

Master's thesis

**Interactive effects of parasitism and predation on the
behaviour of the amphipod *Pallasea quadrispinosa***

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ABSTRACT

Pallasea quadrispinosa is a glacial relict amphipod. The populations in Lake Pääjärvi, Southern Finland, and a nearby spring in which the amphipods have been separated for some 9000 years were studied. In the spring there are no fish. Hence the aims of the study were to firstly determine whether there is any difference in the behaviour of amphipods from Lake Pääjärvi (exposed to fish predation) and amphipods from the spring which have not previously encountered a predator. Secondly to determine how parasitism affects the behaviour of amphipods from the lake. The study had 3 main hypotheses:

1. In lake water without predator scent, neither the uninfected nor the infected amphipods will hide.
2. In predator-conditioned water, uninfected lake amphipods will hide whereas infected lake amphipods will not hide.
3. Uninfected spring amphipods, which have never met a predator, will not react to the addition of predator-conditioned water.

The results showed that there was no statistically significant difference in anti-predatory behaviour between uninfected and infected lake amphipods after the addition of predator-conditioned water, although uninfected amphipods tended to hide more than infected individuals. The marginally significant change in anti-

predatory behaviour after the addition of predator-conditioned water is consistent with the hypothesis that uninfected lake amphipods would hide.

Furthermore, uninfected amphipods spent less time hidden in lake water than in predator-conditioned lake water. There was no difference in the anti-predatory behaviour of uninfected spring amphipods in lake water and in predator-conditioned lake water. There was a difference in anti-predatory behaviour between spring amphipods in lake water and uninfected amphipods in lake water. Spring amphipods hide more so than uninfected amphipods. This experiment provides further evidence for chemical stimuli being important factors in predator-prey, and host-parasite relationships.

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TIIVISTELMÄ

Okakatka, *Pallasea quadrispinosa*, on glasiaalisrelikti, jonka Eteläsuomalaisen Pääjärven, sekä läheisen, kalattoman lähteen populaatiot ovat eriytyneet 9000 vuotta sitten. Kokeen tarkoitus oli tutkia, eroavatko kahden populaation yksilöt käyttäytymisessään kahdella tavalla. Ensiksi, eroaako Pääjärven kalasaalistukselle altistettujen yksilöiden käyttäytyminen lähteen saalistukselle altistumattomien yksilöiden käyttäytymisestä. Toiseksi, kuinka loiset vaikuttavat järvipopulaation yksilöiden käyttäytymiseen. Tutkimuksella oli kolme päähypoteesia:

1. Järvipopulaation, saalistukselle altistetut yksilöt reagoivat loisin pysymällä esillä piiloutumisen sijaan.
2. Saalistajan hajua sisältävässä vedessä ainoastaan ei-loisitut yksilöt piiloutuvat, toisin kuin loisitut yksilöt.
3. Lähdepopulaation ei-loisitut, saalistukselle altistumattomat yksilöt eivät reagoi saalistajan hajuun.

Kokeen tulokset osoittavat että yksilöiden reaktio saalistajien hajuun ei merkitsevästi eroa järvipopulaation loisittujen ja ei-loisittujen yksilöiden välillä, vaikkakin ei-loisituilla yksilöillä oli taipumus piiloutua useammin kuin loisituilla yksilöillä. Marginaalisesti tilastollisesti merkitsevä muutos loisittujen, järvipopulaation yksilöiden käyttäytymisessä sopii esitettyyn hypoteesiin jonka mukaan ei-loisitut yksilöt piiloutuvat saalistajan hajua sisältävässä vedessä. Ei-loisitut yksilöt myös pysyvät piiloutuneina pidemmän ajan vedessä, jossa oli saalistajan hajua, kuin käsittelemättömässä järivedessä. Saalistajan haju ei vaikuttanut ei-loisittujen, lähdepopulaation yksilöiden käyttäytymiseen. Lähde – ja järvipopulaatioiden yksilöt reagoivat eri tavalla saalistajan hajuun. Tämä koe tarjoaa lisätodisteita kemiallisten ärsykkeiden tärkeydestä saalis-saalistaja sekä lois-isäntä – suhteissa.

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1. INTRODUCTION

Predation imposes selection pressures upon organisms, forcing them to develop adaptations to minimise the risks from predation. It is costly to the prey to be predated upon, not just in terms of being eaten but also the energetic costs involved in predator avoidance behaviour, such as hiding. (Lafferty and Morris, 1996).

Parasites are able to alter predatory relationships. Poulin et al (1992) found that the copepod intermediate host when infected by the cestode *Eubothrium salvelini* is more susceptible to fish predation. Barnard and Behkner (1990) demonstrated that the behaviour of parasitised animals differed from that of uninfected animals by manipulating the host decision making directly in the form of altered behaviour. Alternatively, the parasite may influence the host indirectly by changing oxygen consumption rates and activity for example. Bethel and Holmes were amongst the first to properly clarify altered behaviour, especially in acanthocephalans (Bethel and Holmes, 1973; Bethel and Holmes, 1974; Bethel and Holmes, 1977). In these papers, Bethel and Holmes reported the response of amphipods to light when infected by acanthocephalan worms and showed that altered behaviour did indeed occur. The behaviour of the amphipods that Bethel and Holmes studied was altered from being strongly photophobic to photophilic in infected amphipods. Increased sensitivity to disturbance and clinging behaviour was also noted. Moore (1983) studied the parasite *Plagiorhynchus cylindraceus* in its intermediate host *Armidillum vulgare* and predation by its definitive host, the starling. Infected *A. vulgare* were found in areas of less humidity, on light coloured substrate and in unsheltered areas. This altered behaviour may increase the predation pressure.

By inducing altered behaviour in their intermediate hosts, parasites are able to increase their probability of transmission. Lafferty and Morris (1996) noted that transmission depends on predation and they suggested that parasites are able to alter host behaviour and thereby increase the susceptibility of the intermediate hosts to predation by final hosts. There were three main lines of evidence to support this. Hosts infected by transmissible stages of parasites often behave differently in laboratory conditions. They are also more readily eaten by predators than unparasitised hosts. Finally, they are taken more frequently than expected in the wild. Lafferty and Morris (1996) concluded that even though a parasite may cause only a small change in behaviour, this could lead to a large increase in predation. There are many different strategies that parasites employ to ensure transmission success. For example, Seppälä et al - 2004, 2005 (a), 2005 (b) found that eye flukes forming cataracts on the eyes of fish hosts increased susceptibility to predation and therefore transmission.

Acanthocephalans often infect amphipods as their intermediate host. Transmission of the parasite is through ingestion of the intermediate host by a predator. The parasite will grow and reproduce in its final host before releasing eggs in the host faeces. *Pallasea quadrispinosa* is a glacial relict amphipod. The species is found in both Lake Pääjärvi and in a nearby spring in which these two populations having been separated from some 9000 years since the last ice age. *P. quadrispinosa* in Lake Pääjärvi are not only preyed upon but are also prone to infection by the acanthocephalan parasite *Echinorhynchus borealis*. Spring amphipods, however, have not been exposed to predation or parasitism. Hence separation of these amphipods may have led to different behaviours when responding to predation. Therefore it could be predicted that spring amphipods will be unable to detect the chemical stimuli produced by predators and therefore will be more prone to predation. Lake amphipods, however, would be more likely to escape in response to the predatory stimuli. When a predator is not present, there will be no difference in the behaviour between parasitised and unparasitised amphipods simply because there are no chemical cues from the predator.

However, if the parasite induces altered behaviour in the amphipod to increase probability of transmission, when a predator was present, the chemical signal it presents in the water would induce the amphipods to behave in their individual ways: uninfected amphipods would tend to hide and infected amphipods would be less likely to hide and then be preyed upon.

Three main hypotheses were addressed in this study:

1. In lake water without a predator scent, neither the uninfected nor infected amphipods will hide
2. In predator conditioned water, uninfected lake amphipods will hide whereas infected lake amphipods will not hide.
3. Uninfected spring amphipods which have never encountered a predator will not react to the addition of predator-conditioned water.

The first aim of this study was to determine whether there was any difference in the behaviour of amphipods from Lake Pääjärvi infected with an acanthocephalan and those not infected, when exposed to predation. A second aim was to determine if there is a difference in the behaviour of amphipods from Lake Pääjärvi (previously exposed to fish predation) and spring amphipods (not previously exposed to fish predation) when predation pressure is simulated by conditioning the water with predator (fish) scent.

2. MATERIAL AND METHODS

2.1. Study site

Lake Pääjärvi (64° 04'N, 25° 05'E) is a large oligotrophic lake in southern Finland approximately 10 km by 3.8 km with several outlets and a maximum depth of 87 m (Ruuhijärvi, 1974 and Ilmavirta and Kotimaa , 1974). The spring (61° 00'29'' N, 25°11'49'' E) is located approximately 5.5 km southeast from the nearest shoreline of Lake Pääjärvi. It is approximately 8x5 m² though the depth and other features of the spring have been little studied. (K.Salonen- personal communication).

2.2 Collection of amphipods

Samples of *Pallasea quadrispinosa* were collected from Lake Pääjärvi and the nearby spring on 7 November 2005. The amphipods were brought to the University of Jyväskylä on 8 November and the following day amphipods were placed into tanks with 20 to 30 individuals in each tank. There were 2 tanks containing spring amphipods and 3 tanks containing lake amphipods.

2.3 Care of animals

2.3.1 Amphipods

Amphipods from Lake Pääjärvi were kept in tanks containing lake water from Lake Pääjärvi and spring amphipods were kept in water from the spring from which they originated. The temperature in the cold room was maintained at 6°C. After 2 days, the amphipods were taken to a warmer room to acclimate at 10°C for another 2 days and then to the laboratory where the experiments were to take place. The

temperature in the laboratory started at 12°C and increased to 15°C and the amphipods were left to acclimate for 3 days. The room remained at 15 ±1°C throughout the experimental period. Acclimation of amphipods is essential as with any other experiments that involve altering temperatures and habitats.

P.quadrispinosa are known to live in cold deep areas of lakes or springs so it was essential to gradually increase the temperature for the animals to be able to acclimate to the laboratory.

Each tank was aerated using two mouse air pumps (model m-106) connected by lengths of plastic tubing. The water was changed almost weekly, with lake water collected from Jyväsjärvi. Amphipods were fed 2 to 3 times a week with frozen chironomids (Ruto Frozen fish food B.V *Chironomus rosso*). . It was initially observed that if only whole chironomids were provided, larger amphipods consumed them, leaving smaller animals with little or no food available. Therefore small blocks of approximately 3 cm by 3 cm of frozen chironomids were cut into a variety of sizes to ensure that even small amphipods would be able to feed. The same amount of chironomids was replaced when the previous blocks were consumed. If they were not eaten, they were removed to avoid contaminating the water.

2.3.2 Burbot (*Lota lota*)

Three burbot were kept in a large tank with a variety of rocks available for hiding. The tank was aerated using 2 mouse air pumps (Model m-106) connected to 5 tubes with an aeration stone attached to the end. The water flowing through the tank was constant as there was an outlet tube at the base of the tank to be used in future experiments which constantly flowed through the tank. Burbot were fed perch (*Perca fluviatilis*) 2 to 3 times a week which was frozen, thawed and cut into 3 pieces for each burbot. Burbot generally consumed the perch within 24 hours but if there were pieces remaining they were removed to avoid contaminating the water. Tanks were cleaned of excess food and waste regularly.

2.3.3 Roach (*Rutilus rutilus*)

Eleven roach were kept in a similar tank arrangement to that of burbot (see section 2.3.2) and were cared for in a similar manner. Roach were fed chironomids (Ruto Frozen fish food B.V *Chironomus rosso*). A block of frozen chironomids approximately 5 cm by 5 cm was thawed and placed into the tank. Roach usually consumed the chironomids within 24 hours and again any waste was removed regularly. Roach were fed 2 to 3 times a week at the same time as burbot.

2.4 Experimental set up and procedure

Experiments took place in the University of Jyväskylä from 22.11.2005 to 15.12.2005. The evening prior to the experiment, 12 amphipods were randomly chosen by stirring the water and picking amphipods from the 3 spring water tanks and 2 lake water tanks using a small net. Six amphipods were taken from the 3 spring water tanks (2 amphipods from each tank) and 6 amphipods were taken from the 2 lake water tanks (3 amphipods from each tank). Different nets were used for the spring and lake water.

The chosen amphipods were placed in 12 small, non-aerated plastic containers and fed one chironomid each. This step was to reduce any foraging or hunger behaviour during the experiment. The burbot and roach tanks were cleaned and the amphipods were left overnight. The next day, to prepare for the experiment, the water flow in the burbot and roach tanks was turned off 1 hour before the experiment was to begin. This allowed the burbot and roach water to condition, i.e. the predatory scent to accumulate in the water so the amphipods would be able to detect the chemical stimulus. After one hour of conditioning, the lights were switched off and only red light was used. Amphipods tend to inhabit dark areas in the absence of red light, so they should be relatively unresponsive to light at this wavelength. Each of the 12 amphipods were placed individually into small glass tanks containing 1,2L

water from Lake Jyväsjärvi. There was also a small shelter made from black plastic in each tank with 2 nails screwed in to each of the corners which provided a triangular hiding space with an entrance gape of approximately 5mm. A small pebble was placed on top of the shelter to prevent it floating.

When the 12 amphipods were all in the tanks, they were allowed to acclimate in lake water for 10 minutes. After the acclimation time, the behaviour of each amphipod was observed simultaneously every 5 minutes for 1 hour, corresponding to 13 recordings per amphipod per experiment. Behaviour that was recorded was defined as the individual amphipod being either exposed in the water column (recorded as 'E') or under the shelter (recorded as 'U') From the 13 recordings, the response variable was defined as the proportion of time the amphipods spent exposed ('E') in the water column. In total, there were 12 amphipods per trial, and trials were repeated on 9 days. Five-hundred ml of predator conditioned water was added according to the treatments assigned to the individual amphipods and these amphipods were allowed 10 minutes to acclimate. The amphipods were again observed every 5 minutes for 1 hour, corresponding to another 13 recordings per amphipod. Again, the behaviour was defined as the amphipod being either exposed in the water column ('E') or under the shelter ('U'). The amphipods were removed from their individual tanks and placed on petri dishes where they were straightened and measured from the rostrum to the end of the 11th segment. Each amphipod was dissected by removing the head and removing each segment of the amphipod. Any acanthocephalans found were removed, placed into crustacean saline, labeled and refrigerated. Prevalence (%) was also studied and was defined as the percentage of infected amphipods from the total number of amphipods studied.

2.4 Additional data

Some data were also collected from Lake Leppävesi, an oligotrophic lake which is situated about 20 km from Jyväskylä with a maximum depth of 40 m. Amphipods were collected and sampled in the same way as in Lake Pääjärvi. Therefore the data and experiments are comparable. (Benesh. personal comm).

2.6 Analysis of data

2.6.1 Test summary

Data were analysed using SPSS program. The total number of amphipods used in the analysis included my own data and data from previous experiments (Benesh, unpublished data) N=154.

Table 1. The 6 main treatments allocated to *Pallasea quadrispinosa*

Treatments			
Source of amphipod	Addition of water	Infection status	Number of replicates ¹
Lake	Lake	Infected	60 _a
Lake	Lake	Infected	51 _b
Lake	Predator-conditioned	Uninfected	60 _a
Lake	Predator-conditioned	Uninfected	51 _b
Spring	Lake	Uninfected	43 _c
Spring	Predator-conditioned	Uninfected	43 _c

¹ Groups with the same letter are dependent of each other. They were compared using Sign test. All other independent comparisons used 2 sample Kolmogorov-Smirnov test

Table 1 shows the main treatments assigned to amphipods. Treatments were divided into the source of the amphipods, defined as either lake amphipods or spring

amphipods. The lake amphipods were further subdivided by the addition of lake water and predator-conditioned water. They were then separated by their infection status (uninfected and infected), leaving 4 treatments for lake amphipods. The spring source of amphipods was separated by the addition of either lake water or predator-conditioned water and the infection statuses of the spring source of amphipods were both uninfected. This left 2 treatments for the amphipods from the spring therefore resulting in a total of 6 treatments.

2.6.2 Differences and proportions of amphipod behaviour

To determine whether there was a change in behaviour induced by the presence of predator-conditioned water, the number of times each individual amphipod was exposed in lake water was subtracted from the number of times the same amphipod was exposed predator conditioned water. Statistical tests were based on the treatments listed in table 1.

3. RESULTS

3.1 Amphipod data

A total of 154 amphipods were used in the data. Prevalence of infection was 56%, and just over half of *P.quadrspinosa* were infected with 1 or more parasites at a mean intensity of 1.67. The mean length of lake amphipods was 14.85 ± 3.47 mm SD whereas spring amphipods tended to be smaller, measuring 11.89 ± 2.90 mm SD. *Echinorhynchus borealis* were mostly cystacanth stages and the majority of amphipods were infected by only 1 *E.borealis*. In rare instances, there were multiple infections, and the maximum number of parasites found in a single amphipod was 9. Many of the amphipods from Lake Pääjärvi were found to have varying degrees of protozoan infestations on the carapace and swimming appendages. Also, several female spring amphipods were found to be carrying eggs. There were several differences in the behaviour of lake amphipods and spring amphipods. Lake amphipods tended to be more active during the experiments and were more likely to dart in and out of the shelter in an erratic manner. Spring amphipods, however, tended to remain on the bottom of the tank and were less active.

3.1.1 Comparing the changes in behaviour of *P. quadrispinosa*

Treatments were as designated in the materials and methods (See section 2.4) and provided 7 possible comparisons. The hiding behaviour of the amphipods in the different treatments is illustrated graphically (figs 1-3) in which the proportions in the histograms reflect time spent exposed on a scale from hidden throughout the observation period (proportion = 0.00) or exposed during the entire experiment (proportion = 1.00). As there were 7 comparisons and the conventional significance level is set at 0.05, Bonferroni adjustment was used to determine the significance value in these analyses to be $p \leq 0.007$ in order to avoid type 1 error.

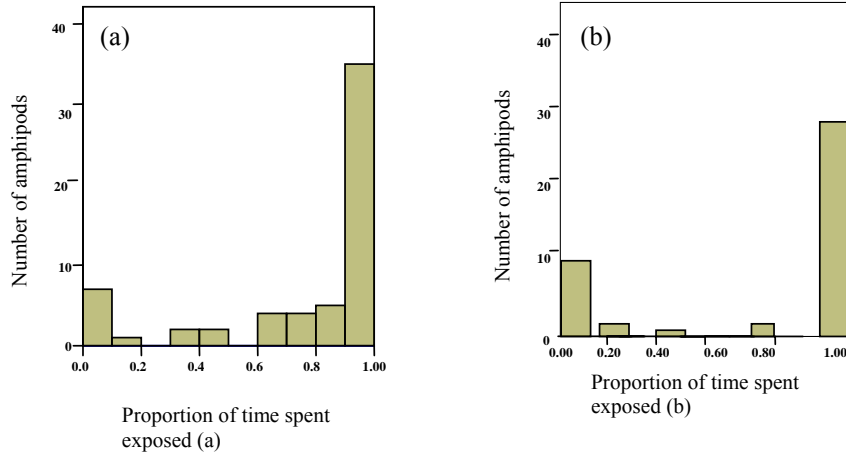


Fig 1: The hiding behaviour of uninfected (a) Mean = 0.78, Std. Dev. = 0.34, N = 60 and infected (b) *Pallasea quadrispinosa* from the lake in normal lake water (not predator-conditioned) Mean = 0.72, Std. Dev. = 0.42, N = 51

Uninfected lake amphipods in lake water (Fig 1a) did not differ significantly, (Kolmogrov Smirnov, $Z = 0.741$, $p=0.642$). Also the means \pm SD of uninfected and infected amphipods were similar which also shows that they did not significantly alter their behaviour in lake water.

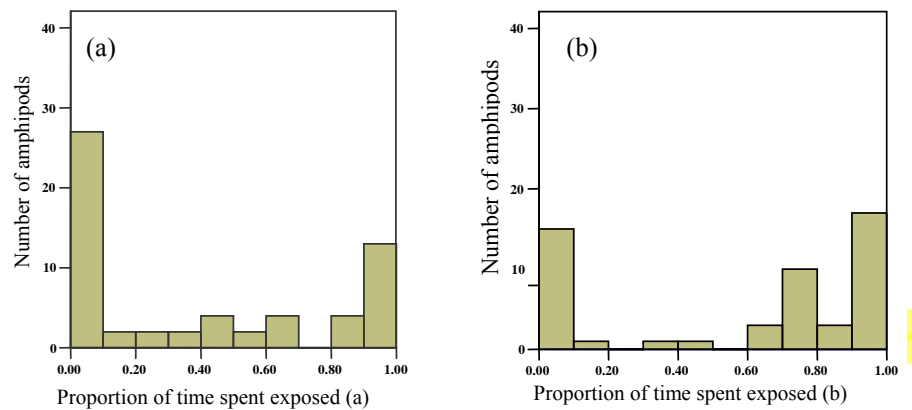


Fig 2: The hiding behaviour of uninfected (a) Mean = 0.39, Std. Dev. = 0.41 N = 60. and infected (b) *Pallasea quadrispinosa* from the lake in predator- conditioned water. Mean = 0.5, Std. Dev. = 0.40, N = 51.

Uninfected and infected lake amphipods did not differ significantly from each other after the addition of predator conditioned water (Kolmogrov Smirnov test $Z = 1.632$ $p= 0.010$) although the level of non significance was marginal. It may be argued that as the Bonferroni correction method is so conservative there may in fact be a significant difference which is not shown in this case. Uninfected amphipods (Fig 2a) clearly hid more than infected amphipods (Fig 2b). Therefore *E.borealis* may have some effect on the behaviour of amphipods in predator conditioned water but the effect cannot be deemed statistically significant.

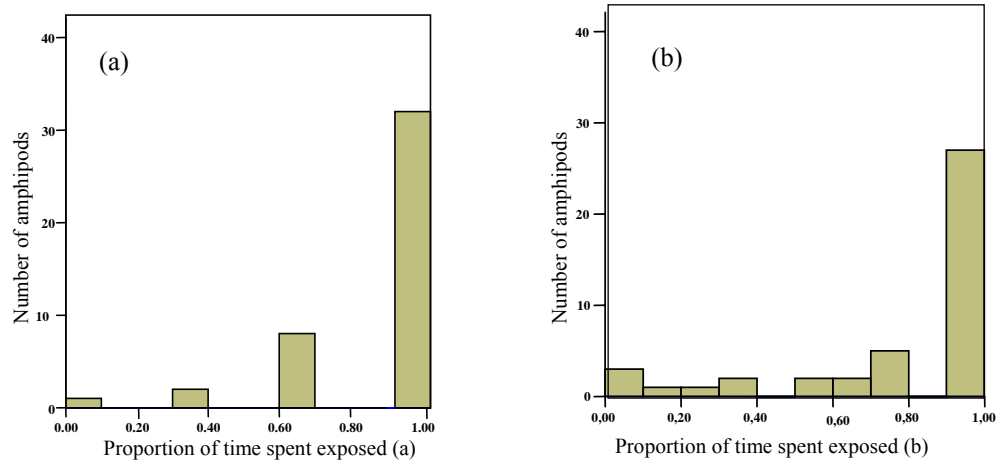


Fig 3: The hiding behaviour of *Pallasea quadrispinosa* from the spring in lake water (a) Mean = 0.97 Std. Dev. = 0.05, N = 43 and predator-conditioned water (b) Mean = 0.79, Std. Dev. = 0.31, N=43.

Uninfected spring amphipods before and after the addition of predator conditioned water did not differ significantly (Sign test $Z = -2.550$ $p = 0.010$). The greatest proportion of the spring amphipods in lake water were exposed (proportion = 0.60- 1.00). After the addition of predator conditioned water (Fig 3b), the proportion of amphipods showing exposed behaviour was similar to that in Fig 3a showing that there is little difference in behaviour.

There was a highly significant difference between uninfected amphipods in lake water (Fig 1a) and uninfected amphipods in predator conditioned water (Fig 2a) Sign test $Z = -5.367$ $p = <0.001$. There was a pronounced shift in behaviour in Fig 1a showing that a large proportion of uninfected amphipods in lake water were exposed (proportion = 1.00). However, uninfected amphipods in predator conditioned water (Fig 2a) showed an opposite trend in behaviour with a higher proportion of amphipods hiding. Furthermore there was a difference in means

between Figs 1a and 2a (0.78 and 0.39 respectively), which further illustrates the differences in behaviour.

There was also a highly significant difference between infected amphipods in lake water (Fig 1b) and infected amphipods in predator conditioned water (Fig 2b) Sign test $Z = -4.057$ $p < 0.001$. There was a clear tendency for infected amphipods in lake water to be exposed (defined as proportion = 1.00). There were also few amphipods hiding, however in Fig 2b exposed behaviour was much less pronounced with similar proportions of amphipods hidden and exposed and the means differ which shows that there was some altered behaviour.

Spring amphipods in lake water (Fig 3a) differ significantly from uninfected amphipods in lake water (Fig 1a) Kolmogorov Smirnov $Z = 1.736$ $p = 0.005$ It is evident that hiding behaviour is more prominent in uninfected lake amphipods than spring amphipods in lake water (as seen in Fig 1a). Spring amphipods in predator conditioned water (Fig 3b) differed significantly from uninfected amphipods in predator conditioned water (Fig 2a) (Kolmogorov Smirnov $Z = 2.372$ $p < 0.001$). It is clearly visible that uninfected amphipods in lake water after the addition of predator conditioned water (Fig 2a) hide more so than spring amphipods after the addition of predator conditioned water (Fig 3b).

5. DISCUSSION

The mean infection rate of amphipods from Lake Pääjärvi is rather high compared to other areas, for example only 0.6% infection in the Bothnian Bay (Valtonen, 1983). Not all the amphipods in Lake Pääjärvi were so heavily infected. Valtonen (1983) found that there was migration of spawning whitefish in the deeper waters of the Bothnian Bay. Interestingly, only spawning fish were parasitised by *Echinorhynchus salmonis* due to the fact that adult fish tended to predate upon

amphipods. Whitefish are definitive hosts of the parasite *E. salmonis*. Under natural conditions, aggregation of intermediate hosts has also been found in molluscs infected with trematodes, in tubificids infected with cestodes and in isopods infected with acanthocephalan species. This may help explain the high infection rate of *Echinorhynchus borealis* in its intermediate host, *Pallasea quadrispinosa*.

The similar behaviour of uninfected and infected lake amphipods in lake water is consistent with the hypothesis that neither uninfected or infected lake amphipods in lake water will not hide (i.e. be more exposed). Lake water lacks strong chemical signals from predators or conspecifics. The amphipods spend the greatest proportion of time exposed in the water column. This suggests that in the absence of predators, amphipods would swim in the water column, and personal observations have shown that the amphipods also spend a large proportion of their time on the bottom of the tank and on the stones supporting the shelter (see materials and methods section 2.4). This behaviour could correspond to what other authors have found, for example, studies by Maynard et al (1998) and MacNiel et al (2003). Maynard et al (1998) found that unparasitised individuals of *Gammarus pulex* spent more time in the substratum and in weeds, possibly corresponding to *Pallasea quadrispinosa* lying on the bottom of the experimental tanks where in its natural benthic habitat there would be plenty of shelter in the form of weeds and leaves. The behaviour of uninfected and infected amphipods was so similar because they do not respond to lake water as it is where they live naturally. Therefore it follows that their behaviour would not change.

There was no significant change in behaviour between uninfected and infected lake amphipods after the addition of predator conditioned water. This did not support the hypothesis that uninfected amphipods would hide more than infected amphipods. Uninfected lake amphipods in predator-conditioned water would tend to hide in the presence of burbot or roach and infected amphipods in predator conditioned water would not hide. It may be predicted that there is some form of behavioural change from the work of previous authors that the addition of predator-conditioned water induces amphipods to alter their behaviour (See Bethel and Holmes 1973, 1974, Lafferty and Morris 1996, Maynard et al. 2003 and Moore.

2002). This behaviour is modified from hidden behaviour to exposed behaviour, as the prey is able to detect the presence of a definitive host or predator by the production of chemical stimuli or perhaps kairomones from the predator. Early authors suggested the presence of allomone like chemicals. Bethel and Holmes (1973) predicted that an allomone mediated system may be involved. Later authors suggested more sophisticated manipulation of hormones and neurochemicals (Lafferty and Morris - 1996). Helluy and Holmes (1990) were some of the first authors to describe a complex manipulation of hormones in Gammarids. They found that serotonin and octapamine altered the behaviour of *Gammarus lacustris*. When injected with serotonin, the amphipods were more likely to exhibit clinging behaviour similar to that of when *G.lacustris* is infected by the parasite *Polymorphus minutus*. However, when octapamine was injected into infected *G.lacustris*, the clinging behaviour ceased. Therefore, Helluy and Holmes (1990) concluded that in infected gammarids, a serotonergic or serotonin-like pathway is activated and acted in antagonism with octapamine pathways. Also for example, altered behaviour may be caused by interference with dopamine such as in the intermediate host *Hemigrapsus crenulatus*, a crab parasitised by the acanthocephalan, *Profilicollis antarcticus*. (Rojas and Ojeda – 2005).

The change in behaviour between uninfected lake amphipods after the addition of predator conditioned water meets with the hypothesis that uninfected lake amphipods would hide i.e. not be exposed. Uninfected amphipods are able to detect the scent of predators. The change in behaviour from lake amphipods in lake water compared to lake amphipods in predator conditioned water should change from the amphipods spending a larger proportion of time exposed in the water column or on the bottom of the tank whereas the addition of predator conditioned water induces the amphipods to spend a higher proportion of time hidden under the shelter. Also from personal observations it was evident that the amphipods spent more time under the shelters. Therefore it could be predicted that the presence of a predator would induce uninfected amphipods to avoid the predator and so would be less likely to succumb to predation or ingestion by a fish predator. *Pallasea quadrispinosa* inhabits the benthic area of lakes, living in the dark, so chemical stimuli may be a reliable strategy to avoid predators. Chivers (1998) suggested that chemical alarm systems are especially common in aquatic environments. Alarm signals are released when a

predator captures its prey or the prey is threatened. Responses to damage release alarm signals have been reported in gastropods, sea urchins and amphibians. Chivers (1998) also found that cross species alarm signalling is thought to be due to the phylogenetic relationships between the two and their ability to detect bufotoxins in tadpoes and in ostoriophysan fish which contain the alarm pheromone hypoxanthine (3)- oxide. Also when damaged conspecifics are present amphipods such as *Gammarus lacustris* exhibited decreased activity and a tendency to seek refuge.

Burks and Lodge (2002) reviewed chemical cues in aquatic environments. They theorised that *Daphnia* respond to fish kaironomes but it is not known whether they can distinguish between different fish species. Behaviourally, *Daphnia* exhibit generalised responses to fish kaironomes but whether they are the same chemical or different is debatable. Chemists argue that it is possible there are many types of kaironomes but the structure of these are largely not studied or identified. Chemical alarm signals are important in aquatic systems but it is important to note that the substratum affects the ability to detect such chemical cues. For example, crayfish located food more quickly in streams with turbulent flow and cobble than sandy areas. When a predator cue was introduced, the crayfish tended to seek shelter and reduce foraging.

The difference in behaviour between infected amphipods in lake water and in predator conditioned water is consistent with the hypothesis that infected amphipods in lake water spend the largest proportion of time exposed compared to the addition of predator conditioned water. Also from personal observations it was apparent that when the predator conditioned water was added there was an increase in erratic behaviour with the amphipods that had been subjected to predator conditioned water spending more time in the upper surfaces of the tank and skimming across the surface of the water.

Such 'skimming' across the surface of the water has been recorded in other species of amphipods during some experiments. Bethel and Holmes (1973) studied the effect of altered behaviour of *Gammarus lacustris* infected with *Lateriporus spp.* They found that infected amphipods were strongly photophilic and also exhibited

clinging behaviour. These combined behaviours would all increase the chance of predation or transmission to the definitive host.

We may predict that the addition of predator conditioned water and the effect of infection by the parasite *E.borealis* induces the amphipods to alter their behaviour and therefore there is an increased chance of susceptibility to predation and ingestion to the definitive host. So parasites are indeed capable of altering the behaviour of the intermediate host to increase the chance of being passed to the definitive host to reproduce (Moore - 1984).

There was no change in the behaviour of uninfected spring amphipods in lake water and predator conditioned water. This is because the spring amphipods have long had no contact with predators and so are unable to distinguish the predatory scent. Therefore it could be predicted that if spring amphipods were subject to a predator, they would be more likely to be preyed upon than amphipods from the lake. The spring amphipods have evolved in geographical isolation and it could be that their ability to detect or respond to predatory cues has been lost.

The difference between spring amphipods in lake water and uninfected amphipods in lake water is consistent with the hypothesis. It is shown that spring amphipods spend a higher proportion of time exposed in the water column or on the bottom of the tank compared to uninfected lake amphipods. Personal observations showed that spring amphipods spent a larger portion of time exposed than uninfected lake amphipods in lake water. This illustrates that spring amphipods are less receptive to predatory stimulus than uninfected lake amphipods. Therefore we may predict that uninfected lake amphipods actively hide and avoid predators whereas spring amphipods do not exhibit a response to predation.

The difference between spring amphipods in predator water and uninfected lake amphipods in predator conditioned water is consistent with the hypothesis that although there is the presence of predator conditioned water, as with the spring amphipods after the addition of lake water, there is no response to predation. As the

spring amphipods have not been in contact with predators they do not react to the addition of predator conditioned water. They spend a higher proportion of time exposed compared to the uninfected lake amphipods.

Therefore, it may be predicted that the uninfected lake amphipods when exposed to predators are less susceptible to predation than the spring amphipods. Also spring amphipods are more likely to succumb to predation, as they are less able to respond or detect predators. Furthermore, many amphipods in spring systems have disappeared post glacially due to the introduction of fish species. This provides evidence that these spring amphipods would indeed suffer if a predator was introduced into this spring system (Ilpo Hakala pers. comm.). Further studies may yield more information regarding spring habitats and amphipod populations.

There are many methods that parasites employ to alter the behaviour of their hosts ranging from altered behaviour in response to light (Bethel and Holmes 1973) to changes in colour (Bakker et al 1997). My results have shown that predation by fish and parasitism by acanthocephalans has an impact on altered behaviour in the amphipod *Pallasea quadrispinosa*. Increased predation due to parasitism is an effective mechanism to ensure the transmission of a parasite to its definitive host.

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