T., Pasternak, A. & Valtonen, E. T. 2004: Seasonal dynamics of egg laying and egg-laying strategy of the ectoparasite *Argulus coregoni* (Crustacea: Branchiura). *Parasitology* **128**: 655-660.
- IV 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.
- V Hakalahti, T., Bandilla, M. & Valtonen, E. T.: Delayed starts during the transmission of a parasite are compensated by accelerated growth. Manuscript, under review for the journal *Parasitology*.

RESPONSIBILITIES OF TEIJA HAKALAHTI IN THE ARTICLES OF THIS THESIS

- Paper I Experiments were planned together with all co-authors. I collected and analysed the parasite samples together with a research trainee. I did the statistical analyses and wrote the paper.
- Paper II Experiments were planned together with all co-authors. I performed the experiments together with MSc student Heli Häkkinen. I did the statistical analyses and wrote the paper.
- Paper III The structure of egg-laying plates was planned together with all co-authors and representatives from the fish farm. I was responsible for planning the experimental procedures. I collected the data together with a research trainee, did the statistical analyses and wrote the paper.
- Paper IV The preliminary idea to do the experiment came from Professor Andrew Dobson. The experiment was planned together with all co-authors. I ran the experiment and sampled the fish together with MSc student Matthias Bandilla and a research trainee. Statistical analyses were performed by M. Bandilla and Peter Hudson. I wrote the paper jointly with M. Bandilla.
- Paper V I both planned the study and ran the experiments together with Matthias Bandilla. I did the statistical analyses. I wrote the paper jointly with M. Bandilla.

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Teija Hakalahti

1 INTRODUCTION

1.1 Life history strategies

Life history theory concerns the dilemma of allocating limited resources into growth, reproduction and maintenance in a way that maximises the fitness of an organism (Roff 1992, Stearns 1992). An organism's life history strategy is the combination of demographic traits such as body size, fecundity, age at maturity, number of reproductive events and offspring size (Stearns 1992). Given limited resources, energy allocated into one life history trait cannot be used for other competing functions, thus creating trade-offs (negative correlations) among the pairs of traits (Roff 1992, Stearns 1992). For example, any investment in reproduction may come at the expense of physiological condition and disease resistance (Festa-Bianchet 1989, Stearns 1992, Dobson & Meagher 1996). Besides trade-offs between the traits, that cannot be maximised simultaneously, the array of life histories available for each group of organisms is also limited by a number of other factors, such as phylogenetic and physiological constraints (Stearns 1992).

Current life history is shaped by external pressures acting on the probability of survival at each stage in the life cycle (Partridge & Harvey 1998, Roff 1992). Consequently, the optimal amount of energy to be spent for different traits varies in the course of an organism's life and is sometimes unpredictable. Organisms are able to vary their allocation pattern by means of phenotypic plasticity (Stearns 1989, 1992), referring to their ability to react to an environmental input with a change in form, state, movement or rate of activity (West-Eberhard 2003). Phenotypic plasticity does not result in a change in genotype frequencies of a population which, on the contrary, is a prerequisite for true evolutionary response and adaptation (Stearns 1992). One classical example of phenotypic plasticity is the cyclomorphosis of *Daphnia*, involving production of elongated head spines as a response to chemical cues released by their predators (e.g. Dzialowski et al. 2003).

Parasites are organisms that live in or on another organism gaining from it part or all of their organic nutrients, and cause some degree of damage to their hosts (Bush et al. 2001). Unlike most free-living species, the geographic range of parasites consists of a set of habitat patches represented by their hosts in an otherwise hostile environment. Given that a parasite's reproduction is dependent on its hosts, parasite fitness is best described by its basic reproductive number, R_0 . For a macroparasite R_0 is determined as the number of new female parasites produced by an average parasite in a fully susceptible host population where there are no density dependent constraints (Anderson & May 1991, Hudson et al. 2002). Although tremendous variability in life history strategies among parasite species and individuals has been reported, relatively few experimental studies on parasite life histories have been conducted (see Poulin 1996, Thomas et al. 2002). The idea that parasites can also exhibit adaptive plasticity in their life history decisions to enhance their fitness is attracting considerable interest at present (e.g. Thomas et al. 2002, Fenton & Rands 2004).

Body size is an important life history trait. As in many free-living organisms (Peters 1983), the body size of female parasite is tightly linked to fecundity, i.e. larger parasite species and individuals produce more offspring (e.g. Skorping et al. 1991, Morand 1996, Timi et al. 2004). Also body size at maturity, in terms of sexual selection and resulting reproductive success, may play an important role in the life histories of animals (Andersson 1994). Larger *Idotea baltica* males were more effective in defending females from other males and also obtained more females (Jormalainen et al. 1994, Jormalainen & Merilaita 1995). Also larger males of the endoparasite *Corynosoma magdaleni* appeared to be the first to approach a non-mated female (Sinisalo et al. 2004). Thus in cases of intense competition for females, selection pressures on male body size might exist (Andersson 1994).

In order to reach a large size at maturity, organisms either have to grow at a fast rate (Metcalf & Monaghan 2001) or grow for a longer time (Roff 1992, Stearns 1992). Postponing maturation, however, may incur costs such as risk of mortality, and that option is traded-off against increased fecundity (Kozłowski & Wiegert 1987, Gemmill et al. 1999). Many animals face unpredictable and sometimes extended periods of food shortages during their growth periods, temporally restricting the growth rates below normal (Metcalf & Monaghan 2001, Ali et al. 2003, Jespersen & Toft 2003). A commonly observed response to a nutrient deficiency is a subsequent period of accelerated growth to regain the original growth trajectory (Arendt 1997, Metcalf & Monaghan 2001). Costs related to immediate and later performance may follow such periods of fast growth (Arendt et al. 2001, Metcalf & Monaghan 2001, Morgan & Metcalf 2001) resulting in organisms not growing at their physiological maximum (Metcalf & Monaghan 2003).

Assuming that a parasite population on a host is not restricted by its density, parasites are unlikely to experience shortages of food. This may enable the high fecundity of parasites compared to their free-living relatives (Jennings & Calow 1975, Calow 1983) and also may weaken some trade offs between life

history traits (Van-Noordwijk & de Jong 1986). One example is a study by Timi et al. (2005) who found no correlation between egg size and numbers in the parasitic copepod *Lernanthropus cynoscicola*, contrasting with similar studies on many free-living animal taxa (e.g. Stearns 1992, Brown 2003).

1.2 Infection strategies

Parasites live in environments where there is a very great risk of failure. Due to the stochastic nature of the external environment and the unpredictable availability of hosts, parasites may experience considerable temporal variation in their transmission success. As such, strategies outside the host may be crucial in determining the overall fitness of a macroparasite (Fenton & Hudson 2002). Since the success of a particular genotype is inherently multiplicative, the appropriate measure of fitness in variable environments is the geometric mean fitness (Gillespie 1977). A geometric mean is calculated by multiplying a series of numbers and taking the n^{th} root of the product, where n is the number of items in the series. This parameter can be maximised by minimizing the variation in fitness over generations (Gillespie 1977). Hence selection in unpredictable environments may result in the fixation of a phenotypic trait whose arithmetic mean fitness is lower than for another trait, if the decreased variance in reproductive success outweighs the cost of reduced arithmetic mean fitness (Seger & Brockman 1987, Philippi & Seger 1989, Hopper 1999).

Extended transmission of each parasite cohort would enable some offspring to endure the unfavourable periods of host absence and decrease the chance of total reproductive failure, thereby decreasing the variance in fitness (Fenton & Hudson 2002). Such a strategy, where one genotype codes for a random set of phenotypes, is known as bet-hedging, and has been proposed for a number of taxa living in unpredictable environments (Cohen 1966, Philippi 1993, Simovich & Hathaway 1997, Danforth 1999, Fenton & Hudson 2002, Soula & Menu 2003). Bet-hedging has its origin in economic theory, and in principle means that if goods are exposed to some danger, it is advisable to divide them in several portions rather than risk them altogether (see Stearns 2000). In parasites this would mean, that each individual parasite produces offspring varying in their timing of infectivity. Theoretical models show that the optimal amount of bet-hedging varies according to predictability of the environment (e.g. Cohen 1966, Fenton & Hudson 2002). At fish farms, where the contact rate between the host and the parasite is high, parasites that infect a host fast are likely to gain a fitness advantage (Fenton & Hudson 2002). In contrast, in nature, where the host populations usually are more sparse, extended periods of recruitment are supposed to play a greater role enabling at least some infection to occur after periodic host absence (see also Pasternak et al. 2000, Fenton & Hudson 2002).

For many animals and plants that have a dormant stage in their life cycle, bet-hedging is manifested as a stochastic relaxation of diapause, such that all

offspring do not emerge at the same time (Cohen 1966, Philippi 1993, Simovich & Hathaway 1997, Danforth 1999, Soula & Menu 2003). Dormancy is a state of inactivity, when metabolic processes are slowed to a minimum, and allows organisms to 'time travel' over unfavourable conditions. Diapause is a genetically determined form of dormancy that can facilitate survival over harsh periods by affording tolerance to extreme environmental conditions (Tauber et al. 1986). Quiescence is a form of dormancy that is an immediate response to a changing environment and which, in contrast to diapause, ends soon after the conditions improve (Tauber et al. 1986).

1.3 Dispersion patterns between hosts

A key feature of the ecology of macroparasites is the distribution pattern of parasite load within a host population (Anderson & May 1991). Generally parasites occur patchily on their host population, so that the majority of hosts harbour none or few parasites while a few hosts have large parasite burdens. In other words, parasite loads are aggregated on their host population (reviewed by Shaw & Dobson 1995, Shaw et al. 1998). Aggregated distributions of parasites can typically be described and modelled using a negative binomial distribution (Anderson & May 1991, Shaw & Dobson 1995). The negative binomial distribution depends on two parameters, its mean and the aggregation index known as k , which is an inverse measure of aggregation (Fisher 1941). For many host parasite systems estimations of k have been below 1 (Shaw & Dobson 1996, Shaw et al. 1998).

There has been extensive research both into biological mechanisms that could drive the observed parasite distribution (e.g. Keymer & Anderson 1978, Karvonen et al. 2004a) and its consequences for population dynamics of hosts and parasites (e.g. Anderson & May 1978, May & Anderson 1978, Grenfell et al. 1995). An aggregated pattern can be generated by a number of mechanisms, although most can be classified as either a consequence of variability in exposure to infection, possibly associated with variable contact rate between a host and its parasites, and/or heterogeneity between hosts in their susceptibility to infection (Shaw & Dobson 1995, Wilson et al. 2002).

Dispersion of parasite infective stages in the environment tends to be heterogeneous. While the host may encounter infective stages at random, the burden associated with an encounter may vary, spatial heterogeneity being reflected in the level of parasite aggregation across hosts (Keymer & Anderson 1978). Also temporal variation in infection pressure appears to be common, particularly in seasonal aquatic systems. For example, numbers of infective stages of *A. coregoni* present in water systems are highly seasonal (Mikheev et al. 2001). Janovy & Kutish (1988) showed that parasites became highly aggregated on their host population when they emerged in discrete pulses.

Even under constant infection pressure, slight differences among individuals in their susceptibility, due either to behavioural or to physiological

responses, may cause heterogeneity in parasite acquisition (Anderson & May 1978, Grenfell et al. 1995, Zelmer & Arai 1998). Variability in host genetic susceptibility reflects heterogeneity in the function of defence mechanisms, including acquired resistance (e.g. Gleeson et al. 2000). Between-species and population level variability in host susceptibility to parasites have commonly been reported (e.g. Bakke et al. 1990, Bakke et al. 1992, Mustafa & MacKinnon 1999). Also parasites themselves may stimulate host susceptibility for further parasitism, for example, by releasing pheromones to attract increasing numbers of parasites to infected hosts (Norval et al. 1989, Ingvarsdóttir et al. 2002, Mordue Lunz 2003).

Aggregation tends to be a dynamic variable, the magnitude of parasite aggregation among hosts changing temporally (Wilson et al. 2002). Demographic mechanisms, such as density dependent mortality acting both in host and parasite populations, result in constant change in the dispersion of parasites among the hosts (Anderson & Gordon 1982). Considering the above-mentioned diversity of mechanism all affecting the level of parasite aggregation, empirical studies aiming to separate the factors behind the patterns are scarce. Recently in aquatic systems both varying exposure (Karvonen et al. 2004a) and susceptibility as dominant mechanisms creating aggregation have received empirical support (Lysne & Skorping 2002).

1.4 Host exploitation rate and host responses

Traditionally macroparasite infections are regarded as a cause of morbidity rather than mortality. Several experimental studies have shown, however, that high parasite burdens may decrease host survival and affect host growth and fecundity, the strength of which tends to be dose-dependent (e.g. Scott & Anderson 1984, Scott 1987). Due to aggregated parasite loads among a host population, these detrimental effects are concentrated on the minority of hosts. Such density dependent effects may be manifested as a regulatory influence of parasites on their host population dynamics (Anderson & May 1978, May & Anderson 1978). One classic example is a study by Hudson et al. (1998) who showed that a nematode parasite was a causative factor behind the population cycles of a game bird.

Typically parasites and their hosts show stable co-existence, with parasite epizootics taking place only when this co-existence is disturbed (Anderson 1979). In aquatic systems, parasite mass infestations have been reported particularly in dense fish rearing units, where the fish are stressed (e.g. Menezes et al. 1990, Northcott et al. 1997, Gault et al. 2002). Poor quality of the environment can affect the physiological condition of the host and such a stress may weaken host defensive mechanisms against parasites (e.g. MacKinnon 1998, Davis et al. 2002). Fish defensive mechanisms to parasites consist of behavioural responses, such as passive and active avoidance behaviour (reviewed by Barber et al. 2000, Karvonen et al. 2004b), and physiological

mechanisms. Physiological mechanisms consist mainly of innate, non-specific resistance, and acquired resistance elicited after a delay from the first encounter with a parasite (Manning 1994).

1.5 *Argulus* ectoparasites

Fish lice in the genus *Argulus* are aquatic crustacean ectoparasites. They are ubiquitous components of parasite communities on fishes in Finnish lakes and in fish farms in both inland (Valtonen et al. 1997, Pasternak et al. 2000, Mikheev et al. 2001) and coastal areas (Valtonen et al. 2001). In this thesis I have studied the species *Argulus coregoni* (Thorell). *A. coregoni* is considered to be a specialist on salmonids, although it has occasionally been found on other fish genera (Shimura 1983). Specificity has been suggested to arise rather from different habitat requirements between fishes, than by incompatibility (Mikheev et al. 2004). *A. coregoni* was able complete its life cycle on roach, *Rutilus rutilus*, and on rainbow trout, *Oncorhynchus mykiss* (Pasternak et al. 2004). The more common species *A. foliaceus* (L.) is a generalist parasite recorded on several fish genera (e.g. Shulman 1970, Valtonen et al. 1997).

Argulids have a direct life cycle and are transmitted via free-swimming metanauplii stages (Shimura 1981, figure 1). Once emerged from eggs, non-feeding metanauplii actively search for a host (Mikheev et al. 2004). Once on a fish, *A. coregoni* feeds on blood and tissues proceeding through 9 developmental stages, after which maturity is attained at a length of about 4 mm (Shimura 1981). Argulids are sexually dimorphic and sexes can be distinguished on the basis of morphological characters of the abdomen. More specifically, females have a pair of seminal receptacles and males have a pair of testes (Shimura 1981). Copulation usually occurs on fish (own personal observations, Pasternak et al. 2000).

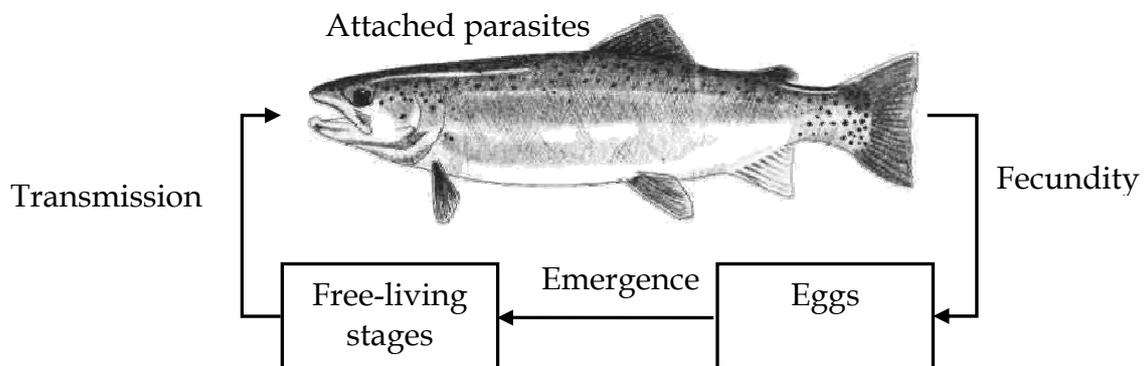


FIGURE 1 *Argulus* spp. life cycle (Fish picture by David L. Bozsik).

After copulation *A. coregoni* continue to grow in size and start to lay their eggs after reaching a length of about 8 mm (Paper III). Batches of eggs which are

fertilized upon being laid (Fryer 1960) are usually laid on solid objects like stones (Mikheev et al. 2001, Paper II). The gelatinous eggs tend to darken and harden soon after deposition, cementing the eggs to the substratum (Shafir & Van As 1986, own personal observations). From time to time attached argulids detach from the fish host and live free, at least during mate finding, egg laying (Shafir & van As 1986, Pasternak et al. 2000, Pasternak et al. 2004) or if the host dies. Pasternak et al. (2004) also noted that host switching by *A. coregoni* occurred more frequently on non-typical roach host than on the salmonid, rainbow trout.

By feeding and injecting a toxic anticoagulant, fish lice erode the skin of the fish and predispose it to bacterial and fungal infections (LaMarre & Cochran 1992, Lester & Roubal 1995). Due to morbidity, like decreased growth and abnormal behaviour, and mortality caused by the parasites (Gross & Stott 1974, Poulin & FizGerald 1987, Lester & Roubal 1995), *Argulus* infestations are of high economic significance. Several *Argulus* spp. outbreaks have been reported throughout Europe (e.g. Menezes et al. 1990, Northcott et al. 1997, Gault et al. 2002).

Fish have been shown to exploit avoidance behaviour to escape *Argulus* infection. Microhabitat choice (Poulin & FizGerald 1988, 1989b) and shoal size of juvenile sticklebacks (*Gasterosteus* spp.) (Poulin & FizGerald 1989c) were mediated by the presence of *A. canadensis*. Both *A. foliaceus* and *A. coregoni* have well-developed eyes for host location (Meyer-Rochow et al. 2001) and the finding that the ability to locate a potential host was impaired in high reflective glass aquaria illustrates the importance of visual cues for this parasite (Mikheev et al. 1998). *A. foliaceus* was shown to employ two distinct host-searching strategies: a sit-and-wait strategy in light and an active cruising strategy in dark, both of which tactics became less stringent as the parasites were starved (Mikheev et al. 2000). Juvenile *A. coregoni* primarily used vision for host searching, whereas other sensory modalities like smell developed later in the ontogeny (Mikheev et al. 2004). They also preferred white areas over dark areas, which behaviour was suggested to facilitate host location, and the behaviour switched towards dark areas at adulthood (Mikheev et al. 2004). Some life history traits of *A. foliaceus* were compared between a lake and a fish farm population (Pasternak et al. 2000). Extended recruitment at the population level was hypothesised to act as a risk-spreading strategy (Pasternak et al. 2000). Previously, only few studies on the life history of *A. coregoni* have been conducted. Those studies mainly concentrated on over-wintering strategies, on hatching patterns of over-wintered eggs (Mikheev et al. 2001) and on seasonal population dynamics (Shimura 1983). Growth and development of *A. coregoni* on a typical salmonid host and on an atypical cyprinid host were also compared (Pasternak et al. 2004).

1.6 Aims of the study

The objective of this thesis was to examine the life history traits of the ectoparasite *Argulus coregoni* with special emphasis on seasonality (I, III), egg-laying behaviour (III), infection and survival strategies (II, IV, V) and ecological factors behind the observed aggregated dispersion pattern among hosts (IV). First, I monitored seasonal population structure, abundance (I) and parasite egg laying at a commercial fish farm (III). Since the recruitment on to the host was shown to be extended through the summer period, a hypothesis concerning the evolutionary mechanism behind the pattern was raised (II). By following emergence patterns of parasite eggs produced by individual females, I examined whether the parasite egg hatching pattern could best be explained by genetic variability, phenotypic plasticity or an adaptive bet-hedging strategy to cope with unpredictability during the transmission phase (II). Here I also obtained estimates of *A. coregoni* fecundity and egg-deposition times (II). In the third paper, I did experiments to monitor egg-laying behaviour of *A. coregoni* in relation to colour, position and depth of the substrate. Since dispersion of parasites among the rainbow trout (*Oncorhynchus mykiss*) population at the fish farm was shown to be aggregated (IV), I aimed to separate experimentally the causative factors behind the observed pattern. More specifically, by undertaking experiments where infection times and exposure periods were manipulated, I examined whether exposure or susceptibility was the dominant factor determining parasite distribution (IV). In the fifth paper, I monitored survival and infectivity in relation to times spent off-host. The possible costs of delayed starts were studied by experimentally withholding metanauplii off-host and later following their survival and growth pattern on hosts (V). The results of this thesis will provide parameter estimates for a detailed population model, which will be constructed in a separate project. The epidemiological model will capture the dynamics of the host-parasite system and can be used to evaluate optimal ways, with respect to both ecological and economical sustainability, to control *A. coregoni* infestations at fish farms.

2 MATERIALS AND METHODS

2.1 Host species (I - V)

During the field sampling (I and IV), parasites were collected from age 2+ rainbow trout (*Oncorhynchus mykiss*) (about 30 - 50 cm in length). Also egg-laying plates for collecting parasite eggs (III) were submerged in places used for rainbow trout farming.

During the laboratory experiments (II, IV and V), rainbow trout were used as a host for *A. coregoni*. Fish used in these experiments were about 6 - 25 cm in length and were obtained from a commercial fish farm located in central Finland. Prior to experiments, fish were acclimated to their environment and were inspected for any attached argulids to make sure that they were uninfected. During the maintenance fish were fed with commercial fish feed (BioMar A/S, Denmark) administered daily.

2.2 Field survey of seasonal population cycle and structure (I and III)

I monitored population abundance, size distribution and sex ratio of *A. coregoni* at a fish farm during two open water periods (I). Weekly parasite samples (~ 100 lice) were collected during early summer of 1999, but later in the season, when the parasite population size decreased, sample sizes were smaller and sampling was performed less frequently. In 2001, sampling was more intense and was performed weekly also in late summer until zero prevalence was observed. On each sampling occasion, all the parasites collected from narcotized (MS-222) fish groups were preserved and pooled, after which the parasites were sexed and total length was measured. In a separate survey, the distribution of *A. coregoni* within a fish population was established by counting the numbers of *A. coregoni* from individually sampled fish (IV).

Simultaneously with monitoring of the population cycle in 2001, the seasonal egg-laying dynamics of *A. coregoni* was monitored at the fish farm (III). I constructed artificial egg-laying plates to capture and collect the eggs (Gault et al. 2002). Eight plates were submerged near the bottom of the 2m-deep flow-through canal. Every week each plate was checked for deposited egg clutches, which were removed and counted, and the plate was cleaned.

2.3 Egg-laying patterns and subsequent hatching (II)

Two sets of mature *A. coregoni* were collected at the fish farm during the July (sample I) and August (sample II) reproductive period of the parasite. Parasites were allocated into 30 aquaria each containing one male and one female *A. coregoni* and one fish as a host for parasites. *A. coregoni* egg laying events and mortalities were recorded every day and deposited eggs were removed and collected individually into 0.5l containers for monitoring of egg hatching. The water temperature and photoperiod were ambient during the breeding.

For comparing the variation in hatching within and between the clutches laid by individual *A. coregoni* females, egg hatching was monitored under constant laboratory conditions over a period of 451 days. Photoperiod was kept at 12h dark: 12h light and the temperature varied between 18 - 20°C. After 2.5 months monitoring at room temperature, egg clutches of sample I and sample II were randomly divided into three treatments: control at room temperature; 2 days cold treatments (1°C) repeated 6 times with an average period of 61 days; and 14 days cold treatments (1°C) repeated 6 times with an average period of 49 days. After the monitoring period the numbers of unhatched eggs were counted.

2.4 Spatial variation in egg deposition (III)

A. coregoni egg laying preferences for various colours of substrates were tested. Three egg laying traps, each consisting of dark (dark wood, black plastic) and light (light wood, white plastic) colour materials, were placed into a fishpond. Numbers of deposited egg clutches on each material were counted and plates were cleaned weekly.

For testing the effect of depth and position of substrate on egg laying, four pairs of traps with a roof-like construction (angle of two adjacent plates 90°) with upper and under surfaces were submerged into separate places in a 2m-deep flow-through canal. Each of the 4 surfaces of the trap (2 under-sides and 2 upper-sides) was divided into three sections according to distance above the bottom of the canal. The distances were 20 - 30 cm, 30 - 40 cm and 40 - 50 cm. Numbers of deposited egg clutches on each surface within each depth section were counted, measured for size and plates were cleaned weekly. To compare

egg-laying preference of *A. coregoni* between stones and the egg traps, nine marked stones were kept in the vicinity of each trap concurrently, and the numbers and relative stone surface area covered by egg clutches determined.

2.5 Parasite aggregation – the role of exposure and susceptibility (IV)

By counting the numbers of *A. coregoni* on individually sampled fish, the distribution of *A. coregoni* within one host population of a fish farm was established. As argulids were aggregated on their host population, laboratory experiments were made to try to identify the mechanisms that could generate this distribution pattern.

Naïve fish ($n = 30$) were selected at random and exposed to 20 newly hatched *A. coregoni* for periods of either 5, 25, 50, 85 or 120 minutes to study the role of varying exposure pressure in shaping the parasite distribution on a fish population. Following 2 hours exposure, each fish was captured and the number of attached metanauplii was estimated by counting the number of unattached parasites in the water. Fish were confined to separate maintenance tanks according to recorded infection level and exposure time. Each of the exposure times had a control unit of 30 fish that were sham-infected, i.e. they were maintained similarly and transferred to exposure tanks but the water was *Argulus* free. One of our objectives was to examine whether a challenge infection by *A. coregoni* would induce defensive responses in rainbow trout when fish were exposed to a second infection and thus create differences in susceptibility. Therefore fish were kept in their tanks at 17°C over a period of 3 weeks to let specific antibodies develop (Aaltonen et al. 1994). After the maintenance, both control and previously-exposed fish were re-exposed to metanauplii and infection success was counted as previously.

2.6 Survival, infectivity and costs for delayed infections (V)

The off-host survival of *A. coregoni* freshly hatched from eggs was monitored every 6 hours at 16.5°C (± 0.5) under laboratory conditions. The effect of starvation period, i.e. time spent host searching, on infectivity of the parasite was tested by exposing individual fish to metanauplii of selected ages and measuring the infection success. Off-host periods between 1 to 180 hours with a step of 20 hours were randomly assigned. The exposures of individual fish to 20 metanauplii ($n = 20$) were conducted at a density of 2 kg m⁻³ at 16°C. Metanauplii were allowed to settle on a host over 2 hours after which the fish was removed. Numbers of attached parasites were determined by counting the number of unattached metanauplii in the water. After the exposure, fish were

confined to separate tanks according to treatment and numbers of parasites on fish were counted after 21, 42 and 50 days maintenance. Each time, 10 randomly chosen female and male *A. coregoni* from each of the tanks were measured for total length.

3 RESULTS AND DISCUSSION

3.1 Seasonal population cycle and structure (I and III)

Strategic life history options for parasites in northern latitudes are largely constrained by seasonal variation in temperature (e.g. Chubb 1980, 1982). The field survey of the present study indicated strong seasonality in the *A. coregoni* infection pattern in terms of population abundance and recruitment, while egg-laying activity and the sex ratio of attached parasites also followed a seasonal pattern. The first juvenile *A. coregoni* were found attached on fish after the water temperature had exceeded 10°C in spring (May). At this time, adult specimens were not recorded on fish, supporting the view that *A. coregoni* only survives the winter as eggs (Shimura 1983, Mikheev et al. 2001). Eggs of *A. foliaceus* cannot hatch at temperatures lower than 10°C (Stammer 1959), although, part of the *A. foliaceus* population can over-winter as inactive stages on fish (Pasternak et al. 2000, Gault et al. 2002).

The highest population abundance of *A. coregoni* was recorded between June and mid-July, after which the population size of attached parasites sharply decreased. However, occasional *A. coregoni* continued to be found on fish until mid-October. The sex ratio of *A. coregoni* was nearly unity until mid-July, after which it became strongly male biased. Concurrent collection of egg clutches with egg-laying traps from early July onwards indicated that females had detached from fish and were laying their eggs. The egg laying period of the *A. coregoni* population extended over 3.5 months. During the early egg laying period, large males exceeding the size of females were recorded on fish, contrasting with findings from earlier studies on sexual dimorphism in size of *A. coregoni* (Shimura 1983) and *A. foliaceus* (Pasternak et al. 2000).

Although recruitment of *A. coregoni* juveniles from eggs was observed until September, it appeared that only one annual generation thrived, because the population size decreased substantially after the females started to lay their

eggs, and no peak in recruitment was observed in autumn. In contrast, *A. foliaceus* proceeds through several generations annually in Finland (Pasternak et al. 2000). Extended hatching at the population level of both *A. coregoni* (Mikheev et al. 2001) and *A. foliaceus* has been proposed to act as an adaptive bet-hedging strategy to cope with unpredictability during the transmission phase (Pasternak et al. 2000). However, the extended pattern could also arise from genetic variability between individuals, or from phenotypic plasticity (Seger & Brockman 1987, Philippi & Seger 1989, Hopper 1999).

3.2 Recruitment pattern at the individual level (II)

Unpredictability in the temporal availability of susceptible hosts for free-living parasite infective stages is likely to act as a selection pressure affecting life history strategies. In conditions where hosts are sparse and host population size fluctuates, genotypes that are able to spread the risk via production of plastic phenotypes resulting in extended transmission are likely to have a selective advantage (Fenton & Hudson 2002). This study has demonstrated one of the first cases of such a bet-hedging strategy for a parasite species.

I found that within a clutch of eggs laid by individual *A. coregoni* females, hatching were more variable than between the clutches. Each clutch of eggs hatched an average after over a period of 7 months and after the monitoring period of 1.2 years all clutches still contained potentially viable eggs. This extended pattern was seen in all egg clutches irrespective of maturation month of the mother or the treatment. These results support the predictions of the diversified bet-hedging strategy in relation to emergence dynamics (Seger & Brockmann 1987, Philippi & Seger 1989). Extended hatching coupled with the long-term survival (>2 years) of diapausing *A. coregoni* eggs (Mikheev et al. 2001) spreads the risk in such a way that a female can ensure that some of her offspring are likely to survive over periods of host absence, thus maximising the geometric mean fitness over generations. In a similar manner the germination of seeds of desert annuals is spread over several years (e.g. Philippi 1993).

Instead of producing phenotypically varying offspring at random, only some of which will be appropriate for current conditions, some organisms respond to environmental variability via phenotypic plasticity triggered by reliable environmental cues (Seger & Brockmann 1987, Philippi & Seger 1989, Meyers & Bull 2002). The cooling treatments of the present study and resulting temperature rise triggered some of the eggs within each clutch to hatch and the relative proportion of eggs hatched was dependent on the length of the cold treatment. Thus the *A. coregoni* egg hatching pattern cannot be explained purely by stochastic bet-hedging or phenotypic plasticity, but rather reflects a combination of both mechanisms. The amount of genotypic variation in hatching dynamics among female parasites is still unknown, but these data suggest that it cannot be the main factor producing the delayed hatching and

recruitment pattern seen at the population level (Pasternak et al. 2000, Mikheev et al. 2001, paper I). The accelerated hatching following the chilling could be adaptive, providing a mechanism to synchronize the life cycle of *A. coregoni* with the short summer period available for growth and breeding (Tauber et al. 1986). Over-wintered eggs of *A. coregoni* collected from fish farm showed a similar hatching pattern, with a high peak followed by a slower rate and delayed hatching (Mikheev et al. 2001).

3.3 Reproduction strategy (II and V)

Male *A. coregoni* grew faster than females during early development under laboratory conditions. At the time of maturation, males were larger (length about 4 mm) than females of the same age (V). In many animals sexual selection favouring bigger males accounts for the sexual dimorphism in growth (Andersson 1994). Such studies were beyond the scope of this thesis, but may provide an interesting research topic for the future. With time, growth rate reversed so that at gravidity females were larger than males (V). Similar dimorphism in growth of *A. coregoni* was observed by Pasternak et al. (2004). Mature *A. foliaceus* males detached more often than females during their search for a mating partner (Pasternak et al. 2000). Thus, the observed sex dimorphism in growth rates following maturation could be due to reproductive behaviour of the parasite, whereby males allocate more energy than females into mate finding instead of using it for growth (see Pasternak et al. 2004).

The length of *A. coregoni* that had finished their egg laying varied from 8.4 mm to 12.2 mm (II). Each female produced an average of 317 eggs and in most cases (80%) all eggs were deposited at one time, followed by the death of the female. Although tremendous variability in the fecundity between parasite females was observed (from 67 to 869 eggs), no correlation between body length and fecundity was found in this study (II).

3.4 Spatial variation in egg deposition (III)

Optimal microhabitat choice maximises acquisition of resources in relation to risks associated with the habitat (e.g. Hugie & Dill 1994). If the needs of an animal with respect to shelter and foraging changes temporally, one might expect microhabitat choice to change over time (Sih 1997). The present study demonstrated microhabitat choice by egg-depositing *A. coregoni* females; most eggs collected during the experiments were deposited on dark substrates or in shadow in the deepest locations of the 2m-deep canal. As both *A. foliaceus* and *A. coregoni* have morphologically well-developed eyes (Meyer-Rochow et al. 2001) and vision was shown to play a key role in host searching (Mikheev et al. 1998, Mikheev et al. 2004), these results suggest that argulids use visual cues

during egg laying. Mikheev et al. (2004) also found that juvenile *A. coregoni* preferred white objects, but their selective behaviour switched towards dark objects later in the ontogeny. Such a negative phototaxis could be adaptive for gravid *A. coregoni* females to avoid visually-hunting salmonids during the vulnerable egg laying period. Free-swimming, gravid parasites usually exceeding size of 8 mm are desirable prey for a fish (personal observation). Poulin & FitzGerald (1989a) found that more female than male *A. canadensis* were eaten by sticklebacks (*Gasterosteus* spp.). Smaller *A. coregoni*, on the contrary, were attracted by light colours, possibly serving as a mechanism to facilitate transmission to fish (Mikheev et al. 2004).

The finding that most *A. coregoni* eggs were deposited on the lowest parts of the traps (20 - 30 cm off the bottom) or stones on the bottom of the canal accord with previous field observations on spatial distribution of parasite eggs (Shimura & Egusa 1980, Mikheev et al. 2001). Some preference for rough substrate in egg deposition of *A. coregoni* has been observed (Shimura & Egusa 1980), which together with deeper location of stones could explain why stones of the present study were more densely covered with parasite eggs than the egg-laying traps. Transmission, which is an essential fitness component of parasites, might also be enhanced via spatial overlap of infective stages with their host niche (Mikheev et al. 2003).

Such a spatial heterogeneity in the distribution of eggs and the following non-random distribution of infective stages (III) coupled with seasonal dynamics of their emergence (I, II) are likely to cause variations in exposure of hosts to parasites. Fish that happen to be near emergence sites of *A. coregoni* during the most intensive spring hatching period are likely to be under the highest exposure pressure. Such variations in exposure potentially produce an aggregated distribution of parasites within a host population (Keymer & Anderson 1978, Janovy & Kutish 1988, Wilson et al. 2002).

3.5 Parasite aggregation - the role of exposure and susceptibility (IV)

Many host-parasite systems are characterised by aggregated dispersion of parasites among their hosts (Shaw & Dobson 1995, Shaw et al. 1998). I showed that juvenile *A. coregoni* (< 2 mm in length) were also aggregated within the host population. Therefore, by experimental manipulations on exposure period and time, and grouping the fish according to infection level, the relative significance of susceptibility and exposure in generating the observed aggregated pattern was explored. During the re-exposure, metanauplii infected the host faster than during the first exposure. Hence challenge infection did not show any development of acquired resistance mechanisms effectively decreasing the attachment of metanauplii. In this respect, this study is in line with most studies on ectoparasitic copepods, where a specific immune response

was absent or did not effectively reduce the level of infection (reviewed by Tully & Nolan 2002).

The infection rate of re-exposed fish did not differ from concurrently maintained and sham-exposed naïve fish. One potentially confounding factor for such an accelerated infection rate regardless of previous infection, may be associated with the fact that fish were older and were kept in captivity for longer. Such a stress may make fish more prone to pathogenic infections (e.g. MacKinnon 1998). On the other hand, activity of fish may be decreased due to stress (Fry 1971). Both fish behaviour (Poulin & Fitzgerald 1988, 1989b,c) and swimming activity of the parasite have been suggested to affect transmission success of argulids (Mikheev et al. 1998, Mikheev et al. 2000). Recent studies, however, have shown that olfactory stimulation plays a less important role than vision in host location by juvenile *A. coregoni* (Mikheev et al. 2004). Although not quantified, it is likely that reduced movement as a consequence of maintenance stress made it easier for metanauplii to attach.

Variable genetic susceptibility to parasitism could also cause an aggregated distribution (Wilson et al. 2002, Lysne & Skorping 2002). No tendency for repeated susceptibility pattern, in terms of infection level among grouped fish, was found during the second exposure to *A. coregoni*, suggesting that rainbow trout were of equal susceptibility. Some protective responses may have been evoked after the settlement of parasites, because only about 40% of attached *A. coregoni* were found on fish during maturation. However, this seemed not to protect fish from further parasitism. Previously, both species and population level variations in fish susceptibility to ectoparasites have been reported (e.g. Bakke et al. 1990, Bakke et al. 1992).

The results of this study suggest that differences in encounter rates (Fryer 1965, Boxshall 1974), duration of exposure to parasite infective stages and host behaviour, especially the ability of host to escape and avoid subsequent movement of argulids, are important in generating the aggregated distribution of argulids observed on a fish population. On the other hand, it seems that transmission of *A. coregoni* is opportunistic and non-selective (see also Mikheev et al. 2004), which would be reasonable since metanauplii carry relatively low energy store, have poorly developed swimming legs (Stammer 1959) and poor sensory repertoire for host location (Mikheev et al. 2004). More developed juveniles could switch to a preferred host and thus increase their level of fitness. *A. foliaceus* were observed to detach from and attach to hosts repeatedly during their ontogeny (Pasternak et al. 2000). Mikheev et al. (2004) also noted that *A. coregoni* host switching occurred more frequently on non-typical roach host than on rainbow trout.

3.6 Survival, infectivity and costs for delayed infections (V)

A. coregoni off-host survival and infectivity were explored by withholding parasites from hosts and then measuring the subsequent infection success.

Costs of late infection were assessed by following parasite growth and survival on fish. Length measurements showed that the length of the off-host period had a negative effect on growth of *A. coregoni*. Mature parasites, that had spent more than 40 hours searching for a host 3 weeks previously, were significantly shorter than parasites that found a host earlier. Although size differences between *A. coregoni* kept off-host for different time periods decreased in magnitude as the parasites developed, a difference was still seen after 6 weeks on a host. Following 7 weeks maintenance on fish, all parasites were of equal size. Such an accelerated growth following prolonged periods of food shortages are known in many free-living animals (Arendt 1997, Metcalfe & Monaghan 2001), but this is, to my knowledge, the first demonstration of a compensatory growth response in a parasite. Such a growth pattern also suggests that growth rates of parasites, although not limited by food availability, may be established at sub-maximal levels.

The off-host survivorship of *A. coregoni metanauplii* at 16.5°C was age-dependent (Anderson & Whitfield 1975); zero mortality was recorded until an age of 90 hours, after which the mortality rate increased exponentially, with the maximum life span being 174 hours. Interestingly, *A. coregoni* that were kept off host over 1 to 140 hours were equally infectious and able to attach fish. The infection and survival patterns of free-living parasite infective stages are generally characterised by temperature dependence, longest survivorships being recorded at low ambient temperatures (e.g. Evans 1985, Thomas & Ollevier 1993, Gannicott & Tinsley 1998, Carthy 1999). As a major part of an *A. coregoni* population recruits in spring at water temperatures around 10°C (I), the effective transmission phases for individual parasites are likely to exceed periods recorded in the present study. Previous detailed studies on infectivity of *Argulus* spp. in relation to time spent host searching are lacking (but see Kollatsch 1959), so temperature dependence of survival remains to be clarified.

Age-dependent mortality and infection patterns have been observed for many other free-living parasite stages (Anderson & Whitfield 1975, Thomas & Ollevier 1993, Gannicott & Tinsley 1998, Karvonen et al. 2003, Whitfield et al. 2003) and are associated with the depletion of finite energy resources (Anderson & Whitfield 1975). This suggests that *A. coregoni metanauplii* carried rather equal amounts of resources for host seeking. Therefore, the temporal heterogeneity in the infectivity of *A. coregoni* is likely to be formed through a variable and extended hatching pattern (Mikheev et al. 2001, paper III) providing a mechanism to cope with the unpredictability during the transmission phase (Seger & Brockmann 1987, Philippi & Seger 1989, Hopper 1999, Fenton & Hudson 2002).

Although not supported by the infectivity data, the expectation that delayed infections in terms of an extended host searching period may pose a fitness cost for a parasite is supported by present observations on survival of parasites after settlement on a host. The survival of *A. coregoni* that had spent more time for host searching seemed to be lower than those that found a host sooner. On average 34% of *A. coregoni* that were kept off-host less than 120 hours reached adulthood, but only 1% of attached parasites kept off-host over

160 hours survived. It appears that there is a cost of delayed transmission and subsequent accelerated growth rates, manifested in survival on host.

4 CONCLUSIONS

The objective of this thesis was two-fold. The first aim was to study the ecology of *Argulus coregoni* by examining its life history tactics. The second aim was to estimate parameters for a detailed host-macroparasite model that will capture parasite population dynamics and provide means to plan cost-effective management practises against *Argulus* ectoparasites that currently cause serious losses in aquaculture throughout the world. This study showed that water temperature was a key determinant of temporal patterns of *A. coregoni* population abundance and dynamics. Neither parasites attached on a host (I), egg-laying (III) nor egg-hatching were observed at temperatures below 10°C (II). Therefore, an *A. coregoni* model has to be run for single seasons by simulating the development of a parasite cohort from hatching to adulthood, and then stopped. Eggs laid by the adult parasite cohort plus any surviving unhatched eggs remaining in the egg-bank from the year before are used as an initial cohort of eggs in the following spring. The *Argulus* model will be based on generic host-macroparasite models by Anderson & May (1978), and flow-chart of the model is represented in figure 2.

One of the key parameters in host-parasite models is the transmission coefficient, β . This instantaneous transmission rate of an *A. coregoni* metanauplius was estimated in a separate experiment by following parasite transmission in relation to host exposure time (Hakalahti, unpublished). Parasite transmission will first be modelled using the law of mass-action, i.e. assuming that parasite transmission is linearly proportional to host density (McCallum 2000). To more exactly estimate β , this experiment should later be repeated under various temperature and host density conditions. Nevertheless, experiments showed that *A. coregoni* transmission was opportunistic and non-selective, and that rainbow trout did not seem to develop effective resistance mechanisms against metanauplii after their first encounters with the parasite (IV). The survival and infectivity of metanauplii were age-dependent, suggesting that individual parasites carried equal amounts of resources for host searching (V). Delayed starts of *A. coregoni* in respect of host searching times

were negatively reflected in the initial growth rate of settled parasites (V). Over time, size differences became less pronounced and eventually all parasites were of equal size. *Argulus* thus appeared to compensate for delayed growth resulting from an extended host searching period by elevated growth rates, although it was shown that nutrient deficiencies coupled with subsequent compensatory growth incurred a cost, through decreased life-expectancy before the reproduction period.

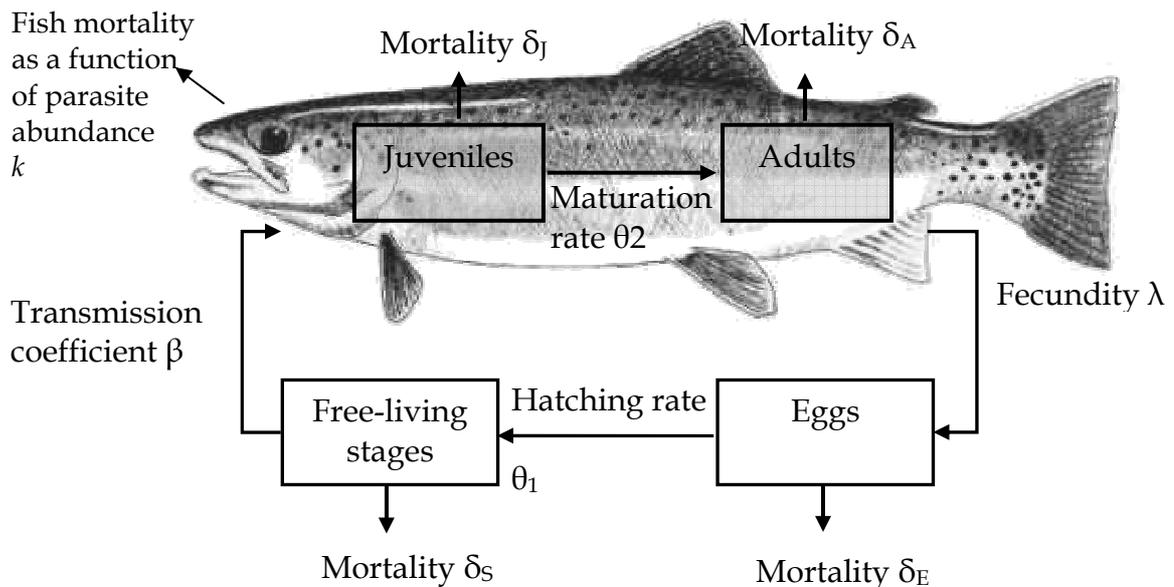


FIGURE 2 A draft *Argulus* model. Summary of gains and losses of individual free-living stages, juveniles, adults and eggs in an *Argulus* population.

Although the majority of the *A. coregoni* population was recruited to fish in spring, the recruitment (I) and the hatching of individual egg clutches were extending over several months (II). The examination of hypotheses concerning the ecological mechanisms behind this extended recruitment pattern supported the predictions of an adaptive bet-hedging strategy in relation to *Argulus* infection strategy (II). Thus, the delayed hatching can be seen as an adaptation to an unpredictable, risk-prone environment. Cooling and subsequent temperature rise triggered high peaks of hatching (II), a phenomenon also seen among the over-wintered eggs (Mikheev et al. 2001). Such a relaxation of diapause during a cold period may have evolved to synchronise the life-cycle of the parasite with the short summer period available for growth and breeding (Tauber et al. 1986). As also shown by the peaked hatching of over-wintered *A. coregoni* eggs collected at a fish farm (Mikheev et al. 2001), relatively little risk-spreading, as expected from mathematical models (Fenton & Hudson 2002), was observed at the high fish densities. Even more extended recruitment is expected under low host densities (Fenton & Hudson 2002).

As in many other host-parasite systems (Shaw & Dobson 1995, Shaw et al. 1998), the *A. coregoni*-rainbow trout system was characterised by aggregated

dispersion of parasites among their hosts (IV). The aggregation seemed to arise through variations in fish exposure to parasites rather than by differences in host susceptibility (IV). Heterogeneity in the exposure could be formed due to observed concomitant spatial (III) and temporal (I and II) variability in the *Argulus* infection pressure. The level of morbidity and mortality caused by a macroparasite is usually a function of the parasite load. The present study gave an estimate of the aggregation index (k) needed for a model to evaluate the potential of parasites to regulate their own population dynamics through density-dependent effects (IV). If parasite fecundity is traded off against virulence, mediated by host survival, parasite growth rates may be constrained by the risk of host mortality. These density-dependent effects remain to be clarified in the *Argulus*-rainbow trout system. However, this cost might be reduced for argulids, because they can detach from a dead fish and find a new host (Pasternak et al. 2000), which is likely to be an advantageous strategy in dense fish populations.

When planning control strategies, an estimate of the basic reproductive ratio of the parasite (R_0) allows evaluation of whether or not to expect parasite epizootics (Hudson et al. 2002). Clearly, when an individual female parasite is not able to replace herself ($R_0 < 1$) the parasite will be lost from the host population. An epidemiological *Argulus* model will be used for testing the effect of various control scenarios on parasite population dynamics. One such example would be simulation of the impact of egg removal on parasite population abundance. A way to remove eggs with artificial egg-laying plates was developed in this project (III).

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YHTEENVETO

Loisen elinkierto-ominaisuudet – perusta tehokkaalle torjunnalle

Loiset ovat eliöitä, jotka ottavat osan tai kaikki resurssinsa toisista elävistä organismeista, loisen isännistä. Loiset elävät suuren osan elämänsä kiinnittyneinä isäntiinsä ja aiheuttavat yleensä haittaa vaikuttaen negatiivisesti isännän elinkierto-ominaisuuksiin. Tästä syystä isännille on kehittynyt erilaisia käyttäytymistapoja välttää loisia tai fysiologisia mekanismeja torjua loiset infektion jälkeen. Isäntä on loisellemme ravintorikas elinympäristö elää ja loisten lisääntyminen on usein tehokkaampaa kuin vapaana elävien, lähisukuisten lajien. Monet suoran kehityksen omaavat loiset leviävät uusiin isäntiin vapaana elävien vaiheiden kautta, jotka ovat lyhytikäisiä. Transmissiovaiheessa (siirtyminen isäntään) tapahtuva suuri kuolleisuus on yksi loispopulaatioiden kasvunopeutta rajoittava tekijä. Loisten transmissiomenukseen vaikuttaa loisellemme herkkien isäntien tiheys loisen ympäristössä. Luonnonoloissa isäntiä on harvassa ja niiden esiintymistä on vaikea ennustaa ja loiset harvoin runsastuvat epidemioiden tasolle. Toisaalta esimerkiksi kalanviljelylaitoksilla loistaudit ovat yleisiä.

Tässä väitöskirjatyössäni tutkin *Argulus coregoni* -kalatäin elinkierto-ominaisuuksia sen elinkierron eri vaiheissa, johon kuuluvat munat, munista kuoriutuvat transmissiovaiheet (metanauplius) ja kalassa elävät aikuistuvat loiset. *A. coregoni* on pääasiassa lohikaloilla esiintyvä laji, jonka isäntänä tutkimuksissa käytin kirjolohta, *Oncorhynchus mykiss*. Kalatäit ovat pintaloisia, jotka ruokailemalla ja liikkumalla kalassa vaurioittavat ihon pintaa. Ihovauriot yhdistettynä sekundäärisiin sien- ja bakteeritauteihin voivat johtaa kalan kuolemaan. Kalatäit muistuttavat elintavoiltaan vapaana eläviä saalistajia, koska ne voivat irrottautua kalasta, elää vapaana ja vaihtaa isäntää. Kalatäiden torjunta on osoittautunut vaikeaksi, ja tehokkaita, ekologisesti kestäviä torjuntamenetelmiä loista vastaan ei ole. Erityisesti *A. coregoni* -lajin ekologian tuntemus on puutteellista. Väitöskirjatyöni pohjalta tullaan kehittämään epidemiologinen lois-isäntämalli, jossa loisen populaatiodynamiikkaa voidaan simuloida erilaisissa tilanteissa. Tämä mahdollistaa erilaisten torjuntakeinojen tehon selvittämisen ja optimoinnin sekä ympäristön että kustannus-tehokkuuden kannalta.

Työssäni I tutkin *A. coregoni* populaation rakennetta ja dynamiikkaa keräämällä kalatäinäytteitä kaloista. Kalatäin aikuis- ja poikasvaiheiden esiintyminen kalanviljelylaitoksella oli vuodenaikaista, ja niitä esiintyi vain yli 10 °C:n lämpötiloissa. Laji talvehti ainoastaan munina. Ensimmäiset kalatäit havaittiin kalojen pinnalla toukokuussa. Loisen munien kuoriutuminen alkoi siten että suuri osa kesän aikana kuoriutuneista loisista kuoriutui ja siirtyi loisimaan kesäkuun puoliväliin mennessä. Osa munista kuoriutui pitkin kesää ja jopa yli usean vuoden. Alkukesästä *A. coregoni* -lajin sukupuolijakauma oli 1:1, mutta muuttui koirasvaltaiseksi heinäkuussa, jolloin naaraat alkoivat irrottautua kaloista munimaan altaiden pohjille. Myös kalatäin populaatiokoko laski syksyä

kohti. Tutkimustulokseni osoittavat, että Keski-Suomessa on vain yksi *A. coregoni* sukupolvi kesän aikana.

Selvitin mekanismeista populaatiotasolla havaitun pitkäkestoisen kuoriutumisen taustalla keräämällä yksittäisten kalatäinaaraiden tuottamia munaryhmiä ja seuraamalla niiden kuoriutumista huoneenlämmössä. 'Bet-hedging' hypoteesin mukaan elinympäristöissä, joissa isäntien esiintyminen on ajallisesti epäenustettavaa, loisten kannattaa tuottaa pitkällä aikavälillä kuoriutuvia jälkeläisiä. Pitkäkestoinen kuoriutuminen populaatiotasolla voi selittyä myös ilmiön joustavuutena (fenotyypinen plastisuus), jos munat altistuvat määrällisesti ja/tai laadullisesti vaihtelevasti kuoriutumista laukaiseville ympäristötekijöille. Tästä syystä osa munaryhmistä altistettiin toistetuille kylmäjaksoille. Tutkimustulokseni tukevat 'bet-hedging' hypoteesin oletuksia suhteessa loisen infektiotaktiikkaan. *A. coregoni* naaraat tuottivat jälkeläisiä, joissa yhdestä munitusta munaryhmästä kuoriutui metanaupliuksia keskimäärin 7 kuukauden ajan. Kylmäjaksot tuottivat kuoriutumispiikkejä huoneenlämpöön siirron jälkeen kaikista munaryhmistä, mutta niiden kokonaiskuoriutumiskausi ei eronnut huoneenlämmössä jatkuvasti pidetyistä munista. Havaittu pitkäkestoinen, jopa usean vuoden ajan jatkuva, transmissio voi varmistaa lohikaloilla loisivien kalatäin elinkierron jatkumisen vesistöissä, joissa yhtäaikainen kuoriutuminen on riski. Myös muninta-ajankohta vaikutti *A. coregoni* munien kuoriutumiseen siten, että ainoastaan pieni osa alkumunintakaudesta (heinäkuu) munituista munista kuoriutui saman vuoden elokuussa. Tämä voi mahdollistaa toisen sukupolven kehittymisen lämpiminä syksyinä.

Kalatäin munien pitkäkestoinen kuoriutuminen ja sitä seuraava pitkäkestoinen kiinnittyminen kaloihin vaikeuttaa loisen torjuntaa perinteisten kemikaalikyöpyjen avulla. Tästä syystä selvitin kalatäin munintakäyttäytymistä ja mahdollisuutta kerätä ja tuhota loisen munia mekaanisin keinoin sijoittamalla keinotekoisia muninta-alustoja kalanviljelyalustoihin. *A. coregoni* -naaraat munivat alustoille runsaasti munia ja valitsivat tummia, syvimmässä vedessä ja virralta suojassa olevia alustojen osia munintapaikoikseen. Altaiden pohjille sijoitettujen kivien pinnoilta kerättiin kuitenkin suhteellisesti enemmän munia kuin alustoilta, joka osoittaa altaiden saneeraamisen (muovitus) tarpeen kalatäinongelmien ehkäisyssä tulevaisuudessa. Edellisessä tutkimuksessa havaittu ajallisesti vaihteleva munien kuoriutuminen yhdistettynä munien epätasaiseen sijoittamiseen vesistöissä aiheuttaa eroja kalayksilöiden välillä metanaupliusaltistuksessa.

A. coregoni kalatäit olivat jakautuneet epätasaisesti yksittäisten kalojen kesken. Ainoastaan pieni osa kalapopulaatiosta oli hyvin voimakkaasti loisittu. Selvitin mekanismeista epätasaisen jakauman taustalla seuraamalla yksittäisten kalojen kalatäininfektiota suhteessa aikaan ja altistuskertoihin laboratorioissa. Loisen epätasainen jakauma kalojen kesken syntyi ainoastaan lyhyillä altistusajoilla. Pitkillä altistusajoilla loiset olivat jakautuneet tasaisesti kalojen kesken. Kaloille ei kehittynyt tehokasta immuunipuolustusta uutta kalatäininfektiota vastaan ensimmäisen altistuksen jälkeen. Altistusväli oli 3 viikkoa. Nämä tulokset tukevat näkemystä, että epätasaisen jakauman taustalla pääsyy-

nä olivat todennäköisemmin erot siinä miten kauan kalayksilöt altistuivat loislle, kuin erot kalojen geneettisessä alttiudessa kalatäille.

Selvitin myös *A. coregoni* metanaupliusten selviytymistä ilman kalaa ja kykyä kiinnittyä kalaan suhteessa kalatäin ikään. Tutkin mahdollisia kustannuksia pidentyneestä isännän etsimisajasta seuraamalla eri-ikäisinä kiinnittyneiden kalatäiden myöhempää kasvua ja selviytymistä kalassa. Kaikki metanaupliukset elivät 90 tuntia (16,5°C) ilman isäntää, jonka jälkeen kuolleisuus kasvoi nopeasti. Pidentynyt kalan etsimisaika oli kalatäille kustannus vaikuttaen negatiivisesti sekä myöhempään kasvuun että selviytymiseen kalassa. Nopeimmin kalaan kiinnittyneet kalatäit kasvoivat alussa nopeammin kuin yksilöt, jotka kiinnittyivät kaloihin myöhemmin. Nämä kuitenkin saavuttivat pituudessa aikaisemmin kiinnittyneet yksilöt. Vastaavia kompensatiokasvumekanismia on aikaisemmin kuvattu vapaana elävillä eliöillä vasteena nälkiintymiseen ja siitä johtuvaan hitaampaan kasvuun.

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