Department of Physics, Chemistry and Biology

Master Thesis

How two different predators affect size distribution and behavior of an aquatic isopod

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Datum
Date

2011-06-03

Språk
Language

☐ Svenska/Swedish
☒ Engelska/English

☐ ________________

Rapporttyp
Report category

☐ Licentiatavhandling
☒ Examensarbete
☐ C-uppsats
☒ D-uppsats
☐ Övrig rapport

☐ ______________

URL för elektronisk version

ISBN
LITH-IFM-A-EX-–—11/2430—SE

ISRN

Serietitel och serienummer **ISSN**
Title of series, numbering

Handledare
Supervisor: Anders Hargeby

Ort
Location: Linköping

Titel
Title:
How two different predators affect the size distribution and behavior of an aquatic isopod

Författare
Author: Johanna Karlsson

Sammanfattning
Abstract:
The aquatic isopod Asellus aquaticus can in some lakes be found as two different ecotypes; one in the habitat dominated by reed and one in stonewort stands. These ecotypes have been shown to differ in size, color and behavior. The reed ecotype is larger, darker and more active compared to the stonewort ecotype. In the two habitats there are different dominating predators: Invertebrate predators in the reed habitat and fish in the stonewort habitat. This project aims to examine how the presence of invertebrate predators and fish affect the two ecotypes of the isopod in regard to behavior and size composition in different substrates. To examine the effect on behavior the activity of isopods collected from Lake Tåkern was measured without and with chemical cues from perch and damselfly larva. The result of the behavior experiment showed no difference between the ecotypes nor the treatments. The lack of differences in the behavior indicates that there could be a variation between lakes. The effect on the size composition was examined by subjecting groups of isopods to predation by perch or damselfly larva in different substrate. The mean length of the group was measured before and after the trials. The size decreased significantly for the reed ecotype in stonewort substrate when subjected to predation by perch. The size for the stonewort ecotype increased significantly in reed substrate with damselfly larva as predator. The effect on size supports that the predators are the cause of the size difference between the ecotypes.

Nyckelord
Keyword:
Asellus aquaticus, behavior, ecotypic divergence, predation, size selection

Contents

1 Abstract

The aquatic isopod *Asellus aquaticus* can in some lakes be found as two different ecotypes; one in the habitat dominated by reed and one in stonewort stands. These ecotypes have been shown to differ in size, color and behavior. The reed ecotype is larger, darker and more active compared to the stonewort ecotype. In the two habitats there are different dominating predators: Invertebrate predators in the reed habitat and fish in the stonewort habitat. This project aims to examine how the presence of invertebrate predators and fish affect the two ecotypes of the isopod in regard to behavior and size composition in different substrates. To examine the effect on behavior the activity of isopods collected from Lake Tåkern was measured without and with chemical cues from perch and damselfly larva. The result of the behavior experiment showed no difference between the ecotypes nor the treatments. The lack of differences in the behavior indicates that there could be a variation between lakes. The effect on the size composition was examined by subjecting groups of isopods to predation by perch or damselfly larva in different substrate. The mean length of the group was measured before and after the trials. The size decreased significantly for the reed ecotype in stonewort substrate when subjected to predation by perch. The size for the stonewort ecotype increased significantly in reed substrate with damselfly larva as predator. The effect on size supports that the predators are the cause of the size difference between the ecotypes.

Keywords: *Asellus aquaticus*, behavior, ecotypic divergence, predation, size selection

2 Introduction

One of the driving forces of evolution is predation: Predators affect the composition of the prey community as well as prey morphology and behavior (Wellborn et al., 1996; Blumenshine et al., 2000; Stoks et al., 2003; Yoshida et al., 2003). But all predators do not hunt in the same way and thus affect the prey in different ways.

In freshwater habitats the two key groups of predators are fish and invertebrate predators (Wellborn et al., 1996). The two predator groups are dominating in different habitats within a lake: Fish in open water and invertebrate predators in dense macrophyte beds, e.g. in the littoral zone (Wellborn et al. 1996; Tolonen et al. 2003). Generally their hunting strategies are different: Fish search for prey actively and use vision to detect it. They can also pursue it at high speed, should their first attempt fail (Wellborn et al., 1996). Invertebrate predators on the other hand often use "sit-and-wait" as hunting tactics; they ambush their prey. Should they fail to capture it in the first attempt their pursuit speed is not as great as the fish's. The invertebrate predators primarily use tactile cues to detect the prey (Wellborn et al., 1996), but they may also use other means of detection such as vision (Rebora et al., 2004).

Another difference between predatory fish and predatory invertebrates is the preferred size of prey. Fish prefer large prey (Wellborn et al. 1996; Blumenshine et al., 2000; Andersson, 2010), while the invertebrates are often more limited by their gape-size and thus feeds on smaller prey (Wellborn et al. 1996; Scott & Murdoch, 1983). This size preference creates a difference in invertebrate prey size between habitats (Tolonen, 2003). In the littoral zone, where invertebrate predators dominate, the community consists of large species. In open water the invertebrate prey are smaller, since fish target large individuals (Wellborn et al. 1996; Blumenshine et al., 2000; Tolonen, 2003; Andersson, 2010).

There are several strategies a prey can use to avoid predation in the presence of predators. One of them is to change behavior (Sih et al., 2000). Some species adapt their behavior depending on what kind of predator that is present and what it eats (Wisenden et al., 1999; Åbjörnsson et al. 2000; Turner et al., 2006). To avoid predation by predators who detect prey with their vision, e.g. fish, low activity is a good strategy (Wellborn et al., 1996). With invertebrate predators low activity is not as beneficial, thus prey living mainly with invertebrate predators has in general higher activity than those living with fish.

An antipredator behavior has a rather high cost so it is important for the prey to be able to identify when a predator is present or not (Sih, 1992). While aquatic predators mainly use vision and tactile cues (Wellborn et al., 1996; Rebora et al., 2004), the most important mean for prey to detect the predators in aquatic environments are chemical cues (Wisenden et al., 1999; Brönmark & Hansson, 2000). There are two types of predator cues: Cues released directly by the predator and cues from injured prey (Brönmark & Hansson, 2000). Prey can identify different predators and what they eat through these cues and so they adapt their behavior (Åbjörnsson et al., 2000; Mortenson & Richardson, 2008). Since predators have different dominating habitats, the prey will behave different depending on where they live (Wellborn, 1996).

When a species occupy several different habitats it can diverge into different ecotypes (Turesson, 1922). An ecotype is ”a distinct form or race of a plant or animal species occupying a particular habitat” (Oxford Dictionary of English, 2011).

The aquatic isopod *Asellus aquaticus* can in some places in Sweden be found as two ecotypes within the same lake (Hargeby et al 2005). One of the ecotypes occurs frequently in habitats dominated by emergent vegetation, such as reed (*Phragmites communis*) (from now on the “Reed habitat”) and has a dark pigmentation and is larger than the other ecotype. The other ecotype is common in habitats where submerged vegetation, particularly stoneworts (*Chara* spp.), is a dominating bottom substrate. In this habitat, the stonewort habitat, the dominating ecotype is lighter pigmented and is smaller than the reed isopods. For two Swedish lakes (L. Krankesjön and L. Tåkern) it has been shown that isopods colonizing recently established stands of stonewort change towards the stonewort ecotype over generations (Hargeby et al. 2004). The ecotypic divergence emerges independently in each lake (Eroukhmanoff et al., 2009a). In both lakes the reed ecotype is the source population for the stonewort ecotype. The dominating predator is likely to differ in the two habitats; in the reed stands the main predators are invertebrates while the main predators in stonewort stands are fish (Hargeby et al., 2005). In addition to the body-size and the cryptic coloring the ecotypes also have other antipredator adaptations (Eroukhmanoff & Svensson, 2009; Eroukhmanoff et al., 2009b). The reed ecotypes body shape is more adapted to fast movements in water and it has a higher endurance and speed than the stonewort ecotype (Eroukhmanoff & Svensson, 2009). In a study of the predator avoidance of the both ecotypes the stonewort ecotype seemed to have lost some of the ability to identify an invertebrate predator as a predator (Eroukhmanoff et al., 2009b), though a similar study with fish has not been done.

Ecotypic divergence is a first step towards speciation (Dhuvetter et al., 2007; Rundle et al., 2000). If we can understand the mechanisms behind the emergence of ecotypes, we will have come one step further towards the understanding of evolution. One of the things that can contribute to the divergence is predation (Nosil & Crespi, 2006).

The aim of the project is to examine how the presence of fish and invertebrate predators affect the two ecotypes of the isopod *Assellus aquaticus* in regard to behavior and size composition of the population in different substrates. I expect that the fish will prey on big isopods and the invertebrate predator will prey upon small. When the ecotypes are in their natural substrate with their natural predator the effect on size will be less obvious, due to better adapted isopods. The stonewort ecotype will not recognize invertebrate predators as predator, while the reed ecotype will recognize both fish and invertebrate predators.

3 Methods

The isopods in the experiments were water sowbugs (*Asellus aquatiqus)*, from *the* reed and stonewort stands in L. Tåkern.

3.1 Predator avoidance behavior

Two different setups were used to examine the effect of chemical cues from perch and damselflies on the isopods' activity. A summary of the number of replicates and isopods in each experiment can be found in Table 1.

To remove predator cues the water used in both setups stood for at least two days in room temperature. All equipment was cleaned with 96% ethanol, before it was used.

The bottom of the isopod arena was covered with a grid of lines 2 cm apart in both setups. When the films were analyzed, the number of lines the isopod had passed fully was counted. When the isopod passed several lines at the same time (e.g. passing over a corner) only one line was counted.

Table 1. Summary of the behavioral experiments preformed.

	Ecotype	**Predator cue**	**Nr of replicates**	**Nr of isopods/replicate**
Setup 1	Reed	Damselfly	10	1
	Stonewort	Damselfly	10	1
	Reed	Perch	10	1
	Stonewort	Perch	10	1
Setup 2	Reed	Damselfly	6	3 – 5
	Stonewort	Damselfly	6	3 – 5
	Reed	Perch	6	3 – 5
	Stonewort	Perch	6	3 – 5

3.1.1 Setup 1

The isopods were collected two days before the experiment and were kept without the presence of predator cues. They were placed individually in a 14x7.5 cm container with 2 dl of water in it. After 1 min of acclimation 3.5 ml of Tåkern water was added as a control. To mix the water in the container with the added water, it was stirred four times. After the disturbance another 5 min of acclimation followed. Then the isopods' movements were recorded in 5 min. A volume of 4 ml of water with cues from either perch or damselfly was added the same way as the control. 5 min after the addition of cues the isopods movements were recorded for another 5 min. Female isopods with eggs were not used in the experiment.

3.1.2 Setup 2: The gradient setup

The isopods were collected two weeks before the experiment and were kept without the presence of predator cues during that time. The isopods were placed in a 20x10 cm arena. The arena was part of a larger container, 50x39 cm, and one side of the arena was connected to the rest of the container by a fine-mesh net, which created a gradient of predator cues (Fig 1). The total amount of water in the container was 9 L. The water was a mix of water from L. Tåkern and declorinated tap water with a ratio of 1:1. In the container there was soft aeration.

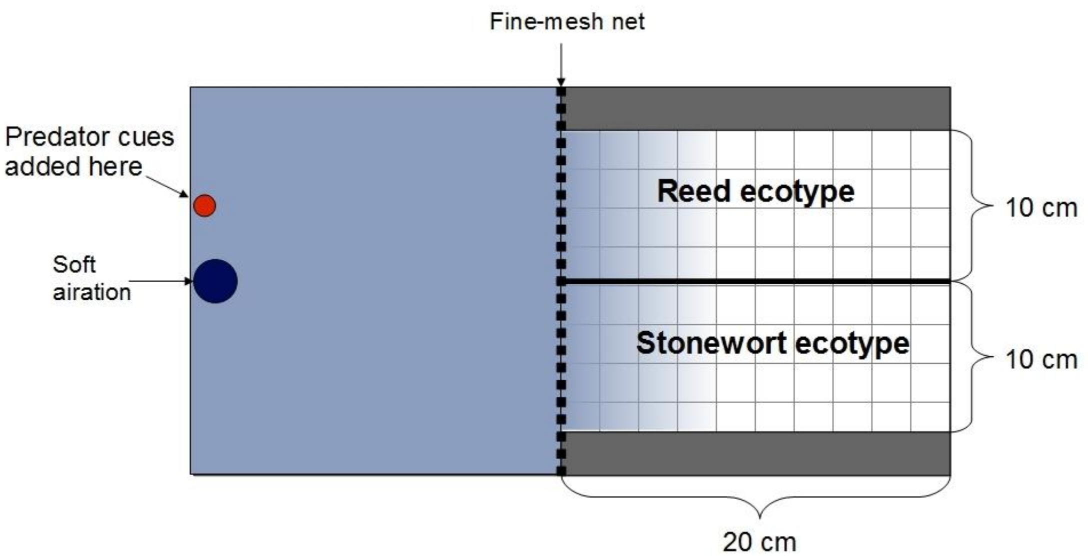

Fig 1. The setup for the behavioral study. The total size was 50x39 cm and was filled up with 9 liters of water. The blue represents the predator cues.

Three to five isopods were used in each trial and treatment. Before the observation started the isopods were allowed to acclimatize to the arena for 30 min. When that time had passed 1.5 dl of declorinated tap water was added to the part of the container farthest away from the isopods' arena as control (Fig 1). 5 min after the water was added the isopods' were recorded during 5 min. Then 1.5 dl water with predator cues from either fish or damselfly larva was added in the same way the tap water was added. With the damselfly cues three damselfly larvae were also added. After 5 minutes from the addition of the predator cues, the isopods were filmed for another 5 min. Female isopods with eggs were not used in the experiment.

3.1.3 Statistical analyzes

In Setup 2 the mean movement in each replicate was used for the statistical analyzes. Otherwise the same statistical analyzes were used for both setups.

A two sample t-test was used to detect any difference in activity before and after the addition of predator cues for each group – reed ecotype with fish cue, reed ecotype with damselflies, stonewort ecotype with fish cue and stonewort ecotype with damselflies.

The difference in movement before and after the addition of predator cues were calculated as 'movement after' – 'movement before'. This difference was used in a two-way ANOVA to analyze the difference between the predator treatments and the ecotypes.

3.2 Survival study

The isopods were subjected for predation by perch and damselfly larva to examine the predators' effect on the size distribution. A summary of the number of replicates and isopods in each experiment can be found in Table 2.

Table 2. Summary of the behavioral experiments preformed.

Predator	Ecotype	Substrate	Nr of replicates	Nr of isopods/replicate	Length study
Damselfly	Reed	No substrate	18	8	Yes
	Stonewort	No substrate	18	8	Yes
	Reed	Reed	4	40	Yes
	Stonewort	Reed	4	40	Yes
	Reed	Stonewort	4	40	No
	Stonewort	Stonewort	4	40	No
Perch	Reed	Reed	4	25	Yes
	Stonewort	Reed	4	25	Yes
	Reed	Stonewort	4	25	Yes
	Stonewort	Stonewort	4	25	Yes

3.2.1 Damselfly without substrate

Eight isopods, of either stonewort or reed ecotype, were photographed and placed together with a single damselfly larva in an open plastic jar with diameter of 10 cm with no substrate. Before the trial the damselfly had been starved for approximately 24 h. After 24 h the damselfly larva was removed and the surviving isopods counted and photographed again. From the photographs the lengths of the isopods were decided and for each replicate a mean value for the isopod length was calculated before and after the trial. A total of 18 damselfly larvae were used with the head width of 4.1±0.3 mm (determined through photographs). Each damselfly larva was used twice, once with the reed ecotype and once with the stonewort ecotype, giving 18 replicates for each ecotype. To compare the number of surviving isopods between the two ecotypes a paired t-test was used, while a two-way ANOVA was used to compare differences between the mean length before and after the trial and the difference between the ecotypes.

3.2.2 Damselfly with substrate

To examine effects of substrate and isopod ecotype on survival when exposed to damselfly larvae, 40 isopods of the same ecotype were put in a 1 L aquarium together with three damselflies for four days. The damselfly larvae had been starved for approximately 24 h before trial. The substrate was, when water was added, an approximately 2 cm layer of either reed or stonewort. In the reed substrate all stem pieces where the isopods could hide inside were removed. Four replicates were done for each group (i.e. the four combinations of ecotype and substrate). After the trial the surviving isopods in each group was counted. To be able to see what effect the damselfly larvae had on the isopod size in the reed substrate, all the isopods in the reed substrate were photographed before and after the trial. Only the reed substrate was chosen for the length study as the damselflies effect in the stonewort is negligible and due to time constraint for the image analyzes. The number of surviving isopod was tested with two-way ANOVA, while the difference in mean length was tested with a paired t-test for each group.

3.2.3 Fish with substrate

A 7,5x14x5 cm container was put in a 17x14.5x5 cm container. The bigger containner was filled with 2 cm light sand and the smaller one with 0,5 cm black sand for the reed substrate and light for the stonewort substrate. The amount substrate in the small container was just enough to cover the bottom and the surrounding bigger container was filled with the same type of substrate as the small container (Fig 2). 25 isopods were placed in the small container. The setup was then placed in a 79x57x42 cm aquarium filled to half with water. In the aquarium there were 5-6 perch (*Perca fluviatilis)* with the length 94±8 mm that had learned to associate the setup with food. The trial was observed the whole time and when the perch had done 13 seemingly successful attacks the trial was finished and the container removed from the aquarium. The length of a trial varied from one to four minutes. Each trial ran in a set of four (all ecotype-substrate combinations) that were all subjected to the same combination of perch individuals. Totally there were four replicates. In every replicate there was a different combination of perch individuals and a different order for which the groups were subjected to the perch. The isopods were photographed before and after the trial to decide the mean length in each replicate before and after the trial. The difference was analyzed with a paired t-test for each ecotype-substrate combination.

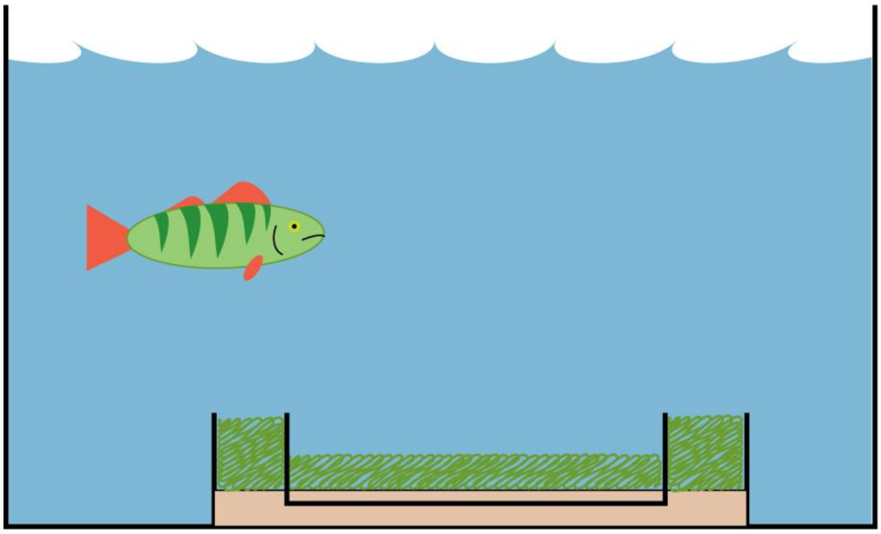

Fig 2. A cross section of the set up for the fish trials. The brown colour represents sand and the green substrate.

3.4 Photographing, image analysis and software

The camera used for filming was a Canon PowerShot SX200IS.

For photographing a Nikon E4500 was used. The isopods were placed individually in petri dishes and each petri dish was placed on a millimeter paper while photographing. The photographs were then used to decide the length of the isopods.

The software used to analyze the pictures were ImageJ 1.43u (NIH, USA, downloaded from http://rsbweb.nih.gov/ij/, December 2010). This program measure, in pixels, the length between two points defined by the user. To transform the pixels to millimeters 10 mm was measured as a reference in one photograph for each set of photographs (i.e. one ecotype, for one treatment, in one trial, either before or after) and used in the entire set. The body lengths of the isopods were measured (Fig 3) and converted to millimeters.

The error in measurement was estimated by measure the length of five randomly selected isopods from each ecotype eight times. The mean standard deviation, 0.26, from these data was the estimated error of measurement.

All statistical analyzes were done in Minitab 15.1.30.0.

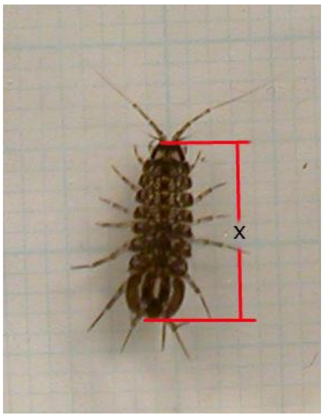

Fig 3. The isopod's body length, x, was measured according to the picture.

4 Results

4.1 Predator avoidance behavior

4.1.1 Setup 1

The activity decreased significantly for the stonewort ecotype when chemical cues from damselfly larva was added (paired t-test: t = 2.42, p = 0.039) (Fig 4). No other changes in activity could be detected.

No difference in activity could be detected for neither the ecotypes nor the predator cues (Table 3).

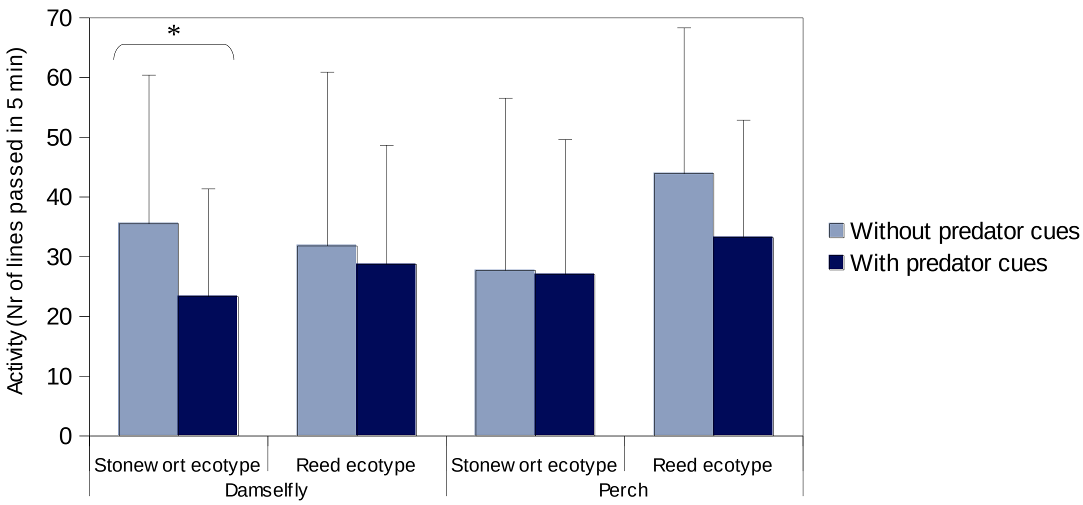

*Fig 4. The activity during 5 min before and after the addition of chemical cues from either damselfly or perch in Setup 1. (n = 10 for all treatments, * denotes p<0.05 tested with paired t-test). Error bars show standard deviation.*

Table 3. Result of two-way ANOVA on activity in number of lines passed in five minutes as depending variable, and ecotype and predator cue as factors for Setup 1.

Source	DF	SS	MS	F	p
Ecotype	1	2	2,025	0	0,947
Predator	1	912	912,025	2,02	0,163
Ecotype x Predator	1	38	38,025	0,08	0,773

4.1.2 Setup 2: The gradient setup

No significant change in activity could be detected with a two sample t-test in any of the treatments after the predator cues were added (Fig 5). Neither could any significant effect of the ecotype nor the predator be detected with a two-way ANOVA (Table 5).

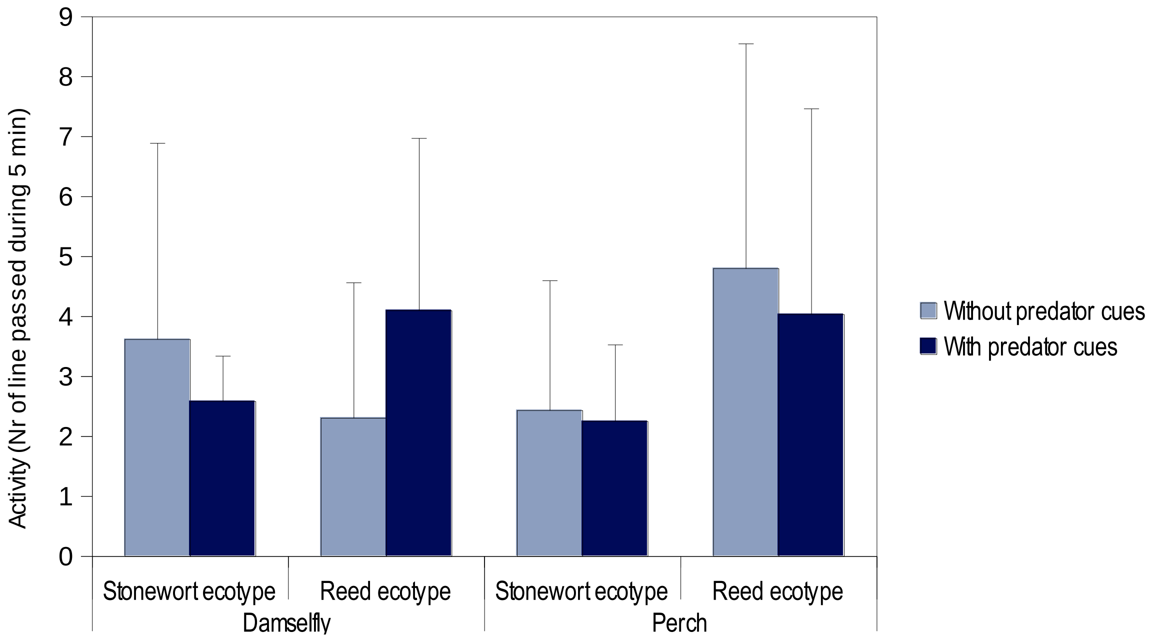

Fig 5. The activity during 5 min before and after the addition of chemical cues from either damselfly or perch in Setup 2 with a gradient of cues. No significant change in activity could be detected (n = 10 for all treatments). Error bars show standard deviation.

Table 4. Result of two-way ANOVA on activity in number of lines passed in five minutes as depending variable, and ecotype and predator cue as factors for Setup 2.

Source	Df	SS	MS	F	p
Ecotype	1	7,526	7,5264	1,45	0,242
Predator	1	4,386	4,3861	0,85	0,369
Ecotype x predator	1	17,442	17,4422	3,37	0,081

4.2 Survival study

4.2.1 Damselfly without substrate

When comparing the survival after 24 h together with a damselfly larva, without any substrate, there was no significant difference between the two ecotypes (paired t-test: t= 1.77, p=0.094)(Fig 6).

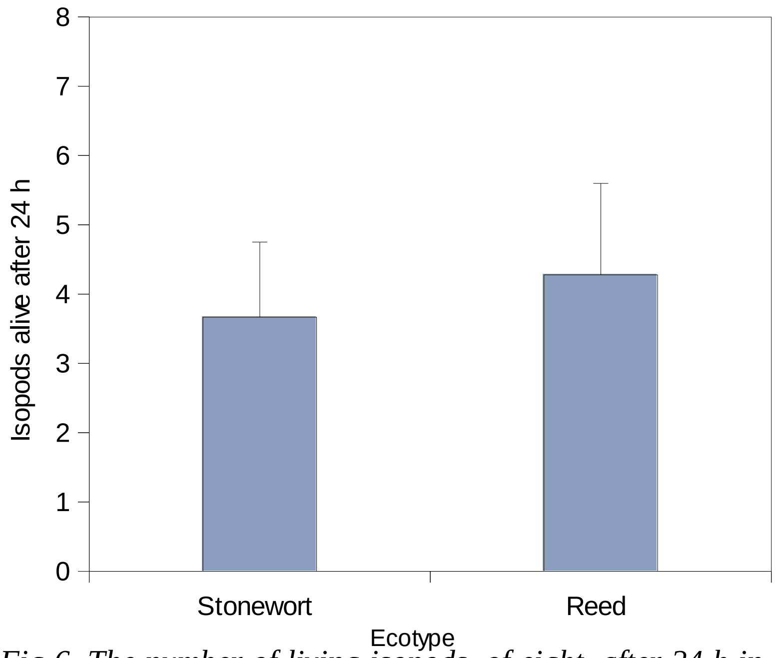

Fig 6. The number of living isopods, of eight, after 24 h in an arena without substrate with a damselfly larva. (n =18).

The isopods' length was measured before and after the trial. The mean length of the isopods was then calculated for before and after (Table 5). To gain a comparison where the size of the damselfly larva was taken in to consideration, the ratio of the head width of the damselfly through the length of the isopod (HWD:LI) was also calculated (Table 5).

Table 5. Mean length of the isopods for each replicate before and after the trial with damselfly and no substrate. It also shows the mean of the ratio Q, which is the head width of the damselfly (HWD) through the length of the isopod (LI), before and after the trial. 'SD min-max' is the span in which the replicates standard deviation was within.

Ecotype	N	Mean length before (mm)	SD before min – max	Mean length after (mm)	SD after min – max	Mean Q before (HWD:LI	SD Q before min – max	Mean Q after (HWD:LI)	SD Q after min – max
Stonewort	18	6.3	1.0 – 1.8	7.3	0.1 – 2.2	0.70	0.13 – 0.23	0.58	0.01 – 0.23
Reed	18	7.1	1.0 – 2.3	8.3	0.3 – 2.2	0.63	0.12 –0.23	0.52	0.02 – 0.14

A two-way ANOVA showed significant increase of the mean length of the isopods before and after the trial (F=131.58, df=1, p<0.001). There was also a significant difference between the ecotypes, with the reed ecotype being larger (F=88.47, df=1, p<0.001), but no interaction between the two factors (F=1.59, df=1, p=0.211) (Table 6).

The difference in the HWD:LI ratio before and after the trials was also analyzed with a two-way ANOVA . It showed significant difference in length both before and after the trial (F=92.51, df=1, p<0.001) and between the ecotypes (F=31.12, df=1, p<0.001) (Table 6). There was no significant interaction between the two factors (F=0.04, df=1, p=0.837).

4.2.2 Damselfly with substrate

The difference in how many isopods, of 40, had survived in each treatment was tested with a two-way ANOVA. The result showed no significant difference between ecotypes (F=0.10, df=1, p=0.757), while there was a significant difference between the substrate (F=1.44, df=1, p=0.005) (Fig 7). There were no significant interaction between the factors (F=0.55, df=1, p=0.474).

In the two groups with reed substrate the isopods length were measured and the mean length before and after the trial were calculated for each replicate. The increase in mean length was significant for the stonewort ecotype, but not for the reed (Table 6).

Table 6. The mean lengths before and after predation trial with damselfly larvae in reed substrate. N is the number of replicates. 'SD min-max' is the span in which the replicates standard deviation was within. The difference between the mean length before the trial and after the trial was tested with a paired t-test.

Ecotype	N	Mean length before (mm)	SD before min – max	Mean length after (mm)	SD after min – max	t-value	p
Stonewort	4	6.3	1.3 – 1.4	6.8	1.2 – 1.4	-4.63	0.019
Reed	4	6.7	1.0 – 1.7	7.0	1.2 – 1.7	-1.8	0.173

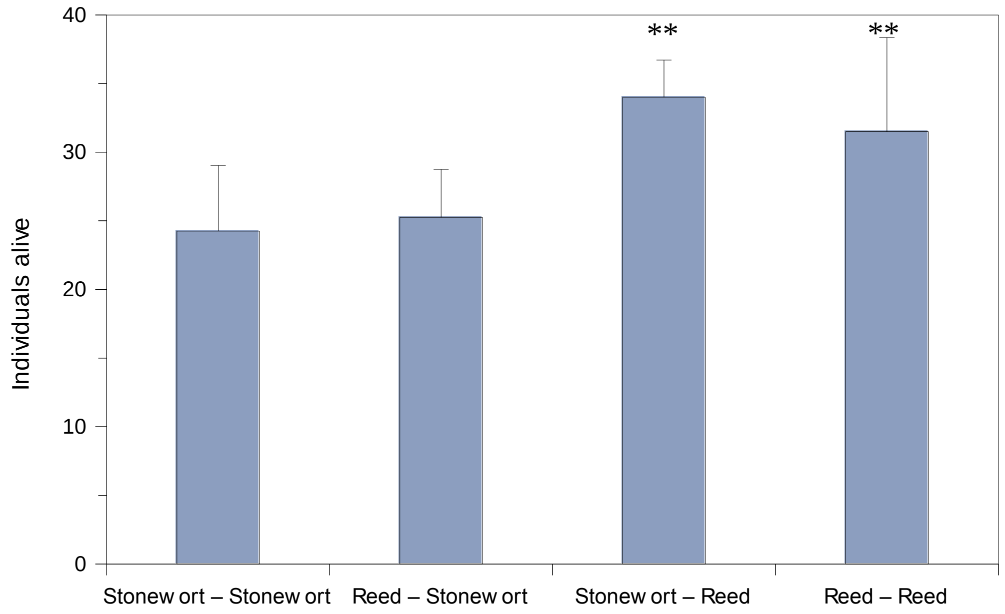

*Fig 7. Number of surviving isopods, of 40, for each group after predation trials with damselfly larvae. Categories are written as"ecotype-substrate" and the error bars show the standard deviation. ** denotes a significantly higher survival ($p<0.005$).*

4.2.3 Perch with substrate

The difference in mean length before and after the trial was tested with a paired t-test for each group. The results showed a significant decrease of the mean length for the reed ecotype in the stonewort substrate (Table 7).

Table 7. The mean lengths before and after predation trial with perch. N is the number of replicates. 'SD min-max' is the span in which the replicates standard deviation was within. The difference between the mean length before the trial and after the trial was tested with a paired t-test.

Ecotype	Substrate	N	Mean length before (mm)	SD before min – max	Mean length after (mm)	SD after min – max	t-value	p
Stonewort	Stonewort	4	6.1	1.1 – 1.5	6.0	1.0 – 1.8	2.83	0.066
Stonewort	Reed	4	6.2	1.1 – 1.3	6.2	1.1 – 1.5	-0.30	0.783
Reed	Stonewort	4	7.4	1.5 – 2.0	7.0	1.4 – 2.0	15.59	0.001
Reed	Reed	4	7.3	1.5 –1.7	7.0	1.6 – 1.9	1.42	0.250

5 Discussion

5.1 Antipredator behavior

The activity did not differ between the two ecotypes nor between the predator cue treatments. It was expected that the reed ecotype would have higher activity over all since other studies have shown that there is a difference between them in terms of physical performance, exploratory behavior and predator recognition (Eroukhmanoff & Svensson, 2009; Eroukhmanoff et al, 2009b). While physical performance was tested on isopods from both L. Krankesjön and L. Tåkern, exploratory behavior and predator recognition has only been tested on animals from L. Krankesjön.

The lack of reaction of the cues from predators might be because they're a part of the isopods everyday life and thus don't think they're strange or something to be alarmed of: Antipredator behavior do have a high cost (Sih, 1992). The stonewort ecotype did react to the damselfly cues and this supports this theory since damselfly larvae are not abundant in the stonewort habitat. This new”scent” could have made the stonewort isopods more vary.

Although if it only were a lack in response to predator cues the reed ecotype would still have been more active than the stonewort according to earlier studies (Eroukhmanoff & Svensson, 2009; Eroukhmanoff et al, 2009b). The earlier behavior studies have been done on isopods from L. Krankesjön. As the ecotypes emerge independently in each lake (Eroukhmanoff et al., 2009a) there might be a difference in behavior – and other attributes – between the isopods in the lakes due to historical differences.

Another thing that could have affected the results is that the isopods were not able to see the predators. It might be that the isopods need visual contact to realize the threat. Although this is unlikely since much communication in aquatic systems happens through chemical cues (Brönmark & Hansson, 2000). But as stated before; even if this has an effect it should only have affected the difference between the treatments, not between the ecotypes before any predator cues were added.

5.2 Survival with damselflies

When the damselflies had a smorgasbord of isopods to choose from, without any substrate where the isopods could hide, they foremost choose the smaller individuals as prey. Since many invertebrate predators are gape-limited (Scott & Murdoch, 1983), as are the damselflies (unpublished data), this was expected. The isopods' relative size, measured with the ”head width damselfly: isopod length”-ratio, gave an insight in how large isopod the damselfly larvae can handle (unpublished data).

In a habitat where the dominating predators are gape-limited, such as the reed habitat, it is possible to grow into a size-refuge as it reduce the risk of becoming prey to those predators (Persson et al, 1996; Wong et al, 2010). In contrast, the isopods in the stonewort habitat cannot outgrow their main predator and hence size is not as important.

There was a tendency to a difference in number of individuals eaten in each ecotype, with more individuals eaten of the stonewort ecotype. This is most likely connected to the size difference between the two ecotypes.

The results were different in the experiments with substrate. In the reed substrate only the stonewort ecotype showed a difference in size. Since size differed for both ecotypes without any substrate we can conclude that something makes it easier for the damselflies to choose prey more discriminative. If the behavior between the two ecotypes differed it might give a different encounter rate for the two. Although the behavior study showed no difference in activity, there are other factors to take into consideration, such as predator recognition. The stonewort ecotype has failed to identify a dragonfly larva, another invertebrate predator present in reed habitat, as a predator in a previous study (Eroukmanoff et al. 2009b). This leads to that the stonewort isopod will go closer to the predator than the reed isopod. The reed ecotype is also faster and has higher endurance (Eroukhmanoff & Svensson, 2009) which could grant them a higher escape rate during an attack.

Another factor that could play a part in the size selection is visibility: The reed substrate is dark and while the reed ecotype has cryptic coloring for it and the stonewort ecotype has not (Hargeby et al, 2005). The class Odonata, to which the damselflies belong, have good vision (Land, 1997) and the larvae of a species belonging to the class has been showed to use both mechanical and visual cues to catch their prey (Rebora et al., 2004). This speaks for that the damselfly larvae also use vision as a cue when they hunt and that the isopods visibility is important.

There was no difference in survival between the ecotypes, but survival was generally higher in the reed substrate. This substrate gives the isopods more hiding spaces and they can sit tight to the substrate (personal observation). The stonewort substrate, on the other hand, makes the isopods sit very exposed. In the wild, invertebrate predators do not have that a big impact on the prey in the stonewort stands, since they are not abundant there (Tolonen et al., 2003).

5.3 Survival with perch

In the perch experiment the mean length decreased for the reed ecotype in the stonewort substrate. The most energy efficient for a perch is to take a big isopod rather than a small one (Persson & Greenberg, 1990). The visibility of the isopods is the most probable explanation to the results in this experiment: The reed isopods lack cryptic coloring for the stonewort habitat and hence become more exposed to the perch, which search for their prey visually (Wellborn et al. 1996). The behavior of the isopods can be disregarded since each trial lasted for a short amount of time (< 4 min) and the perch search actively for the prey.

5.4 Conclusion

The behavior, measured in activity, did not differ between the ecotypes. This makes it possible to conclude that the predators do not affect a difference in behavior as the difference isn't there. This also indicates that there may be a difference between ecotypes in different lakes.

In their original substrate the predators affect the size of the non-native ecotype in a way that drives it towards the size of the native ecotype. This size pattern, with large individuals in among the reed habitat and smaller in more open water, is not only limited to the freshwater isopod. It can be seen with different species where the large species lives among the emerge macrophytes and the smaller in more open water (Wellborn et al. 1996; Blumenshine et al., 2000; Tolonen, 2003). This study supports the theory that this pattern is, at least partly, caused by predators.

6 Acknowledgements

I want to thank my supervisor Anders Hargeby for guiding and helping me on this long and sometimes difficult trek. I also like to thank Fabrice Eroukhmanoff for lending me equipment and sharing his expertise.

Two other people who have been a great help on my metaphorical travel to greater knowledge are Olle Samuelsson and Karin Lundengård. Olle and I literally travelled miles, some of them in a row boat, to get my isopods. Without him the trips to Lake Tåkern would have been both difficult and boring.

Karin helped me through the most tricky and treacherous paths that is statistics and also had a lot of good inputs on my writing.

Thanks go also to my examiner Karl-Olof Bergman and my two opponents Helena Johansson and Anna Senior for their helpful input. During a pause in the trek, you got me inspired to keep going again.

During the last steep path I had a lot of help from my sister, especially in the visual parts not shown in this report (The poster, leaflet and presentation).

I also want to thank Jim Butcher, JK Rowling and Credissa Cowell for being such awesome authors. Without the company of their audiobooks I would've gone insane during the long hours in the laboratory.

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