

Matthias Bandilla

Transmission and Host and  
Mate Location in the Fish  
Louse *Argulus coregoni* and its  
Link with Bacterial Disease in Fish



Matthias Bandilla

Transmission and Host and Mate Location in the  
Fish Louse *Argulus coregoni* and its Link with  
Bacterial Disease in Fish

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella  
julkisesti tarkastettavaksi Konneveden tutkimusasemalla  
kesäkuun 16. päivänä 2007 kello 14.

Academic dissertation to be publicly discussed, by permission of  
the Faculty of Mathematics and Science of the University of Jyväskylä,  
in Konnevesi Research Station, on June 16, 2007 at 2 p.m.



UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2007

Transmission and Host and Mate Location in the  
Fish Louse *Argulus coregoni* and its Link with  
Bacterial Disease in Fish

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 179

Matthias Bandilla

Transmission and Host and Mate Location in the  
Fish Louse *Argulus coregoni* and its Link with  
Bacterial Disease in Fish



UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2007

Editors

Timo Marjomäki

Department of Biological and Environmental Science, University of Jyväskylä

Irene Ylönen, Marja-Leena Tynkkynen

Publishing Unit, University Library of Jyväskylä

Jyväskylä Studies in Biological and Environmental Science

Editorial Board

Jari Haimi, Anssi Lensu, Timo Marjomäki, Varpu Marjomäki

Department of Biological and Environmental Science, University of Jyväskylä

Cover picture by Suvianna Huikuri

URN:ISBN:9789513928575

ISBN 978-951-39-2857-5 (PDF)

ISBN 978-951-39-2818-6 (nid.)

ISSN 1456-9701

Copyright © 2007, by University of Jyväskylä

Jyväskylä University Printing House, Jyväskylä 2007

## ABSTRACT

Bandilla, Matthias

Transmission and host and mate location in the fish louse *Argulus coregoni* and its link with bacterial disease in fish

Jyväskylä: University of Jyväskylä, 2007, 40 p.

(Jyväskylä Studies in Biological and Environmental Science  
ISSN 1456-9701; 179)

ISBN 978-951-39-2857-5 (PDF), 978-951-39-2818-6 (nid.)

Yhteenveto: *Argulus coregoni* -kalatäin siirtyminen kalaisäntään, isännän ja parittelukumppanin paikallistaminen sekä loisinnan yhteys kalan bakteeritautiin

Diss.

*Argulus coregoni* is an ectoparasite preferentially infecting salmonid fish. In this thesis I focussed on aspects of transmission, host and mate location of *A. coregoni* and its link with secondary diseases in fish. Under fish farming conditions, metanauplii and juvenile stages of the parasite were aggregated among the host population; i.e. a relatively small proportion of the host population carried the majority of the parasite population. In laboratory experiments I showed that such aggregated distribution was likely to be driven by differences in exposure to the parasite and not by differences in susceptibility or by acquired immunity. The transmission of *A. coregoni* metanauplii in the laboratory was highly successful, with 80% attaching to the host within 1-2 h. Extended transmission periods due to host absence did not decrease the infectivity of the parasite, but some consequences of a prolonged transmission process became apparent during the early development; parasites that experienced long transmission periods were smaller compared to argulids with short transmission time. However, in late phases of development the smaller parasites compensated their decreased size by an increased growth rate. Since *A. coregoni* is a sexually reproducing species, such "catch-up" growth might be beneficial in terms of reaching the optimal mating size, higher fecundity and sexual selection. In Y-maze experiments it was shown that visual cues are most important for host finding, however the parasite could also detect fish odour which might act as an auxiliary cue to find a host. Males responded to female odour but not vice versa, indicating that two different reproductive strategies exist, where males are the active mate searchers while females are rather stationary on the host and wait for partners. Host switching experiments support this view since adult males were more active in switching between hosts than were adult females. Knowledge gained in this study elucidates the sophisticated behavioural patterns of *A. coregoni* and can be used for developing sustainable control strategies at fish farms, which is important since this thesis showed that argulids can enhance the susceptibility of fish to *Flavobacterium columnare*, the causative agent of columnaris disease.

Keywords: Aggregation; *Argulus coregoni*; ectoparasite; transmission; hierarchical response; host searching; host switching; mate searching; susceptibility to bacteria.

Matthias Bandilla, University of Jyväskylä, Department of Biological and Environmental Science, P. O. Box 35, FI-40014 University of Jyväskylä, Finland

**Author's address** Matthias Bandilla  
Department of Biological and Environmental Science  
University of Jyväskylä  
P.O. Box 35  
FI-40014 University of Jyväskylä, Finland  
Email: matband@bytl.jyu.fi

**Supervisors** Professor E. Tellervo Valtonen  
Department of Biological and Environmental Science  
University of Jyväskylä  
P.O. Box 35  
FI-40014 University of Jyväskylä, Finland

Dr. Teija Hakalahti  
Department of Biological and Environmental Science  
University of Jyväskylä  
P.O. Box 35  
FI-40014 University of Jyväskylä, Finland

**Reviewers** Professor Robert Poulin  
Department of Zoology  
University of Otago  
King Street 340  
P.O. Box 56  
Dunedin, New Zealand

Dr. Victor Mikheev  
Institute of Ecology and Evolution  
Russian Academy of Sciences  
33 Leninskii Prospekt  
119071 Moscow, Russia

**Opponent** Professor Christina Sommerville  
Institute of Aquaculture  
Faculty of Natural Sciences  
University of Stirling  
Stirling, FK9 4LA, Scotland

# CONTENTS

## LIST OF ORIGINAL PUBLICATIONS

1	INTRODUCTION.....	7
	1.1 General introduction.....	7
	1.2 Fish ectoparasites.....	8
	1.3 The study species and aims of the study.....	9
2	MATERIALS AND METHODS.....	12
	2.1 Host species (I-V).....	12
	2.2 Parasite rearing (I-V).....	12
	2.3 Transmission of <i>A. coregoni</i> to fish: Distribution patterns and the effects of off-host periods (I and II).....	13
	2.4 Host and mate location mechanisms (III).....	14
	2.5 Host switching (IV).....	14
	2.6 The link between ectoparasites and bacterial disease in fish (V).....	15
3	RESULTS AND DISCUSSION.....	16
	3.1 Transmission of <i>A. coregoni</i> to fish: Distribution patterns and the effects of off-host periods (I and II).....	16
	3.2 Host and mate location mechanisms (III).....	18
	3.3 Host switching (IV).....	20
	3.4 The link between ectoparasites and bacterial disease in fish (V).....	21
4	CONCLUSIONS.....	22
	<i>Acknowledgements</i> .....	25
	YHTEENVETO (RÉSUMÉ IN FINNISH).....	26
	ZUSAMMENFASSUNG (RÉSUMÉ IN GERMAN).....	29
	REFERENCES.....	33



## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on five original papers, which will be referred to in the text by their Roman numerals (I-V). I performed a major part of planning and data collection in each paper with the support of Teija Hakalahti (I-IV), Heli Häkkinen (I) and Lotta-Riina Suomalainen (V). I did the statistical analyses in papers III & IV. Papers I & V were analysed jointly with the co-authors and paper II was analysed by Teija Hakalahti. The co-authors contributed to writing the manuscripts but I carried the main responsibility for writing the papers where I am the first author (I, III, IV & V). I co-authored paper II which was the main responsibility of Teija Hakalahti-Sirén.

- I Bandilla, M., Hakalahti, T., Hudson, P. J. & Valtonen, E. T. 2005. Aggregation in *Argulus coregoni* (Crustacea: Branchiura): a consequence of host susceptibility or exposure? *Parasitology* 130: 169-176.
- II Hakalahti, T., Bandilla, M. & Valtonen, E. T. 2005. Delayed transmission of a parasite is compensated by accelerated growth. *Parasitology* 131: 647-656.
- III Bandilla, M., Hakalahti-Sirén T. & Valtonen, E. T. 2007. Experimental evidence for a hierarchy of mate- and host-induced cues in a fish ectoparasite, *Argulus coregoni* (Crustacea: Branchiura). *International Journal for Parasitology*, in press.
- IV Bandilla, M., Hakalahti-Sirén T. & Valtonen, E. T. Exploring costs and benefits of host switching using the fish ectoparasite, *Argulus coregoni* (Crustacea: Branchiura). (under review in "Behavioral Ecology and Sociobiology").
- V Bandilla, M., Valtonen, E. T., Suomalainen L-R., Aphalo, P. J. & Hakalahti, T. 2006. A link between ectoparasite infection and susceptibility to bacterial disease in rainbow trout. *International Journal for Parasitology* 36: 987-991.

# 1 INTRODUCTION

## 1.1 General introduction

Parasitic life forms obtain energy and shelter from another living organism termed the host. Exploiting the resources of other organisms has proved a highly successful strategy in evolutionary terms: it has been estimated that at least half of all living organisms are obligatory parasites or resemble a parasitic life in some parts during their ontogeny (Price 1980, Windsor 1998).

The benefits that come with a parasitic life style include the availability of large amounts of resources like food and living habitat generally due to the small size of the parasite compared to its host. Moreover the host can buffer effects of the highly variable external environment making it easier for the parasite to flourish in a rather protected microhabitat. However, there is an intrinsic negative effect that a parasite exerts on the exploited organism. Eventually this so called virulence (Day 2003, de Roode et al. 2005) can trigger the evolution of antagonistic mechanisms against the parasite visible, for example, in specific immune responses of the host (e.g. Wakelin 1996, Claerebout & Vercruyssen 2000). In response to such developments the parasite is selected to overcome these obstacles potentially leading to a co-evolutionary arms race between host and parasite (Dawkins & Krebs 1979).

Besides the challenges for a parasite connected to immunity of the host there are also other problems inherent in a parasitic way of life. The host may learn how to decrease the contact risk with harmful pathogens by avoiding certain habitats during selected time periods or adopt behaviour like group forming to dilute the transmission of a parasite (Poulin & FitzGerald 1989a, b, Hutchings et al. 1998, Wilson et al. 2002). Also, free-living infective stages of parasites are often confronted with sparse host populations making it unpredictable when a chance for transmission occurs (Fenton & Hudson 2002). Thus, the transmission of infective parasite stages from one host to another is a critical parameter for the fitness of a parasite, and transmission patterns of pathogens have received considerable interest by the scientific community (e.g. Anderson & May 1991, Hudson et al. 2002, Karvonen et al. 2003). Parasites have

developed several ways to optimize their transmission such as synchronized emergence of infective stages with maximum host availability (Kearn 1986; Gannicott & Tinsley 1997) and the ability to detect visual, physical and chemical cues connected to the host which leads to rapid host finding (Mikheev et al. 1998, 2000, Devine et al. 2000). Some parasites might even manipulate the behaviour of their host species in order to enhance the effectiveness of transmission (Poulin et al. 2003, Seppälä et al. 2005). At a more strategic level, the development of complex life cycles that exploit several host species in succession during the life of a parasite has been identified as one way to increase the transmission success (Poulin et al. 1998, Parker et al. 2003). Other parasites may enhance their transmission success by producing eggs which are highly variable in terms of hatching time (Hakalahti et al. 2004a); this “bet hedging” phenomenon is advantageous when host availability is heterogeneous and highly unpredictable (Fenton & Hudson 2002).

In the past, parasites in general have been considered as degenerate organisms which are simple reproductive machines (see Thomas et al. 2002) and behavioural studies on parasites are still fairly rare. However, in addition to those problems for parasites inherent to their parasitic life style, they are also confronted with challenges that are usually only considered for free-living species. Sexually reproducing parasites, for example, need to search for and identify appropriate mating partners. From a scientific point of view it appears very interesting to strive for understanding how parasites trade-off their parasitic life against other needs like, for example, mate searching. Moreover, understanding parasite behaviour can have direct applied benefits for controlling parasites relevant to intensive animal cultures (Gault et al. 2002, Hakalahti et al. 2004c, Fenton et al. 2006, Larsen 2006), especially since parasites may promote secondary infections by bacteria, fungi or viruses due to their potential as vectors of diseases or through their negative impact on body organs and the host immune system in general (e.g. Cusack & Cone 1986, Singhal et al. 1990, Woo 1992, Grenfell & Dobson 1995, Randolph 2004, Pylkkö et al. 2006, Smith et al. 2007).

## 1.2 Fish ectoparasites

Fish ectoparasites attach themselves to the skin and gills of the host. The outer surfaces of fish are exploited by a huge diversity of parasites including protozoans, monogeneans, leeches, molluscs, crustaceans and even other fish (Kearn 2004). Often the fish can deal adequately with the damage that the ectoparasites inflict on the skin tissue so that low infections generally do not lead to fish mortality. However, higher infection levels of ectoparasites can have severe effects on fish populations especially, under conditions of high host densities as in fish farms. Some of the most notorious fish ectoparasites are *Gyrodactylus salaris* on salmon gills (Bakke & Harris 1998) and fish lice like

*Lepeoptheirus salmonis* and *Caligus* spp. (Wootten et al. 1982, Costello 2006), which have led to huge fish population losses in nature and aquaculture. High numbers and epizootics of fish lice belonging to the genus *Argulus* spp. have also been reported (e.g. Cross and Stott 1974, Northcott et al. 1997, Hakalahti et al. 2004b, Taylor et al. 2006). By damaging the fish skin and mucus, ectoparasites can directly affect fish health for example by decreasing the immunocompetence and disrupting osmoregulatory mechanisms (Ruane et al. 1999, Tully Nolan 2002). Moreover, the fish skin and mucus act as an important barrier against secondary disease (Hellio et al. 2002); thus ectoparasites possibly enhance the susceptibility of fish to co-existing fungal and microbial diseases (Cusack & Cone 1986).

There is growing scientific interest in ectoparasites, partly due to their importance in terms of fish farming, but also because of their complex behavioural strategies including transmission, reproduction and the interaction with other pathogens. Fundamental knowledge gained from research on the ecology of fish ectoparasites can subsequently be used to develop ecologically sustainable control strategies (Bron et al. 1993, Hakalahti et al. 2004c, Fenton et al. 2006).

### 1.3 The study species and aims of the study

*Argulus coregoni* Thorell (Crustacea: Branchiura) is a fish ectoparasite preferentially infecting salmonids and is distributed throughout the boreal hemisphere (Shimura 1983, Hakalahti & Valtonen 2003). The direct life cycle of this parasite (Fig.1) is triggered in spring when the water temperature exceeds 10 °C (Hakalahti & Valtonen 2003). Metanauplii of *A. coregoni* (~ 0.7 mm) hatch from egg clutches that are laid at the bottom of a water body preferably on hard substrata like stones and actively search for a host. After attaching themselves to fish they undergo nine moulting stages until they reach adulthood at a length of about 4 mm (Shimura 1981), which takes approximately 4-6 weeks depending on the water temperature. They may grow to a length of up to 14 mm (Hakalahti & Valtonen 2003). Many hooks and spines and a pair of powerful suckers beneath the carapace enable *A. coregoni* to adhere to the fish and scuttle rapidly over the host's skin. *Argulus coregoni* pierce the skin of the host with a slender stylet and feed on the blood and/or skin tissue by using its mouth tube and mandibles (Bower-Shore 1940, Cressey 1983, Kearn 2004). Mature male and female argulids need to locate each other and usually mate on the fish (Pasternak et al. 2000), although off-host copulation has been also observed (Stammer 1959, Walker 2004). More specifically, with males and females attached together, females receive sperm via two seminal receptacles. The female carries the sperm inside her body for several days and the eggs are only fertilised during deposition (Kearn 2004). Mechanisms involved in mate finding and pair formation of argulids are not well understood, but it has been

assumed that males and females can make multiple matings with different partners (Kollatsch 1959). Gravid females lay egg batches containing approximately 300 eggs preferably on rough substrata like stones, and most *A. coregoni* females die after egg deposition (Hakalahti et al. 2004a, c). In Finland, during winter only the egg stages of *A. coregoni* survive and all hatched parasites will perish (Hakalahti & Valtonen 2003). After overwintering, new *A. coregoni* will emerge and currently there is only one generation of this parasite per year in Finland. However, increased temperatures due to global warming may trigger the emergence of a second generation of *A. coregoni* in Finland increasing the parasite pressure on natural fish populations and on fish in farms (Hakalahti et al. 2006).

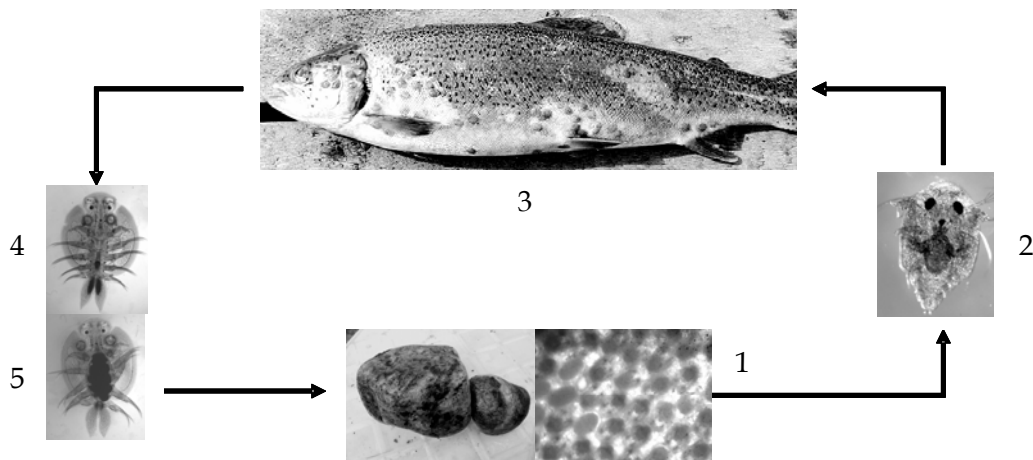


FIGURE 1 The life cycle of *Argulus coregoni* (pictures not to scale); metanauplii hatch from eggs on stones (1); free-swimming metanauplii search for a host (2); attached argulids grow on fish (3); adult males (4) and females (5) mate on fish after which the female lays eggs on stones; (pictures 1, 3 & 4 by T. Hakalahti-Sirén).

In this thesis I investigated aspects of transmission, host and mate finding of *A. coregoni* and the link with secondary diseases in fish. In papers I and II, I focussed on transmission of *A. coregoni*. More specifically I examined whether *A. coregoni* metanauplii in a fish farm are transmitted in such a way as to generate an aggregated distribution of the parasite on its host (I). An aggregated distribution is a characteristic of most parasites and means that a small proportion of the host population is infested by the majority of the parasite population (Shaw & Dobson 1995, Poulin 2007). On the basis of the distribution of *A. coregoni* at the fish farm, I addressed a further question in the first paper by using experimental exposures: Is the distribution of *A. coregoni* on rainbow trout (*Oncorhynchus mykiss*) a consequence of host susceptibility or is the exposure time more important?

In paper II, I studied how long the infective metanauplii stages of the parasite may survive without a host and how the transmission success is affected by the time the metanauplii are off-host. Moreover I followed the

growth of *A. coregoni* on the host over time and examined how the handicap of a prolonged transmission process may affect the subsequent development of the parasite.

The aims in papers III and IV were to shed more light on mate and host location of *A. coregoni*. I tested whether the argulids could respond to mate and host related chemical and visual cues and if a hierarchy exists within these signals (III). In paper IV, I investigated how mate searching may influence the switching of the parasite between individual hosts.

Paper V was concerned with the interactions between parasites and concurrent bacterial pathogens. Pylkkö et al. (2006) found enhanced bacterial invasion (*Aeromonas salmonicida*) during a trematode infection (*Diplostomum spathaceum*) in European grayling (*Thymallus thymallus*), but they did not record increased disease or mortality among fish infected concomitantly with the bacterium and the parasite. No existing studies have clearly shown that parasites increase the susceptibility of fish to bacterial pathogens (see Busch et al. 2003). I tested whether *A. coregoni* enhances the susceptibility of fish to "columnaris disease" due to *Flavobacterium columnare* infection. *Flavobacterium columnare* has been identified as one of the most problematic pathogens in the Finnish fish farming industry (Suomalainen 2005). Characteristic signs of columnaris disease are "saddleback" lesions (i.e. skin necrosis around the dorsal fin; Bader 2003), and erosion of skin and tail leading to osmotic and electrolyte imbalances and eventually to death of the infected fish (Tripathi et al. 2005). Since *F. columnare* is usually restricted to the fish epidermis (Tripathi et al. 2005) it is likely that the skin grazing activity of ectoparasites like *A. coregoni* can enhance the adherence of the bacterium to the fish skin (Decostere et al. 1999) and thus lead to severe outbreaks of columnaris disease.

This thesis was born out of a larger project which aimed to understand important biological parameters of fish farming pathogens. The knowledge gained from these studies could be used to find ecologically sustainable ways for parasite control at fish farms. Some of the understanding gained during my studies has complemented the construction of a mathematical model of *A. coregoni*, which is a first step towards the goal of alternative control strategies (Fenton et al. 2006).

## **2 MATERIALS AND METHODS**

### **2.1 Host species (I-V)**

To determine the *A. coregoni* distribution under aquaculture conditions I sampled rainbow trout of age 2+ with an average length of 40 cm from a fish farm channel (I). In the experimental work performed in the laboratory (I-V) I used rainbow trout of age 0+ and 1+ ranging in size between 14-20 cm and 42-100 g. All fish were obtained from a commercial fish farm in Central Finland. Before the start of the experiments the fish were acclimated to laboratory conditions in flow-through tanks and were fed daily with commercial fish feed pellets (Biomar/Denmark). The skin of the fish was checked visually to be sure that they were not infested with ectoparasites and the fish came from fish farm rearing sites which had no *Argulus* outbreaks in the same year. Fish that had obvious signs of disease during the experiments were removed and killed.

### **2.2 Parasite rearing (I-V)**

All parasites used in my studies originated from a fish farm in Central Finland. Stones carrying egg batches of *A. coregoni* were collected from the bottom of the fish farming channels. The egg clutches were scraped off the stones, immersed in water and kept in the dark at 4 °C to prevent egg hatching. Prior to the experiments, the egg clutches were transferred to room temperature (21 °C) and were allowed to acclimate to their new temperature environment naturally. Eggs were exposed to continuous light to induce the hatching. The hatched metanauplii (~ 0.7 mm in length) were collected with a pipette and then used for experimental infection of fish (I-V) or for studying the off-host survival of metanauplii (II).

### 2.3 Transmission of *A. coregoni* to fish: Distribution patterns and the effects of off-host periods (I and II)

The distribution of *A. coregoni* metanauplii among rainbow trout under aquaculture conditions was analyzed by counting argulids on 2+ fish at a fish farm in Central Finland between May and June 2002 (I). I used experimental exposures in the laboratory to define the relative importance of host susceptibility and exposure time in relation to the observed *Argulus* distribution pattern at the fish farm (I). Rainbow trout were individually exposed to 20 *A. coregoni* metanauplii for selected periods of time. Following the exposure, the infection success of the parasite was estimated by counting the unattached metanauplii in the water. Similarly, control fish were sham-treated, i.e. they were exposed to parasite-free water. The infected and control fish were divided into groups according to their exposure time and infection level. They were then transferred to 300 l flow-through tanks (17 °C) for a maintenance period of three weeks to allow the development of possible protective resistance against the parasite. After the maintenance period the developed argulids were removed and the experiment was repeated. The same fish were individually exposed to 20 *A. coregoni* metanauplii for the same selected time periods as in the first exposure; this time the control fish were also exposed to argulids. The infection success of the argulids from the first exposure was compared to that of the second exposure. Moreover it was checked if fish varied in their susceptibility to *A. coregoni* or if the transmission of the metanauplii was rather opportunistic and non-selective.

The off-host survival of *A. coregoni* metanauplii and their transmission potential in relation to off-host periods were examined (II). For the survival experiment, groups of 10 metanauplii were kept in plastic containers filled with lake water. The mortality was checked every 6 h and the data were compared to an age-independent and age-dependent survivorship model (Anderson & Whitfield 1975). The influence of off-host periods on the transmission success was analysed by withholding freshly hatched metanauplii from host-attachment for selected times ranging between 1-140 h. Following the off-host periods the metanauplii were allowed to attach to individual fish and their infection success was determined by counting the unattached *A. coregoni* in the water. The parasites that had attached were allowed to grow for 50 days on the fish. During the 50 days growing period the length of the argulids was measured after 21, 42 and 50 days to determine whether delayed transmission during the metanauplii stage is reflected in the development of the parasite.



## 2.4 Host and mate location mechanisms (III)

A Y-maze consisting of a main leg (20 cm in length) with two side arms (each 12 cm in length) at an angle of 90° was constructed out of water-tight ply wood. Lake water passed through the two side arms and exited in the main leg in a unidirectional way.

I tested the directional response of free-swimming argulids towards 3 different odour treatments: female *A. coregoni* odour, male *A. coregoni* odour or fish odour. In each trial the odour of 10 adult females, 10 adult males or 5 rainbow trout was linked to a selected side arm. The other arm received untreated water. I also tested whether *A. coregoni* respond towards light since it has been suggested that photic cues are the most important for location of a host by juvenile *A. coregoni* (Mikheev et al. 2004).

During each trial, a single virgin adult male or female *A. coregoni* was placed into the base of the Y-maze and was allowed to swim up the main leg. I recorded into which arm the parasite swam and the time that it took for each parasite to enter one side arm was measured. Subsequently I tested whether a hierarchy existed among those cues, which had a significant influence on the directional response of *A. coregoni*, by pitting these stimuli against one another in the Y-maze.

In exposure experiments I examined whether adult virgin female *A. coregoni* on fish might attract free-swimming adult male conspecifics in a semi-natural environment. One infected fish carrying five females and an uninfected fish were transferred into a tank supplied with aerated lake water. Artificial plastic plants in the tank gave hiding opportunities for the fish. During each trial a single adult virgin male *A. coregoni* was introduced to the tank. After 2 h exposure time, the fish were caught individually with a dip net and anaesthetized with MS-222 to record whether the free-swimming male parasite attached to the previously infected or to the control fish.

## 2.5 Host switching (IV)

I determined whether the sex, age and the presence of mating partners influenced the host switching activity of *A. coregoni*. In the laboratory individual rainbow trout were infected with a group of eight male or eight female *A. coregoni*. One individual infected fish was kept in a 50 l flow-through tank together with a) an uninfected fish, b) a fish that carried the same sex of parasites or c) a fish that carried the opposite sex of parasites. After a selected period, the fish were individually anaesthetized and the parasites that had switched from one host to another were counted. Also, two fish that both carried a mixed population of four males and four females were maintained together to analyze whether host switching of the parasite occurs during the

presence of the opposite sex on the same host. The two fish in each experimental unit could be distinguished by distinctively cutting the adipose fin. Half of the parasites were stained with neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) prior to the experiment to distinguish whether they switched from their original host (Anstensrud 1989).

In another experimental set-up I examined whether rainbow trout pose a threat to host-switching parasites through predation. Two fish were kept together in a 50 l flow-through tank together with a single free-swimming parasite. After a 2 h exposure, fish were caught individually with a dip net and anaesthetized with MS-222 to record whether the free-swimming parasite had attached to the fish. If the parasite was neither found on the skin nor in the water, the oesophagus and stomach of the fish were opened to analyse whether the fish had eaten the argulid.

## **2.6 The link between ectoparasites and bacterial disease in fish (V)**

The aim was to elucidate whether infections with *A. coregoni* lead to higher susceptibility of fish to bacterial disease. Rainbow trout were infected with *A. coregoni* metanauplii and kept in maintenance tanks to allow parasite growth. After three weeks, when the parasites had reached an average length of 4 mm, the fish were concomitantly infected with *Flavobacterium columnare* and maintained in 30 l flow-through tanks. Over a period of 21 days at 12 h intervals their survival was monitored and compared with that of fish infected with either the parasite or the bacterium alone. Sham-treated fish that were neither infected with the parasite nor with the bacterium were used as a control. Dead and moribund fish were removed from the tanks and skin samples were taken to determine if they were infected with *F. columnare* using PCR analyses with species-specific primers.

### 3 RESULTS AND DISCUSSION

#### 3.1 Transmission of *A. coregoni* to fish: Distribution patterns and the effects of off-host periods (I and II)

The time after egg-hatching is a crucial period for *A. coregoni* metanauplii. Transmission to a host must occur relatively rapidly, since the free-living parasite depends on its own finite nutrient reserves. I observed how the transmission success of metanauplii was reflected in the parasite distribution among rainbow trout at a fish farm. Metanauplii and juvenile *A. coregoni* showed an aggregated distribution within their host population (I). Although some parasites species may be non-aggregated within a host population (Anderson 1974, Johnson 1992), the finding in this study is in line with a widespread pattern among parasites species: generally a disproportionately small segment of the host population is infected by the majority of the parasite population (Shaw & Dobson 1995, Shaw et al. 1998, Poulin 2007). The reasons underlying such aggregated distribution are considered to be mainly connected to heterogeneities in exposure; e.g. due to behavioural differences between hosts or differences in susceptibility generated for example by immune responses (Anderson et al. 1978, Wassom et al. 1986, Poulin & FitzGerald 1989a, b, Wilson et al. 2002, Dalgaard et al. 2003).

To clarify how the aggregated distribution of argulids among rainbow trout at the fish farm may have developed experimental exposures of fish to *A. coregoni* metanauplii were undertaken. Two repeated challenge infections within three weeks showed that there was no protective acquired resistance against metanauplii. On the contrary, fish were even more susceptible to argulids 3 weeks after the first exposure. Control fish, which were not exposed to argulids during the first challenge-infection, gained similar infection rates compared to fish challenged twice indicating that there was no immunosuppressive mechanism induced by argulids. Glover et al. (2004) demonstrated comparable results with the salmon louse (*Lepeoptheirus salmonis*) on Atlantic salmon (*Salmo salar*). They found that relative infection level in a first parasite challenge was a poor predictor of its relative infection in a second

challenge. However, I used rainbow trout as the fish model species, which is not native to Finnish waters but was introduced from North America very recently. Therefore it is possible that native Finnish salmonid populations such as brown trout (*Salmo trutta*) which have co-evolved with *A. coregoni* for thousands or millions of years might have acquired the ability to develop immune responses against this parasite and this remains to be studied.

Prior to the second challenge infection it was noticed that only 38% of the parasites were found attached on the fish originating from the first exposure three weeks earlier. The loss of the parasites can be attributed to the fish handling, natural mortality, antiparasitic behaviour of the fish like rubbing the skin against hard material (Walker 2004) and predation of fish on detached parasites (Poulin & Fitzgerald 1989c, Pasternak et al. 2004, IV). The increased infection success of argulids during the second exposure is most likely associated with the maintenance period between the two challenge infections. Handling of fish and confinement periods in the laboratory are known to stress fish (Salonius & Iwama 1993). Activity of fish is decreased after stress situations (Fry 1971) and variation in host behaviour can influence infection rates of ectoparasites (Poulin et al. 1991). Thus it appears that stress during the maintenance and handling reduced the activity of fish, making it easier for *A. coregoni* metanauplii to infect the fish. Indeed, Mikheev et al. (2000) emphasized the importance of fish swimming activity in *A. foliaceus* attachment success. Moreover, argulids do not rely on random encounters with hosts but rather may be described as “micropredators”. They can respond to host-related cues and are swimming actively to find a fish host (Mikheev et al. 2000, III). Naturally, the infection of rather inactive hosts in the vicinity of metanauplii appears to be easier compared to hosts that are changing their position more frequently, leading to the enhanced infection success observed during the second exposure.

The repeated exposure experiment showed that there was no tendency for infected fish to display a similar relative infection on re-exposure (I). This means that the transmission of *A. coregoni* metanauplii was non-selective and that all fish were similarly susceptible to the parasite. Pasternak et al. (2004) found that *A. coregoni* metanauplii seem to be extremely flexible when it comes to infecting a potential host. *Argulus coregoni* is a specific ectoparasite of salmonids but occasionally other host species, for example roach (*Rutilus rutilus*), can be infected, even though growth of the parasite on the alternative host is lower compared to growth on the preferred salmonid fish (Pasternak et al. 2004). The opportunistic and non-selective infection strategy of *A. coregoni* metanauplii might be a mirror of their natural habitat in which the availability of potential hosts can be a very stochastic event (Hakalahti et al. 2004a). The transmission of metanauplii to a fish in close vicinity to emerging sites of *A. coregoni* was highly successful as the experimental exposures showed, where metanauplii had an infection success of approximately 80 % within 1-2 h (I, II). Thus, variation in exposure time and not susceptibility differences among the host individuals seems to be the key factor generating the aggregated distribution of *A. coregoni* on rainbow trout at the fish farm (I).

Compared to the relatively high fish densities at fish farms, the host availability in nature can be rather sparse, which might lead to extended host searching periods after *A. coregoni* metanauplii hatch from their eggs. The survival and transmission success of *A. coregoni* metanauplii deprived of a host for selected times was tested (II). Metanauplii survived a maximum 174 h at 16.5 °C without a host and first mortalities occurred 90 h after hatching. An age-dependent model provided a good fit to the survival data suggesting that all metanauplii carried similar energy resources (Thomas & Ollevier 1993, Cable et al. 2002, Whitfield et al. 2003).

Interestingly, the off-host period of metanauplii had no effect on transmission: metanauplii that were kept off-host over 1-140 h were all equally infectious. However, the effect of the extended off-host period and the associated enhanced energy depletion (Patel et al. 1997) was visible in the parasite growth. The body length of attached *A. coregoni* was negatively correlated with the time spent host-searching. Surprisingly, over time, this correlation diminished and when the parasites reached maturity at an age of 50 days all were of similar size. Analyses of the growth rates revealed that parasites experiencing long off-host periods increased their growth rate compared to argulids that attached to the host soon after hatching.

This so-called compensatory growth has been extensively studied in fish (e.g. Ali et al. 2003) and has also been shown to exist in crustaceans (Arendt 1997, Wu and Dong 2002, Jespersen and Toft 2003). Since *A. coregoni* is a sexually reproducing species, the benefits of compensatory growth might be connected to sexual selection and mate choice. In the animal kingdom larger sized individuals are often preferred by mating partners compared to small ones and have advantages when competing for mates (Reznick 1985, Andersson 1994, Maklakov 2003). Also, a larger size at maturity is linked to higher gamete production and therefore increased fitness, as found in parasitic copepods (Timi et al. 2005). Moreover, the transmission of *A. coregoni* peaks during a relatively short period in spring (Hakalahti and Valtonen 2003) which makes compensatory growth beneficial for argulids to reach an optimal mating size when availability of mating partners is high. Despite the possible benefits of compensatory growth in *A. coregoni*, it can be expected that some costs are involved since preliminary observations in this study showed that accelerated growth had an effect on parasite survival at late developmental stages of the parasite.

### **3.2 Host and mate location mechanisms (III)**

The results of the Y-maze experiments showed that both sexes were attracted to light and fish odour. Free-swimming *A. coregoni* males responded to chemical cues produced by adult females but not vice versa. I analyzed the hierarchy of these stimuli by pitting the cues against each other in the Y-maze and showed

that light was the most salient stimulus for both male and female parasites. Also, male parasites were attracted more strongly towards fish odour than female odour.

The high visual sensitivity of *A. coregoni* is not surprising considering that they possess highly developed compound eyes (Meyer-Rochow et al. 2001). *Argulus coregoni* inhabits well-oxygenated clear running water and it appears that vision plays the leading role for host-finding purposes as an adaptation to their habitat (Mikheev et al. 2004). However, in this study it was also shown that the parasite can detect chemical cues related to host location supporting the results of Mikheev et al. (2000) who noted increased swimming activity of *A. foliaceus* in the presence of fish odour. The ability to detect fish odour might be an auxiliary function to support the primary visual sense and in situations when the light is crepuscular.

Since *A. coregoni* is a dioecious species, the sexes need to recognize each other for reproduction. However, only males were attracted to female odour and not vice versa. That can be explained by a trade-off between being stationary but excreting costly sex pheromones (Kotiahho 2001) and being motile but allocating resources to active mate searching (Proctor 1992, Byers et al. 2005). Previous observations have indicated that activity differences between male and female argulids exist (Pasternak et al. 2000, 2004) and indeed in paper IV it was found that male and female *A. coregoni* pursue different strategies for mate-searching. However, it remains to be studied over which scale pheromones might be detected by male argulids and whether such chemical signals also carry qualitative information about an individual. Even though females did not respond to male odour it is possible that they become more sensitive to mate-related chemical cues at later developmental stages.

In this study mature argulids were of 6 mm length but they have the capacity to reach lengths up to 14 mm (Hakalahti & Valtonen 2003). Thus, the argulids used in the experiments could still grow for some time. Moreover, they were intentionally removed from their hosts to perform the experiments. Therefore it appears plausible that a hierarchy existed in the Y-maze experiment where male parasites reacted stronger towards host related cues; the primary target for off-host males seems to be re-attaching to a host while the location of a mating partner is as yet of secondary importance. The response hierarchy in the Y-maze experiment is supported by the exposure experiment. Free-swimming adult male *A. coregoni* did not preferentially infest fish infected with female parasites over parasite-free fish. However, cues connected to mate finding may become a priority at late adult stages and/or once the parasite has attached to the host.

### 3.3 Host switching (IV)

Rainbow trout were experimentally infected with *A. coregoni* to determine how sex, age and the presence of mating partners influenced the host switching activity of the parasite. Host switching by juvenile and pre-adult parasites of both sexes was low in all experiments. Also host switching by adult females was on a low level; however, adult male *A. coregoni* showed increased host switching activity when females were on a different host and a similar increased host switching pattern when females were absent from the system.

There are several reasons that could explain the detachment of *Argulus* from its host. Antiparasitic behaviour of fish, like rubbing the skin against hard material has been observed (Walker et al. 2004) and might be the main reason for detachment, especially of parasites in the juvenile and pre-adult stage. Another factor that can lead to detachment from the host is the development of an immune response by the fish. However, no effective immune response against *A. coregoni metanauplii* was identified earlier (I) and immune responses of fish against crustacean ectoparasites in general seem to be of little importance (reviewed by Tully & Nolan 2002). Moreover, an immune response of fish could not explain the differences of host switching between the sexes; adult male *A. coregoni* switched the host more actively than adult females.

It appears that the increased host switching by adult male *A. coregoni* was influenced by the absence of mating partners and that males and females pursue different strategies connected to reproduction; adult males represent active mate searchers whereas females remain rather stationary on a host. There is theoretical and experimental evidence for the view that in sexually reproducing species generally only one sex searches actively for a mating partner while the other remains stationary (Hammerstein & Parker 1987, Gibson & Langen 1996, Nishida et al. 1996). Moreover, male *A. coregoni* grew faster during early life stages than females but with the start of sexual maturity this pattern altered and females were much bigger at late adult stages (II). Pasternak et al. 2004 found a similar sexual dimorphic growth pattern in *A. coregoni*. This dynamic sexual size dimorphism might develop because males need to grow fast at early stages to attain maturity quickly. Adult males may allocate much of their energy resources into mate searching and cannot invest as much energy and time into body growth and feeding (Proctor 1992, Byers et al. 2005). Adult females on the other hand would remain stationary on the host and grow at a faster pace than males to reach a larger size which is often closely correlated with fecundity (e.g. Timi et al. 2005).

Adult male *A. coregoni* switched their hosts significantly more in the absence of females from the experimental system but the switching was not influenced when a second host was introduced that carried female parasites. Thus, in combination with the findings of study III it appears that chemical cues emitted by females seem to act on a short scale and not over wide distances, but this needs to be substantiated in more specific experiments.

Besides possible costs of mate searching in terms of body growth, there is a direct predation risk involved. A substantial amount of free-swimming parasites in the exposure experiment (22%) had been eaten by the fish.

### **3.4 The link between ectoparasites and bacterial disease in fish (V)**

Although several studies have emphasized the possible role of parasites in enhancing infections of fish with secondary bacterial pathogens (e.g. Cusack and Cone 1986, Busch et al. 2003, Gustafson et al. 2005) there is only scarce empirical support for this hypothesis (Pylkkö et al., 2006). In this study, the mortality of fish challenged with *A. coregoni* was negligible while infection with *F. columnare* alone led to significantly lower survival. Compared to single infections, the mortality was significantly higher and the onset of columnaris disease was earlier among fish which were concomitantly infected by *A. coregoni* and *F. columnare*. Dead fish showed skin necrosis around the tail and dorsal fin typical for columnaris disease (Bader et al. 2003) and the PCR analyses confirmed that fish were infected by *F. columnare*.

Hence, moderate infections with *A. coregoni* as in this work (7 adult parasites/fish) have no direct effect on fish mortality but they can be lethal in combination with other pathogens like *F. columnare*. This interaction is important for aquaculture considering that the co-occurrence of bacterial and parasitic diseases is the rule rather than the exception (Rintamäki-Kinnunen 1997, Buchmann & Bresciani 1997). However, the mechanism through which *A. coregoni* enhances the invasion of bacteria into fish remains to be studied. The argulids might have opened adherence sites for the bacterium due to the damage inflicted on the skin and mucus (Madetoja et al. 2000, Bader et al. 2003). On the other hand, the argulids might have induced stress (van der Salm et al. 2000) making fish indirectly more prone to *F. columnare* infection due to decreased immunocompetence (Salonius and Iwama 1993, Engelsma et al. 2003).



## 4 CONCLUSIONS

In this thesis I studied various aspects of the biology of the fish louse *A. coregoni* including transmission (I, II), host and mate location (III, IV) and the link with secondary diseases in fish (V). Under aquaculture conditions *A. coregoni* was aggregated amongst the host population (I) and this is in line with almost all parasitic species (Shaw & Dobson 1995, Poulin 1998, Shaw et al. 1998, Poulin 2007). The key factor leading to aggregation of argulids seemed to be the exposure time of fish to the parasite (I). However, as shown in the fourth paper, *A. coregoni* is relatively mobile and especially adult males switch their host frequently. Therefore it can be expected that the distribution pattern of argulids is highly dynamic.

In the laboratory the transmission of *A. coregoni* metanauplii was highly successful (I, II) but they may experience prolonged host searching periods especially in nature due to scarcity of fish leading to heterogeneities among parasite sizes. It was shown that a catch-up growth mechanism in *A. coregoni* exists that allowed parasites that experienced extended off-host periods to compensate their initial small size. Such compensatory growth was previously only known to exist in free-living species (e.g. Arendt 1997, Wu & Dong 2002, Jespersen & Toft 2003). Catch-up growth in *A. coregoni* may have developed for reasons connected to sexual selection and to optimize mating size. However, delayed costs due to catch up growth might be visible in subsequent performance like survival (II) and fecundity (Metcalf & Monaghan 2001).

The compensatory growth of *A. coregoni* due to prolonged transmission periods (II) can be described as a state-dependent response (Thomas et al. 2002) which refers to the ability of an organism to react to fluctuations in the environment through phenotypic plasticity. The compensatory growth response (II) and the hierarchical response of argulids, first to host related and then to mate related chemical cues (III) indicate that this parasite indeed perceives a large set of environmental parameters. This illustrates the high level of sophistication of argulids in reacting to heterogeneities in the environment with the ultimate aim of increasing their fitness.

The differences of host switching between male and female argulids (IV), the sexual size dimorphism (II) and the ability of males to detect chemical cues of females (III) can be explained in evolutionary terms. Evolution can select for small sized males to increase mobility which enhances the probability to locate females (Berry & Shine 1980). On the other hand, evolution might select for increased female size to increase fecundity, especially since it is expected that parasites have a relatively low probability of transmission (Poulin 1996). Relatively complex mating behaviour, including mate guarding and mate choice, is known to exist in some crustacean parasites (Anstensrud 1990, 1992, Todd et al. 2005) and it would be worthwhile to investigate further sexual selection in *A. coregoni*.

Like the findings in paper IV, host switching ability was also noted in other fish ectoparasites like *Lepeoptheirus salmonis* (Ritchie 1997, Hull et al. 1998) and *Caligus elongatus* (Pike 1989, Stuart 1990). Apart from this thesis (IV) there are no specific studies that have tried to elucidate why the inter-host transfer of lice between fish seems to be more widespread than previously thought. However, Jaworski & Holm (1992) found that male *L. salmonis* are more active than females and postulated that host switching could be stimulated by the absence of receptive females on the host. Pasternak et al. (2004) recorded increased activity of *A. coregoni* on roach (*Rutilus rutilus*) which is considered a potential but not optimal host for *A. coregoni* indicating that host specificity issues might also be involved in host switching activity of argulids. The implications of host switching on the spread of fish lice between cages in aquacultures might be severe, especially since it was shown that ectoparasites can enhance the susceptibility of fish to bacterial pathogens (V).

In this work I studied the interaction of ectoparasites and bacteria and their combined effect on fish (V). The direct effect of argulids on host mortality was negligible but when *F. columnare* was introduced as a second stressor the negative impact of the parasite was visible as enhanced fish mortality. However, even though *A. coregoni* alone had no influence on fish mortality it is possible that other host parameters like growth have been negatively affected (Ruane et al. 1999). Studies that concentrate on more than just one parasitic species and the effect on its host are rare due to inherent practical and theoretical complications. However, in nature, outside the controlled environment of a laboratory, a broad variety of parasitic species is acting in concert on the shared hosts (Buchmann & Bresciani 1997, Rintamäki-Kinnunen 1997).

As shown in paper IV argulids and especially adult males are able to switch between host individuals. Due to host switching *A. coregoni* might negatively affect a large part of a fish population by inducing pathogenic diseases. Argulids might work as vectors of bacteria between fish since it is known that they can spread the virus causing spring viraemia (Pfeil-Putzien 1977, Ahne 1985). Nematodes parasitic on fish have been found in the haemocoel of argulids indicating that argulids might even act as intermediate hosts of other parasites (Molnár & Székely 1998, Moravec et al. 1999). For future studies it will be fruitful to investigate further the vector abilities of *A. coregoni*,

particularly if attempts are made that include a representative parasitic fauna of the host.

Studies like those in this thesis can be of great economic and environmental importance if the knowledge gained is appropriately processed. Fish farms suffer from losses due to the invasion of pathogens (e.g. Wootten et al. 1982, Dalsgaard & Madsen 2000, Costello 2006). Integrated pest management strategies that combine the power of chemical medication (Stone et al. 2000, Shinn et al. 2003, Hakalahti et al. 2004b) with knowledge of the biology of the pathogens (Gault et al. 2002, Hakalahti et al. 2004c) can substantially decrease the impact of pathogens with less negative impact on the environment. In fact, better understanding of the biology of *A. coregoni* has led to the construction of a population model (Fenton et al. 2006) which is the first step to sophisticated alternative *Argulus* control at fish farms.

## *Acknowledgements*

I would like to thank my supervisor Professor E. Tellervo Valtonen for guidance and the possibility to benefit from her wealth of experience that helped me to pursue these studies. Warmest thanks are due to my colleague and later on supervisor Dr. Teija Hakalahti-Sirén who shared with me hundreds of hours in the laboratory and could answer so many questions that I had concerning this PhD thesis.

I would like to acknowledge my co-authors Lotta-Riina Suomalainen for introducing me into the world of DNA analyses, Pedro Aphalo for essential statistical help, Peter Hudson for many helpful comments and ideas concerning the first manuscript and Päivi Rintamäki-Kinnunen for providing bacterial strains needed in this thesis. Roger Jones and Dan Benesh kindly helped whenever I had questions regarding the English.

I am thankful to Savon Taimen OY and Yriö Lankinen for supplying me with fish material and the easy co-operation throughout the last years. Heli Häkkinen helped during field and experimental work in the first paper. The laboratory work has been performed at the Konnevesi Research station and I wish to thank the staff especially Janne Koskinen, Jyrki Raatikainen and Risto Latvanen for arranging the experimental set ups and for taking care of the fish when I was not there. I am grateful to Lenka Trebatická for taking care of the parasites eggs when needed and for valuable discussions concerning the manuscripts. There are several people that accompanied me throughout my time as a PhD student and that helped me through some difficult periods; especially I would like to thank Christian, Jonna, Riikka and Tuomas.

I am indebted to my parents and sisters for all their support and encouragement throughout this whole project. Maila and Vihtori Huikuri helped me to adjust and settle into Finland which was essential to make this project successful. Above all, I am grateful to Suvianna for all her love during these years. Without you this thesis would have never been possible.

This work was funded by the University of Jyväskylä (Rector's grant), the Graduate School of Biological Interactions (University of Turku) and the Academy of Finland (SUNARE project). I also got financial support from the Ellen & Arturi Nyyssönen foundation.

## YHTEENVETO (RÉSUMÉ IN FINNISH)

### *Argulus coregoni* -kalatäin siirtyminen kalaisäntään, isännän ja parittelukumppanin paikallistaminen sekä loisinnan yhteys kalan bakteeritautiin

Loiset saavat suojan ja energian toiselta elävältä eliöltä, jota kutsutaan loisen isännäksi. Evolutiivisessa mielessä toisen eliön resurssien hyväksikäyttö on ollut erittäin menestyksenkäs strategia: on arvioitu, että ainakin puolet kaikista eliöistä on elintavaltaan täysin riippuvaisia loisinnasta tai jokin niiden elinvaihe on loisiva. Loistutkimuksen alkuaikoina loisten ajateltiin olevan alkeellisia eliöitä, yksinkertaisia lisääntymiskoneita, ja vasta viime vuosina loisilla tehdyt käyttäytymiskokeet ovat yleistyneet. Monet loiseliöt tuottavat suuria taloudellisia tappioita kaupallisissa eläinkasvattamoissa, joten niiden käyttäytymisen ja ekologian tunteminen tuo suoraa käytännön hyötyjä niiden torjuntaan. Loisinta vaikuttaa isäntiin haitallisesti, joko suorasti tai epäsuorasti. Loisen voi vaikuttaa isännän kasvuun, lisääntymiseen tai esimerkiksi altistaa isännän sekundaarisille bakteeri-, sieni- tai virusinfektioille. Isäntiä vaihtavat loiset voivat myös toimia tautien siirtäjinä isäntäpopulaatioissa.

Tämän väitöskirjatutkimuksen tutkimuslaji on *Argulus coregoni* -kalatäi. *Argulus coregoni* on pääasiassa lohikaloilla esiintyvä äyriäispintaloinen, jonka levinneisyys ulottuu koko borealiselle vyöhykkeelle. Kalatäin elinkierto on suora ja alkaa keväällä kun veden lämpötila ylittää +10 °C. Kalatäin metanauplius-toukat (pituus ~0.7 mm) kuoriutuvat vesistöjen pohjakivien pinnoille munituista munista, ja ne etsivät kalaisäntää aktiivisesti. Kalaan kiinnittymisen jälkeen ne läpikäyvät yhdeksän kehitysvaihetta, kunnes saavuttavat aikuisuuden noin 4 mm pituisina. Kasvunopeus riippuu suoraan veden lämpötilasta, mutta Suomen lämpötiloissa aikuistuminen saavutetaan noin 4-6 viikon kuluessa. *Argulus coregoni* voi kasvaa noin 14 mm pituiseksi. Kalatäi on sopeutunut elämään kalan pinnalla: sen ruumiin pinnalla on erilaisia väkäsiä, koukkuja ja kaksi voimasta imukuppia, joiden avulla se liikkuu nopeasti kalan pinnalla. Kalatäi syö kalan verta ja kudoksia läpäisevän imukärsän ja suuosien avulla. Sukukypsyyden saavuttamisen jälkeen kalatäikoiraat etsivät naaraan ja parittelevat yleensä kalan pinnalla. Naaraat irrottautuvat kalasta ja munivat keskimäärin 300 munaa. Kalojen pinnoilla loisivat *A. coregoni* kalatäit kuolevat avovesikauden loppuun mennessä, ja laji talvehtii ainoastaan munina. Suomessa *A. coregoni* -lajilla on yksi sukupolvi vuoden aikana. Aikaisempien tutkimustulosten perusteella toinen loissukupolvi voi olla mahdollinen myös Suomessa, jos ilmasto lämpenee. Tämä johtaisi väistämättä lisääntyneisiin kalatäiongelmiin kalankasvatuksessa.

Tässä väitöskirjassa tutkin *A. coregoni* -lajin ekologiaa, erityisesti sen isäntään siirtymisvaiheessa ja parittelukumppanin etsimisvaiheessa. Tutkin myös kalatäiloisinnan vaikutusta bakteeritaudille altistavana tekijänä kalalle. Osajulkaisuissa I ja II selvitin *A. coregoni* kalatäin munista kuoriutuneiden metanaupliusien siirtymistä kirjolohiin. Aluksi selvitin loisinnan voimakkuuden jakaumaa kirjolohien (*Oncorhynchus mykiss*) kesken kalanviljelylaitoksella (I). *Ar-*

*gulus coregoni* metanaupliukset ja poikasvaiheet olivat jakautuneet kalojen kesken epätasaisesti siten, että pieni osa kalayksilöistä kantoi suurimman osan loistaakasta. Tällainen aggregoitunut loisten jakauma isäntäpopulaatiossa on hyvin usein havaittu ilmiö loistutkimuksissa. Selvitin laboratorioskokeilla syytä epätasaiseen jakaumaan esittäen kysymyksen: oliko *A. coregoni* kalatäin aggregoitunut jakauma kalayksilöiden välillä seuraus kalayksilöiden alttiuseroista loisinnalle vai eroista altistumisajoissa? Tutkimustulokseni tukevat näkemystä, että erot kalojen altistumisessa loisinnalle on tärkeämpi tekijä aggregoituneen jakauman taustalla kuin erot kalojen alttiudessa loisinnalle.

Osajulkaisussa II selvitin, kuinka kauan *A. coregoni* metanauplius-toukat selviytyvät ilman kalaa ja miten isännän paikallistamiseen käytetty aika vaikuttaa loisen kiinnittymismenestykseen. Tutkin myös, kuinka viivästynyt isäntään kiinnittyminen vaikuttaa kalatäin myöhempään kehitykseen ja selviytymiseen. Tulosteni perusteella *A. coregoni* -kalatäin isäntään siirtyminen on tehokasta, koska noin 80% loisista kiinnittyi kaloihin 1-2 tunnin sisällä. Täiden elinaika ilman isäntää oli noin viikko. Pidemmät isännänetsimisajat eivät vaikuttaneet haitallisesti kalatäin kiinnittymismenestykseen, mutta negatiivinen vaikutus havaittiin myöhemmissä kehitysvaiheissa. Kalatäin kasvunopeuden ja isännänetsimisajan välillä havaittiin negatiivinen korrelaatio. Kuitenkin ne kalatäit, jotka kasvoivat alussa hitaimmin, ottivat kasvussa kiinni alussa nopeimmin kasvaneet yksilöt. Sukukypsät kalatäit olivat samankokoisia riippumatta isännänetsimiseen käytetystä ajasta. Havaittua kompensatiokasvua ei ole aikaisemmin kuvattu loisilla. Kompensatiokasvu oli kustannus kalatäille, koska isännänetsimisajan pituus korreloi negatiivisesti poikasvaiheiden selviytymisen kanssa kaloissa.

Osajulkaisuissa III ja IV tutkin *A. coregoni* -kalatäin isännän ja parittelukumppanin paikallistamista. Valintakokeiden avulla (Y-käytävä) testasin reagoivatko kalatäit kaloista ja toisista kalatäiyksilöistä erityyppisiin kemiallisiin signaaleihin ja valon lähteeseen, jota käytettiin matkimaan lohikalojen ruumiin tuottamia valonheijastuksia (III). Tässä tutkimuksessa selvitin myös näiden signaalien välistä hierarkiaa. Osajulkaisussa IV seurasin kalatäikoiraiden ja -naaraiden isännänvaihtoa loisen eri kehitysvaiheissa. Tulokseni osoittivat, että visuaaliset signaalit ovat *A. coregoni* kalatäille tärkeämpiä kuin kemialliset signaalit isännän paikallistamisessa. Kalatäit kuitenkin reagoivat myös kalan hajuun. Kalatäikoiraat reagoivat myös kalatäinaaraiden tuottamaan hajuun, kun taas kalatäinaaraat eivät reagoineet toisten loisyksilöiden tuottamaan hajuun. Näiden tulosten perusteella kalatäikoirailla ja -naarailla on erilaiset lisääntymisstrategiat: koiraat toimivat aktiivisina parittelukumppanin etsijöinä ja naaraat ovat passiivisempia pysytellen kalan pinnalla. Kalatäillä tehdyt isännänvaihtamiskokeet tukivat tätä näkemystä, koska sukukypsät koiraat vaihtoivat isäntää useammin kuin naaraat. Kalatäiden irrottautuminen kaloista ja isännänvaihto ennen sukukypsyyden saavuttamista oli harvinaista.

Osajulkaisussa V tutkin kalatäi- ja bakteeri-infektion välistä vuorovaikutusta kirjolohella. Selvitin kokeellisesti, lisääkö *A. coregoni* -loisinta kalan alttiutta *Flavobacterium columnare* infektiolle. *F. columnare* on tällä hetkellä yksi ongelmallisimmista bakteeritaudeista Suomalaisessa kalanviljelyssä. Taudin tyypilli-

set oireet ovat kuoliot ja syöpyvät kalan iholla, ja tauti johtaa usein kalan kuolemaan. Tulosteni mukaan *A. coregoni* infektiota (7 aikuista/kala) ei vaikuta kalojen kuolleisuuteen, mutta yhdessä *F. columnare* altistuksen kanssa se voi olla tappava. Loiselle ja bakteerille yhteisaltistettujen kalojen kuolleisuus oli korkeampi kuin pelkästään bakteerille altistettujen kalojen kuolleisuus. Tämä on ensimmäinen kokeellinen tutkimus, joka osoittaa selvän yhteyden kalan pintaloisen ja bakteeritaudin puhkeamisen välillä. Vastaavien vuorovaikutusten ymmärtäminen olisi tärkeää vesiviljelyn kannalta, koska erilaisten mikrobien aiheuttaminen tautien ja loisten yhteisesiintymisen kalaviljelylaitoksilla on ennemminkin sääntö kuin poikkeus.

Tämä väitöskirjatyö on auttanut eräiden loistutkimuksissa tyypillisten ilmiöiden ymmärtämisessä (loisten aggregoituminen; julkaisu I), mutta myös tuonut esiin uusia mielenkiintoisia ilmiöitä, joiden tutkiminen loisilla on ollut puutteellista (kompensaatiokasvu, parittelukumppanin etsiminen, loisten ja infektiotautien välinen yhteys; julkaisu II-V). Tämän väitöskirjatutkimuksen kaltainen tutkimustyö on tärkeää sekä taloudellisesti että ympäristön kannalta. Loiset ja erilaiset taudinaiheuttajat aiheuttavat huomattavia tappioita kalaviljelyelinkeinoille. Torjuntastrategiat, joissa yhdistetään tehokkaiden kemiallisten torjunta-aineiden käyttöä ja loiseläiden biologian tuntemista, voivat vähentää tappioita ja ympäristövaikutuksia. Itse asiassa *A. coregoni* kalatäin biologian tutkimus on johtanut lois-isäntäpopulaatiomallin kehittämiseen, joka on ensimmäinen askel kestävän kalatäitorjunnan kehittämiseksi kalaviljelylaitoksilla.

## ZUSAMMENFASSUNG (RÉSUMÉ IN GERMAN)

### **Die Fischlaus *Argulus coregoni*: Transmissionsaspekte, Wirts- & Partnersuche und die Verbindung mit bakteriellen Infektionen in Fischen**

Die Ausbeutung von Ressourcen anderer Lebewesen ist in der Evolution eine höchst erfolgreiche Strategie: Schätzungen haben ergeben, dass mindestens die Hälfte aller Organismen obligatorische Parasiten sind bzw. einen Teil ihrer Entwicklung in einem parasitären Lebensstil vollziehen. In der Vergangenheit wurden Parasiten generell als rückständige und unterentwickelte Organismen angesehen und als einfache Reproduktionsmaschinen dargestellt. Das führte dazu, dass bis zum jetzigen Zeitpunkt verhaltensbiologische Untersuchungen von Parasiten relativ selten sind. Zusätzlich zu den Herausforderungen an ein parasitäres Leben, wie z.B. eine immunologische Abwehrreaktion des Wirts oder antiparasitäre Verhaltensweisen, sind Parasiten mit Problemen konfrontiert, die oft nur für frei lebende Lebewesen in Betracht genommen werden. Ein Beispiel ist die Partnersuche bei geschlechtlichen Parasiten. Das Verstehen parasitären Verhaltens kann dazu beitragen, die Ausbreitung von Parasiten in der Nutztierhaltung zu mindern, allzumal Parasiten der Herd für sekundäre Infektionen von Pilzen, Bakterien oder Viren sein können.

In dieser Dissertation habe ich mich mit der Fischlaus *Argulus coregoni* (Crustacea: Branchiura) beschäftigt, die in der Vergangenheit vor allem den Bestand von gezüchtete Fischen in intensiven Aquakulturen beeinträchtigt hat. *Argulus coregoni* ist ein Ektoparasit vorzugsweise von lachsartigen Fischen mit einem Verbreitungsgebiet, das sich über Süßwasserhabitate der kühl gemäßigten Breitengrade erstreckt. Der direkte Lebenszyklus von *A. Coregoni* wird durch das Schlüpfen von kleinen larvenartigen Metanauplii (~ 0.7 mm) aus Eigelegen am Gewässergrund eingeleitet, wenn die Wassertemperatur im Frühjahr über 10 °C steigt. Die Metanauplii sind umgehend infektiös und suchen aktiv schwimmend nach einem geeigneten Fischwirt. Nach Anheftung an die Fischhaut werden je nach Wassertemperatur in 4-6 Wochen neun Häutungsstadien durchlaufen und der Parasit erreicht die adulte Form mit einer Länge von ca. 4 mm. Während des fortlaufenden Wachstums können Maximallängen von bis zu 14 mm erreicht werden. Zahlreiche Klammerhaken und ein Paar Saugnäpfe an der Unterseite des Körpers (Carapax) ermöglichen es *A. Coregoni*, sich dauerhaft am Fisch anzuheften und gleichzeitig über die Haut zu gleiten. Mit Hilfe eines schlanken Stachels durchbricht der Parasit die Fischepidermis und ernährt sich von Blut und/oder verschiedenen Hautpartikeln. Fischhaut- und mukus werden durch die Nahrungsaufnahme, aber auch durch die Haftungsorgane von *A. Coregoni* geschädigt. Adulte männliche und weibliche Arguliden müssen sich zur Paarung lokalisieren, die gewöhnlich auf dem Fischkörper vollzogen wird, obwohl auch Paarungen abseits des Wirts beobachtet wurden. Trächtige Weibchen positionieren Gelege von ca. 300 Eiern vorzugsweise auf Steinen am Gewässergrund und sterben gewöhnlicherweise nach der Eiablage im Spätsommer oder Herbst. Nur die



abgelegten Eier von *A. coregoni* überleben den Winter in Finnland; alle geschlüpften Arguliden sterben. Nach dem Überwintern geht eine neue Generation von *A. coregoni* aus den Eigelegenen hervor. Gegenwärtig existiert nur eine Generation von *A. coregoni* in finnischen Gewässern, jedoch können erhöhte Temperaturen aufgrund der globalen Erwärmung eine zweite Argulidengeneration in Finnland hervorrufen, was den Parasitendruck auf wilde Fischpopulationen und vor allem auf Fischzuchtbetriebe erheblich erhöhen kann.

Der Fokus dieser Dissertation lag auf Aspekten der Transmission und der Wirts- und Partnersuche von *A. coregoni* sowie der Interaktion dieser Fischlaus mit bakteriellen Sekundärinfektionen in Fischen. Die Transmission, d.h. der Vorgang der Infektion eines Wirts, ist ein kritischer Parameter der die Fitness eines Parasiten stark beeinflusst, was zur Folge hatte, dass die Transmission starkes Interesse in der Wissenschaft geweckt hat. In den Veröffentlichungen I und II habe ich die Transmission von frisch geschlüpften *A. coregoni* Metanauplii auf Regenbogenforellen (*Oncorhynchus mykiss*) untersucht. Im Besonderen habe ich geprüft, ob *A. coregoni* Metanauplii in einer Fischzuchtanstalt in solcher Weise übertragen werden, dass eine aggregierte Verteilung des Parasiten innerhalb der Fischpopulation generiert wird. Eine aggregierte Verteilung ist typisch für fast alle Parasiten und bedeutet, dass ein relative kleiner Teil der Wirtspopulation von einer überproportional großen Masse der jeweiligen Parasitenart infiziert ist. Der größte Teil der Wirtsindividuen wird von einem speziellen Parasiten also nur relativ gering parasitiert. Die in der Fischzuchtanstalt erhobenen zeigten, dass auch im Fall von *A. coregoni* eine aggregierte Verteilung vorlag (I). Auf der Grundlage der aggregierten Verteilung von Arguliden in der Fischzuchtanstalt entstand eine weitere Frage: Ist die Verteilung von *A. coregoni* innerhalb einer Gruppe von Regenbogenforellen die Konsequenz von unterschiedlichen Anfälligkeiten zwischen den Fischen, oder ist eher die Exposition bzw. die Dauer der Aussetzung der treibende Faktor? Durch experimentelle Infektionsversuche im Labor konnte ich zeigen, dass nicht unterschiedliche Anfälligkeiten individueller Fische oder die Entwicklung einer speziellen Immunreaktion, sondern eher die Aussetzungsdauer der bestimmende Faktor für die aggregierte Verteilung von *A. coregoni* innerhalb der Fischpopulation in der Fischzuchtanstalt war.

In Veröffentlichung II wurde untersucht wie lange infektiöse *A. coregoni* Metanauplii ohne einen Wirt überleben können und wie der Transmissionserfolg von Metanauplii durch eine verlängerte Wirtsabwesenheit beeinflusst wird. Darüber hinaus habe ich das Wachstum von *A. coregoni* auf dem Wirt verfolgt und untersucht wie der Nachteil einer verlängerten Wirtsabwesenheit während des Metanuplius Stadiums die nachfolgende Entwicklung des Parasiten beeinflusst. Der Transmissionserfolg der Metanauplii im Labor war relativ hoch, denn ca. 80 % setzten sich an einem Wirt innerhalb von 1-2 Std. fest. Verlängerte Wirtssuchzeiten durch experimentelles Zurückhalten der Wirtsfische verminderte überraschenderweise nicht die Infektivität der Metanauplii, jedoch wurde das

Handikap längerer Wirtsabwesenheit in der frühen Entwicklung sichtbar. Arguliden mit längeren Transmissionszeiten waren von kürzerer Körperlänge, verglichen mit Parasiten die ein Wirtstier schneller infizieren konnten. Erstaunlicherweise wurde dieser Längenunterschied von den benachteiligten Parasiten mit fortschreitender Entwicklung durch deutlich erhöhte Wachstumsraten kompensiert. Da sich *A. coregoni* geschlechtlich fortpflanzt, könnte ein so genanntes „Kompensationswachstum“ vorteilhaft im Hinblick auf optimale Fortpflanzungsgröße, gesteigerte Fruchtbarkeit und sexuelle Selektion sein.

Die Untersuchungen in den Manuskripten III und IV sollten Aufschlüsse über Aspekte der Partner- und Wirtssuche von *A. coregoni* geben. In so genannten binären „Y-Auswahltests“ konnten einzelne schwimmende Arguliden ihre Schwimmrichtung zwischen links oder rechts auswählen (III). In diesen Tests habe ich die Reaktion von erwachsenen Arguliden zu potentiellen chemischen und visuellen Signalen von Wirts- bzw. Partnerorganismen untersucht. Außerdem wurde geprüft ob eine Hierarchie innerhalb dieser Signale existiert. Die Untersuchungen zeigten, dass visuelle Signale, die in Verbindung mit Lichtreflexionen von den Fischschuppen stehen, am wichtigsten für die Wirtsfindung von *A. coregoni* sind. Die Arguliden waren aber auch für Fischgerüche empfänglich, die als unterstützendes Signal zum Auffinden von einem geeigneten Wirt benutzt werden könnten. Männliche *A. coregoni* reagierten positiv auf chemische Signale von Weibchen, aber die Weibchen zeigten keine Anzeichen für die Wahrnehmung von männlichen Signalen chemischer Natur. Diese Resultate deuten an, dass zwei verschiedene Reproduktionsstrategien existieren, wobei männliche *A. coregoni* aktive Partnersucher sind, während die Weibchen ihre Energie eher in Körperwachstum investieren und dadurch eine erhöhte Fruchtbarkeit erlangen. Die Wirtswechsel-Experimente in Manuskript IV erhärteten diese Hypothese, denn die männlichen Fischläuse waren sehr aktiv in Bezug auf einen Wirtswechsel, wenn keine potenziellen Fortpflanzungspartner auf dem gleichen Fisch vorzufinden waren. Weibliche Fischläuse waren relativ passiv beim Wirtswechsel und blieben stationär auf einem Fisch, auch wenn keine Partner zur Verfügung standen.

In Veröffentlichung V habe ich die Wechselbeziehung zwischen Parasiten und Bakterien in Fischen untersucht. Obwohl in der Literatur die Hypothese von erhöhter Anfälligkeit von parasitierten Fischen für bakterielle Infektionen wiederholt gestellt worden ist, konnte dies nicht eindeutig bewiesen werden. In meinen Studien habe ich geprüft ob Regenbogenforellen, die von *A. coregoni* parasitiert werden, eine erhöhte Anfälligkeit für die „Columnaris-Krankheit“ zeigen, die durch das Bakterium *Flavobacterium columnare* hervorgerufen wird. Es handelt sich dabei um einen äußerst problematischen Krankheitserreger innerhalb der Fischzuchtindustrie. Charakteristische Anzeichen der „Columnaris-Krankheit“ sind das Absterben und die Erosion von Hautzellen vor allem im Bereich der Rücken- und Schwanzflosse, was in drastischen Fällen sogar zum Zelltod bis hinein ins Rückgrat führen kann. Letztendlich führen diese Verletzungen mit den verbundenen osmotischen und

elektrolytischen Komplikationen zum Tod des infizierten Fisches. Die Resultate meiner Versuche konnten eindeutig belegen, dass eine moderate Infektion mit *A. coregoni* allein (~ 7 erwachsene Arguliden/Fisch) nicht direkt zum Tod der Regenbogenforellen führte, aber eine erhöhte Mortalität konnte verzeichnet werden, als die Argulus Infektion mit einer *F. columnare* Infektion gekoppelt wurde. Im Hinblick auf die intensive Produktion von Fischen in Zuchtanstalten erscheint dieses Ergebnis sehr wichtig, denn in der Regel besteht eine große Diversität von bakteriellen und parasitären Krankheitserregern in Zuchtgewässern.

Die Resultate dieser Dissertation konnten dazu beitragen, einige typische Merkmale im Bereich der Parasitologie zu verstehen (Veröffentlichung I). Darüber hinaus führten die Ergebnisse der Veröffentlichungen II-V zu neuen Erkenntnissen von Ektoparasiten, die bisher unverständlich bzw. unbekannt waren. Studien wie in dieser Dissertation können von enormer wirtschaftlicher und umweltpolitischer Bedeutung sein, wenn die neuen Erkenntnisse adäquat angewandt werden. Die intensive Produktion in Fischzuchtanstalten leidet an Verlusten, die hervorgerufen wird durch die Invasion von verschiedenartigen Krankheitserregern. Eine integrierte Strategie, welche die Effizienz von chemischen Applikationen und das Fachwissen über die Biologie der Krankheitserreger kombiniert, kann die Auswirkungen von Krankheiten in der Fischzucht drastisch reduzieren, ohne die Umwelt zu stark zu belasten. In der Tat konnten Erkenntnisse über die Biologie von *A. coregoni* dazu beitragen, ein Populationsmodell dieser Fischlaus zu erstellen, was der erste Schritt in Richtung einer alternativen Parasitenbekämpfungsmethode ist.

## REFERENCES

- Ahne, W. 1985. *Argulus foliaceus* L. and *Piscicola geometra* L. as mechanical vectors of spring viraemia of carp virus (SVCV). *J. Fish Dis.* 8: 241-242.
- Ali, M., Nieceza, A. & Wootton, R. J. 2003. Compensatory growth in fishes: a response to growth depression. *Fish Fish.* 4: 147-190.
- Anderson, R. M. 1974. An analysis of the influence of host morphometric features on the population dynamics of *Diplozoon paradoxum* (Nordman, 1832). *J. Anim. Ecol.* 43: 873-887.
- Anderson, R. M. & May, R. M. 1991. *Infectious disease of humans: dynamics and control.* 757 p., Oxford University Press, Oxford.
- Anderson, R. M. & Whitfield, P. J. 1975. Survival characteristics of the free-living cercarial population of the ectoparasitic digenean *Transversotrema patialense* (Soparker, 1924). *Parasitology* 70: 295-310.
- Anderson, R. M., Whitfield, P. J. & Dobson, A. P. 1978. Experimental studies of infection dynamics: infection of the definitive host by the cercariae of *Transversotrema patialense*. *Parasitology* 77: 189-200.
- Andersson, M. 1994. *Sexual selection.* 624 p., Princeton University Press, Princeton, New Jersey.
- Anstensrud, M. 1989. A vital stain for studies of behaviour and ecology of the parasitic copepod *Lernaeocera branchialis* (Pennellidae). *Mar. Ecol. Prog. Ser.* 53: 47-50.
- Anstensrud, M. 1990. Moulting and mating in *Lepeoptheirus pectoralis* (Copepoda, Caligidae). *J. Mar. Biol. Ass. U. K.* 70: 269-281.
- Anstensrud, M. 1992. Mate guarding and mate choice in two copepods, *Lernaeocera Branchialis* (L.) (Pennellidae) and *Lepeoptheirus pectoralis* (Müller) (Caligidae), parasitic on flounder. *J. Crust. Biol.* 12: 31-40.
- Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. *Q. Rev. Biol.* 2: 149-177.
- Bader, J. A., Nusbaum, K. E. & Shoemaker, C. A. 2003. Comparative challenge model of *Flavobacterium columnare* using abraded and unabraded channel catfish, *Ictalurus punctatus* (Rafinesque). *J. Fish Dis.* 26: 461-467.
- Bakke, T. A. & Harris, T. A. 1998. Diseases and parasites in wild Atlantic salmon (*Salmo salar*) populations. *Can. J. Fish. Aquat. Sci.* 55 (Suppl. 1): 247-266.
- Berry, J. F. & Shine, R. 1980. Sexual size dimorphism and sexual selection in turtles (order testudines). *Oecologia* 44: 185-191.
- Bower-Shore, C. 1940. An investigation of the common fish louse, *Argulus foliaceus* (Linn.). *Parasitology* 32: 361-371.
- Bron, J. E., Sommerville, C. Wootton, R. & Rae, G. H. 1993. Following of marine Atlantic salmon, *Salmo salar* L., farms as a method for the control of sea lice, *Lepeoptheirus salmonis* (Kroyer, 1837). *J. Fish Dis.* 16: 487-493.
- Buchmann, K. & Bresciani, J. 1997. Parasitic infections in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. *Dis. Aquat. Org.* 28: 125-138.

- Busch, S., Dalsgaard, I. & Buchmann, K. 2003. Concomitant exposure of rainbow trout fry to *Gyrodactylus derjavini* and *Flavobacterium psychrophilum*: effects on infection and mortality of host. *Vet. Parasitol.* 117: 117-122.
- Byers, J., Wiseman, P., Jones, L. & Roffe, T. 2005. A large cost of female mate sampling in pronghorn. *Am. Nat.* 166: 661-668.
- Cable, J., Tinsley, R. C. & Harris, P. D. 2002. Survival, feeding and embryo development of *Gyrodactylus gasterostei* (Monogenea: Gyrodactylidae). *Parasitology* 124: 53-68.
- Claerebout, E. & Vercruyse, J. 2000. The immune response and the evaluation of acquired immunity against gastrointestinal nematodes in cattle: a review. *Parasitology* 120 (suppl.): S25-S42.
- Costello, M. J. 2006. Ecology of sea lice parasitic on farmed and wild fish. *Trends Parasitol.* 22: 475-483.
- Cressey, R. F. 1983. Crustaceans as parasites of other organisms. In: Provenzano, A. J. Jr. (ed.), *The biology of crustacea*. Vol. 6: 251-273. Academic Press, New York.
- Cross, D. G. & Stott, B. 1974. The effect of *Argulus foliaceus* L. on the growth and mortality of a grass carp population. *J. Inst. Fish. Manag.* 5: 39-42.
- Cusack, R., Cone, D. K. 1986. A review of parasites as vectors of viral and bacterial diseases of fish. *J. Fish Dis.* 9: 169-171.
- Dalgaard, M. B., Nielsen, C. V. & Buchmann, K. 2003. Comparative susceptibility of two races of *Salmo salar* (Baltic Lule river and Atlantic Conon river strains) to infection with *Gyrodactylus salaris*. *Dis. Aquat. Org.* 53: 173-176.
- Dalsgaard, I. & Madsen, L. 2000. Bacterial pathogens in rainbow trout, *Oncorhynchus mykiss* (Walbaum), reared at Danish freshwater farms. *J. Fish Dis.* 23: 199-209.
- Dawkins, R. & Krebs, J. R. 1979. Arms races between and within species. *Proc. R. Soc. Lond. B* 205: 489-511.
- Day, T. 2003. Virulence evolution and the timing of disease life-history events. *Trends Ecol. Evol.* 18: 113-118.
- Decostere, A., Haesebrouk, F., Charlier, G. & Ducatelle, R. 1999. Characterization of four *Flavobacterium columnare* (*Flexibacter columnaris*) strains isolated from tropical fish. *Vet. Microbiol.* 62: 35-45.
- de Roode, J. C., Pansini, R., Cheesman, S. J., Helinski, M. E. H., Huijben, S., Wargo, A. R., Bell, A. S., Chan, B. H. K., Walliker, D. & Read, A. F. 2005. Virulence and competitive ability in genetically diverse malaria infections. *Proc. Natl. Acad. Sci. USA* 102: 7624-7628.
- Devine, J. G., Ingvarsdóttir, A., Mordue, W., Pike, A. W., Pickett, J., Duce, I. & Mordue (Luntz) J. 2000. Salmon lice, *Lepeophtheirus salmonis*, exhibit specific chemotactic responses to semiochemicals originating from the salmonid, *Salmo salar*. *J. Chem. Ecol.* 26: 1833-1847.
- Engelsma, M. Y., Hougee, S., Nap, D., Hofenk, M., Rombout, J. H. W. M., van Muiswinkel, W. B., & Verburg-van Kemenade, B. M. L. 2003. Multiple acute temperature stress affects leucocyte populations and antibody

- responses in common carp, *Cyprinus carpio* L. *Fish Shellfish Immunol.* 15: 397-410.
- 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. *J. Appl. Ecol.* 43: 660-668.
- Fenton, A. & Hudson, P. J. 2002. Optimal infection strategies: should macroparasites hedge their bets? *Oikos* 96: 92-101.
- Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. In: Hoar, W. S. & Randall, D. J. (eds.), *Fish Physiology*: 1-98. Academic Press, London, New York.
- Gannicott, A. M. & Tinsley, R. C. 1997. Egg hatching in the monogenean gill parasite *Discocotyle sagittata* from the rainbow trout (*Oncorhynchus mykiss*). *Parasitology* 114: 569-579.
- Gault, N. F. S., Kilpatrick D. J. & Stewart, M. T. 2002. Biological control of the fish louse in a rainbow trout fishery. *J. Fish Biol.* 60: 226-237.
- Gibson, R. M. & Langen, T. A. 1996. How do animals choose their mates? *Trends Ecol. Evol.* 11: 468-470.
- Glover, K. A., Nilsen, F. & Skaala, O. 2004. Individual variation in sea lice (*Lepeophtheirus salmonis*) infection on Atlantic salmon (*Salmo salar*). *Aquaculture* 241: 701-709.
- Grenfell, B. T. & Dobson, A. P. 1995. *Ecology of infectious diseases*. 536 p., Cambridge University Press, Cambridge.
- Gustafson, L. L., Ellis, S. K. & Bartlett, C. A. 2005. Using expert opinion to identify risk factors important to infectious salmon-anemia (ISA) outbreaks on salmon farms in Maine, USA and New Brunswick, Canada. *Prev. Vet. Med.* 70: 17-28.
- Hakalahti, T., Häkkinen, H. & Valtonen, E. T. 2004a. Ectoparasitic *Argulus coregoni* (Crustacea: Branchiura) hedge their bets - studies on egg hatching dynamics. *Oikos* 107: 295-302.
- Hakalahti, T., Karvonen, A. & Valtonen, E. T. 2006. Climate warming and disease risks in temperate regions - *Argulus coregoni* and *Diplostomum spathaceum* as case studies. *J. Helminthol.* 80: 93-98.
- Hakalahti, T., Lankinen, Y. & Valtonen, E. T. 2004b. Efficacy of emamectin benzoate in the control of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout *Oncorhynchus mykiss*. *Dis. Aquat. Organ.* 60: 197-204.
- Hakalahti, T., Pasternak, A. F. & Valtonen, E. T. 2004c. Seasonal dynamics of egg laying and egg-laying strategy of the ectoparasite *Argulus coregoni* (Crustacea: Branchiura). *Parasitology* 128: 655-660.
- 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.
- Hammerstein, P. & Parker, G. A. 1987. Sexual selection: games between the sexes. In: Bradbury, J. W. & Andersson, M. B. (eds.), *Sexual selection: testing the alternatives*: 119-142. John Wiley & Sons, New York.

- Hellio, C., Pons, A. M., Beaupoil, C., Bourgougnon, N. & Gal, Y. L. 2002. Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. *Int. J. Antimicrob. Agents* 20: 214-219.
- Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeck, H. & Dobson, A. P. 2002. *The ecology of wildlife diseases*. 197 p., Oxford University Press, Oxford.
- Hull, M. Q., Pike, A. W., Mordue (Luntz) A. J. & Rae G. H. 1998. Patterns of pair formation and mating in an ectoparasitic caligid copepod *Lepeoptheirus salmonis* (Krøyer 1837): implications for its sensory and mating biology. *Phil. Trans. R. Soc. Lond. B* 353: 753-764.
- Hutchings, M. R., Kyriazakis, I., Anderson, D. H., Gordon, I. J. & Coop, R. L. 1998. Behavioural strategies used by parasitized and non-parasitized sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces. *Anim. Sci.* 67: 97-106.
- Jaworski, A. & Holm, J. C. 1992. The distribution and structure of the population of sea lice (*Lepeoptheirus salmonis* Krøyer) on Atlantic salmon (*Salmo salar* L.) under typical rearing conditions. *Aquacult. Fish. Mgmt.* 23: 577-589.
- Jespersen, L. B. & Toft, S. 2003. Compensatory growth following early nutritional stress in the Wolf Spider *Pardosa Prativaga*. *Funct. Ecol.* 17: 737-746.
- Johnson, S. G. 1992. Parasite-induced parthenogenesis in a freshwater snail: stable, persistent patterns of parasitism. *Oecologia* 89: 533-541.
- Karvonen, A., Pauku, S., Valtonen, E. T. & Hudson, P. J. 2003. Transmission, infectivity and survival of *Diplostomum spathaceum* cercariae. *Parasitology* 127: 217-224.
- Kearn, G. C. 1986. The eggs of monogeneans. *Adv. Parasitol.* 25: 175-273.
- Kearn, G. C. 2004. *Leeches, lice and lampreys*. 432 p., Springer, Dordrecht, The Netherlands.
- Kollatsch, D. 1959. Untersuchungen über die Biologie und Ökologie der Karpfenlaus (*Argulus foliaceus* L.). *Zool. Beitr.* 5: 1-36.
- Kotiaho, J. S. 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biol. Rev.* 76: 365-376.
- Larsen, M. 2006. Biological control of nematode parasites in sheep. *J. Anim. Sci.* 84: 133-139.
- Madetoja, J., Nyman, P. & Wiklund, T. 2000. *Flavobacterium psychrophilum*, invasion into and shedding by rainbow trout *Oncorhynchus mykiss*. *Dis. Aquat. Organ.* 43: 27-38.
- Maklakov, A. A., Bilde, T. & Lubin, Y. 2004. Sexual selection for increased male body size and protandry in a spider. *Anim. Behav.* 68: 1041-1048.
- Metcalfe, N. B. & Monaghan, P. 2001. Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* 16: 254-260.
- Meyer-Rochow, V. B., Au, D. & Keskinen, E. 2001. Photoreception in fish lice (*Branchiura*): The eyes of *Argulus foliaceus* Linné, 1758 and *A. coregoni* Thorell, 1865. *Acta Parasitol.* 46: 321-331.

- Mikheev, V. N., Mikheev, A. V., Pasternak, A. F. & Valtonen, E. T. 2000. Light-mediated host searching strategies in a fish ectoparasite, *Argulus foliaceus* L. (Crustacea: Branchiura). *Parasitology* 120: 409-416.
- Mikheev, V. N., Pasternak, A. F. & Valtonen, E. T. 2004. Tuning host specificity during the ontogeny of a fish ectoparasite: behavioural responses to host-induced cues. *Parasitol. Res.* 92: 220-224.
- Mikheev, V. N., Valtonen, E. T. & Rintamäki-Kinnunen, P. 1998. Host searching in *Argulus foliaceus* L. (Crustacea: Branchiura): the role of vision and selectivity. *Parasitology* 116: 425-430.
- Molnár, K. & Székely, C. 1998. Occurrence of skrjabillanid nematodes in fishes of Hungary and in the intermediate host, *Argulus foliaceus* L. *Acta Vet. Hung.* 46: 451-463.
- Moravec, F., Vidal-Martinez, V. & Aguirre-Macedo, L. 1999. Branchiurids (*Argulus*) as intermediate hosts of the daniconematid nematode *Mexiconema Cichlasomae*. *Fol. Parasitol.* 46: 79.
- Nishida, R., Schulz, S., Kim, C. S., Fukami, H., Kuwahara, Y., Honda, K. & Hayashi, N. 1996. Male sex pheromone of a giant danaine butterfly, *Idea leuconoe*. *J. Chem. Ecol.* 22: 949-972.
- Northcott, S. J., Lyndon, A. R. & Campbell, A. D. 1997. An outbreak of freshwater fish lice, *Argulus foliaceus* L., seriously affecting a Scottish stillwater fishery. *Fisheries Manag. Ecol.* 4: 73-75.
- Parker, G. A., Chubb, J. C., Ball, M. A. & Roberts, G. N. 2003. Evolution of complex life cycles in helminth parasites. *Nature* 425: 480-484.
- Pasternak, A. F., Mikheev, V. N. & Valtonen, E. T. 2000. Life history characteristics of *Argulus foliaceus* L. (Crustacea: Branchiura) populations in Central Finland. *Ann. Zool. Fennici* 37: 25-35.
- Pasternak, A., Mikheev, V. & Valtonen, E. T. 2004. Growth and development of *Argulus coregoni* (Crustacea: Branchiura) on salmonid and cyprinid hosts. *Dis. Aquat. Organ.* 58: 203-207.
- Patel, M. N., Stolinski, M. & Wright, D. J. 1997. Neutral lipids and the assessment of infectivity in entomopathogenic nematodes: observations of four *Steinernema* species. *Parasitology* 114: 489-496.
- Pfeil-Putzien, C. 1977. Experimentelle Übertragung der Frühjahrsvirämie (spring viraemia) der Karpfen durch Karpfenläuse (*Argulus foliaceus*). *Zentralbl. Veterinaermed. Reihe B* 25: 319-323.
- Pike, A. W. 1989. Sea lice – major pathogens of farmed Atlantic salmon. *Parasitol. Today* 5: 291-297.
- Poulin, R. 1996. Sexual size dimorphism and transition to parasitism in copepods. *Evol.* 50: 2520-2523.
- Poulin, R. 1998. Evolutionary ecology of parasites: from individual to communities. 212 p., Chapman & Hall, London.
- Poulin, R. 2007. Are there general laws in parasite ecology? *Parasitology*, in press.
- Poulin, R., Nichol, K. & Latham, A. D. A. 2003. Host sharing and host manipulation by larval helminths in shore crabs: cooperation or conflict? *Int. J. Parasitol.* 33: 425-433.



- Poulin, R. & FitzGerald, G. J. 1989a. Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). *Behav. Ecol. Sociobiol.* 24: 251-255.
- Poulin, R. & FitzGerald, G. J. 1989b. Risk of parasitism and microhabitat selection in juvenile sticklebacks. *Can. J. Zool.* 67: 14-18.
- Poulin, R. & FitzGerald, G. J. 1989c. Male-biased sex ratio in *Argulus canadensis* Wilson, 1916 (Crustacea: Branchiura) ectoparasitic on sticklebacks. *Can. J. Zool.* 67: 2078-2080.
- Poulin, R., Rau, M. E. & Curtis, M. A. 1991. Infection of brook trout fry, *Salvelinus fontinalis*, by ectoparasitic copepods: the role of host behaviour and initial parasite load. *Anim. Behav.* 41: 467-476.
- Proctor, H. C. 1992. Effect of food-deprivation on mate searching and spermatophore production in male water mites (Acari, Unionicolidae). *Funct. Ecol.* 53: 1987-1994.
- Price, P. W. 1980. *Evolutionary biology of parasites.* 237 p., Princeton University Press, Princeton, New Jersey.
- Pyökkö, P., Suomalainen, L-R., Tirola, M. & Valtonen, E. T. 2006. Evidence of enhanced bacterial invasion during *Diplostomum spathaceum* infection in European grayling, *Thymallus Thymallus* (L.). *J. Fish Dis.* 29: 79-86.
- Randolph, S. E. 2004. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology* 129 (suppl.): S37-S65.
- Reznick, D. 1985. Cost of reproduction: an evaluation of the empirical evidence. *Oikos* 44: 257-267.
- Rintamäki-Kinnunen, P. 1997. Parasitic and bacterial diseases at salmonid fish farms in Northern Finland. Department of Biology. *Acta Univ. Ouluensis, Scientiae Rerum Naturalium A* 294: 1-43.
- Ritchie, G. 1997 The host transfer ability of *Lepeoptheirus salmonis* (Copepoda: Caligidae) from farmed Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* 20: 153-157.
- Ruane, N. M., Nolan, D. T., Rotllant, J., Tort, L., Balm, P. H. M. & Wendelaar Bonga, S. E. 1999. Modulation of the response of rainbow trout (*Oncorhynchus mykiss* Walbaum) to confinement, by an ectoparasitic (*Argulus foliaceus* L.) infestation and cortisol feeding. *Fish Physiol. Biochem.* 20: 43-51.
- Salonius, K. & Iwama, G. K. 1993. Effects of early rearing environment on stress response, immune function, and disease resistance in juvenile coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*). *Can. J. Fish Aquat. Sci.* 50: 759-766.
- Seppälä, O., Karvonen, A. & Valtonen, E. T. 2005. Manipulation of fish host by eye flukes in relation to cataract formation and parasite infectivity. *Anim. Behav.* 70: 889-894.
- Shaw, D. J. & Dobson, A. P. 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* 117: 597-610.

- Shaw, D. J., Grenfell, B. T. & Dobson, A. P. 1998. Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* 177: 597-610.
- Shinn, A. P., Wootten, R., Côté, I. & Sommerville, C. 2003. Efficacy of selected oral chemotherapeutants against *Ichthyophthirius multifiliis* (Ciliophera: Ophryoglenidae) infecting rainbow trout *Oncorhynchus mykiss*. *Dis. Aquat. Organ.* 55: 17-22.
- Shimura, S. 1981. The larval development of *Argulus coregoni* Thorell (Crustacea: Branchiura). *J. Nat. Hist.* 15: 331-348.
- Shimura, S. 1983. Seasonal occurrence, sex ratio and site preference of *Argulus coregoni* Thorell (Crustacea: Branchiura) parasitic on cultured freshwater salmonids in Japan. *Parasitology* 86: 537-552.
- Singhal, R. N., Jeet, S. & Davies, W. 1990. The effects of argulosis-saprolehniasis on the growth and production of *Cyprinus carpio*. *Hydrobiologia* 202: 27-31.
- Smith, J. L., Wootten, R. & Sommerville C. 2007. The pathology of the early stages of the crustacean parasite, *Lernaeocera branchialis* (L.), on Atlantic cod, *Gadus morhua*. *L. J. Fish Dis.* 30: 1-11.
- Stammer, H. J. 1959. Beiträge zur Morphologie, Biologie und Bekämpfung der Karpfenläuse. *Z. Parasitenkd.* 19: 135-208.
- Stone, J., Sutherland, I. H., Sommerville, C., Richards, R. H. & Varma, K. J. 2000. Field trials to evaluate the efficacy of emamectin benzoate in the control of sea lice, *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann, infestations in Atlantic salmon *Salmo salar* L. *Aquaculture* 186: 205-219
- Stuart, R. 1990. Sea lice, a maritime perspective. *Bull. Aquacult. Assoc. Can.* 1: 18-24.
- Suomalainen, L-R. 2005. *Flavobacterium columnare* in Finnish Fish Farming: Characterisation and putative disease management strategies. *Jyväskylä studies in biological and environmental science* 155.
- Taylor, N. G. H., Sommerville, C. & Wootten, R. 2006. The epidemiology of *Argulus* spp. (Crustacea: Branchiura) infections in stillwater trout fisheries. *J. Fish Dis.* 29: 193-200.
- Thomas, F., Brown, S. P., Sukhdeo, M. & Renaud, F. 2002. Understanding parasite strategies: a state-dependent approach? *Trends Parasitol.* 18: 387-390.
- Thomas, K. & Ollevier, F. 1993. Hatching, survival, activity and penetration efficiency of second-stage larvae of *Anguillicola crassus* (Nematoda). *Parasitology* 107: 211-217.
- Timi, J. T., Lanfranchi, A. L. & Poulin, R. 2005. Is there a trade-off between fecundity and egg volume in parasitic copepod *Lernanthropus cynoscicola*? *Parasitol. Res.* 95: 1-4.
- Todd, C. D., Stevenson, R. J., Reinardy, H. & Ritchie, M. G. 2005. Polyandry in the ectoparasitic copepod *Lepeophtheirus salmonis* despite complex precopulatory and postcopulatory mate-guarding. *Mar. Ecol. Prog. Ser.* 303: 225-234.

- Tripathi, N. K., Latimer, K. S., Gregory, C. R., Ritchie, B. W., Wooley, R. E. & Walker, R. L. 2005. Development and evaluation of an experimental model of cutaneous columnaris disease in koi *Cyprinus carpio*. *J. Vet. Diagn. Invest.* 17: 45-54.
- Tully, O. & Nolan, D. T. 2002. A review of the population biology and host-parasite interactions of the sea louse *Lepeoptheirus salmonis* (Copepoda: Caligidae). *Parasitology* 124 (suppl.): S165-S182.
- van der Salm, A. L., Nolan, D. T., Spanings, F. A. T. & Wendelaar Bonga, S.E. 2000. Effects of infection with the ectoparasite *Argulus japonicus* (Thiele) and administration of cortisol on cellular proliferation and apoptosis in the epidermis of common carp, *Cyprinus carpio* L., skin. *J. Fish Dis.* 23: 173-184.
- Wakelin, D. 1996. *Immunity to parasites*. 224 p., Cambridge University Press, Cambridge.
- Walker, P. D., Flik, G. & Wendelaar Bonga, S. E. 2004. The biology of parasites from the genus *Argulus* and a review of the interactions with its host. In: Wiegertjes, G. F. & Flik, G. (eds.), *Host-parasite interactions*: 107-129. SEB Symposium Series 55, Garland Science/BIOS Scientific Publishers.
- Wassom, D. L., Dick, T. A., Arnason, N., Strickland, D. & Grundmann, A. W. 1986. Host genetics: A key factor in regulating the distribution of parasites in natural host populations. *J. Parasitol.* 72: 334-337.
- Whitfield, P. J., Bartlett, A., Khammo, N. & Clothier, R. H. 2003. Age-dependent survival and infectivity of *Schistosoma mansoni* cercariae. *Parasitology* 127: 29-35.
- Wilson, K., Bjørnstad, O. N., Dobson, A. P., Merler, S., Poglajen, G., Randolph, S. E., Read, A. F. & Skorping, A. 2002. Heterogeneities in macroparasite infections: patterns and processes. In: Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeek, H. & Dobson, A. P. (eds.), *The ecology of wildlife diseases*: 6-44. Oxford University Press, Oxford.
- Windsor, D. A. 1998. Most of the species on earth are parasites. *Int. J. Parasitol.* 28: 1939-1941.
- Woo, P. T. K. 1992. Immunological responses of fish to parasitic organisms. *Annu. Rev. Fish Dis.* 339-366.
- Wootton, R., Smith, W. & Needham, E. A. 1982. Aspects of the biology of the parasitic copepods *Lepeoptheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. *P. Roy. Soc. Edinb. B.* 81: 185-197.
- Wu, L. & Dong, S. 2002. Compensatory growth responses in juvenile Chinese shrimp, *Fenneropenaeus chinensis*, at different temperatures. *J. Crust. Biol.* 22: 511-520.