Selective predation by perch (*Perca fluviatilis*) on a freshwater isopod, in two macrophyte substrates

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<td>Selective predation by perch (Perca fluviatilis) on a freshwater isopod, in two macrophyte substrates. Recent studies show that populations of the freshwater isopod Asellus aquaticus L. can rapidly become locally differentiated when submerged stonewort (Chara spp.) vegetation expands in lakes. In the novel Chara habitat, isopods become lighter pigmented and smaller than in the ancestral reed stands. In this study, I used laboratory experiments to investigate if selective predation by fish could be a possible explanation for these phenotypic changes. Predation from fish is generally considered to be a strong selective force on macroinvertebrate traits. In the first experiment I measured perch handling time of three size classes of Asellus to see which size used in the experiments that would be the most profitable to feed upon. No difference in handling time was detected between prey sizes, hence the largest size would be the most beneficial to feed upon. In the second experiment I let perch (Perca fluviatilis L.) feed on a mixture of Asellus phenotypes in aquaria manipulated to mimic the substrates in either the Chara or the reed habitats. Remaining isopods were significantly smaller and lighter pigmented in the fish aquaria than in the controls, showing that the perch preferred to feed on large and dark individuals. In the Chara habitat, selection on isopod pigmentation was according to what could be expected from background matching, but in the reed habitat selection was quite the opposite. These results support the hypothesis that predation from fish is a strong selective force behind the rapid local adaptation seen in Asellus populations in the novel Chara habitat.</td>
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Abstract

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References
Selective predation by Perch (*Perca fluviatilis*) on a freshwater isopod, in two macrophyte substrates.

1 Abstract
Recent studies show that populations of the freshwater isopod *Asellus aquaticus* L. can rapidly become locally differentiated when submerged stonewort (*Chara* spp.) vegetation expands in lakes. In the novel *Chara* habitat, isopods become lighter pigmented and smaller than in the ancestral reed stands. In this study, I used laboratory experiments to investigate if selective predation by fish could be a possible explanation for these phenotypic changes.

Predation from fish is generally considered to be a strong selective force on macroinvertebrate traits. In the first experiment I measured perch handling time of three size classes of *Asellus* to see which size used in the experiments that would be the most profitable to feed upon. No difference in handling time was detected between prey sizes, hence the largest size would be the most beneficial to feed upon. In the second experiment I let perch (*Perca fluviatilis* L.) feed on a mixture of *Asellus* phenotypes in aquaria manipulated to mimic the substrates in either the *Chara* or the reed habitats. Remaining isopods were significantly smaller and lighter pigmented in the fish aquaria than in the controls, showing that the perch preferred to feed on large and dark individuals. In the *Chara* habitat, selection on isopod pigmentation was according to what could be expected from background matching, but in the reed habitat selection was quite the opposite. These results support the hypothesis that predation from fish is a strong selective force behind the rapid local adaptation seen in *Asellus* populations in the novel *Chara* habitat.

Key words: fish predation, divergent selection, habitat-specific adaptation, background matching.

2 Introduction
Predation from fish is an important factor behind the composition of species and phenotypes we find in an invertebrate community. Especially taxa that are large, active and free-swimming are sensitive to fish predation, and therefore tend to be fewer in fish containing than in fishless lakes (Wellborn et al. 1996, Schilling et al. 2009). Depending on which habitat we study, certain species and phenotypes tend to dominate by numbers. The complexity of the habitat is probably important for this. In unstructured habitats the fish is an effective predator and has no problem in gathering information on which type of prey it can choose from (Diehl 1992). Both efficiency and information on prey abundance are crucial in determining how selective the predator can afford to be. Research regarding fish predation on invertebrate prey addresses both ecological questions with applications in conservation and fishing (Schilling et al. 2009), as well as evolutionary questions concerning natural selection (Rundle et al. 2003).

Selective forces, like predation, can create rapid local adaptation. One such example is the differences observed between different populations of the benthic freshwater isopod *Asellus aquaticus* within two Swedish lakes-, Lake Tåkern (58°21’ N, 14°50’ E) and Lake Krankesjön (55°42’ N, 13°28’ E). Both lakes are shallow and have experienced dramatic shifts from a phytoplankton dominated state towards a macrophyte dominated state during the last 25 years (Hargeby et al. 2007). These shifts have led to the colonization of previously bare sediment areas by submerged macrophytes, particularly the stoneworts (*Chara tomentosa* L.). This novel habitat quickly became colonized by *A. aquaticus* that most likely migrated from the reed (*Phragmites australis*) stands in both lakes. After colonization a rapid habitat specific adaptation in the newly established *Asellus* populations were observed. Isopods in the novel stonewort habitat were brighter and smaller.
than in the reed source habitat (Hargeby et al. 2004, 2005). These differences presumably are the result of different selective pressures in the two habitats (Eroukhmanoff et al. 2009). Similar habitat specific adaptations are known in North American *Hyalella* amphipods, and are thought to have arisen due to alternative predatory regimes (Wellborn et al. 2005).

Aquatic isopods are preyed upon by invertebrate predators, as well as by waterfowl and fish (Persson 1983, Rask and Hiisiuori 1985, Jormalainen and Merilaita 1995, Wagner and Hansson 1998). Invertebrate predators often have tactile sensory systems and are less efficient in handling large prey. Fish on the other hand are in most cases visually oriented and less gape limited when feeding on invertebrate prey, resulting in selection for smaller reproductive size in invertebrate prey (Wellborn et al. 1996, Schilling et al. 2009). Considering these two groups of predators and the observed size difference in isopods between the two habitats one can hypothesize that predation by fish is more intense in the stonewort habitat. This seems to be supported by several studies that found an increasing gradient of fish predation from shore-bound reed stands to open water areas (Wagner and Hansson 1998, Blumenshine et al. 2000, Tolonen et al. 2003).

In the colour polymorphic isopod *Idotea baltica*, phenotypes resembling the background more closely were significantly less susceptible to predation by perch (Merilaita 2001). Perch is a common species in Lake Tåkern and Lake Kränkesjön, and is known to feed on isopods (Rask and Hiisiuori 1985). The species is also known to be an efficient forager in dense vegetation (Diehl 1988). Cryptic pigmentation as a defense from visually hunting fishes also fit the different dominating color morphs in *A. aquaticus*, since the bottom in the reed stands is dark and the stonewort vegetation is light green or yellow.

To test if selective predation by fish is the likely force behind the observed habitat-specific adaptations in these *Asellus* populations I decided to do a laboratory study. In this study I did experiments with perch as predator and *Asellus* as prey in aquaria manipulated to mimic the substrate in the two habitats.

I also did an experiment where I measured perch handling time for three size classes of *Asellus*. In this experiment I wanted to see which size that would be preferable for the perch to feed upon from an optimal foraging point of view. Optimal foraging theory states that foragers should prefer prey that yield more energy per unit time (Sih and Christensen 2001). Basically this involves two steps, the search time and the handling time. Search time is the time it takes a predator to find a new prey since its last prey was devoured, and handling time is the time it takes from the beginning of an attack until the prey is swallowed and the predator is ready to find a new prey (Diehl 1988).

The aim of this study was (1) to find out if perch (*Perca fluviatilis* L.) feed selectively on different phenotypes of the benthic isopod (*Asellus aquaticus* L.), and (2) if this selectivity differs between two different macrophyte habitats, reed stands (*P. australis*) and stands of submerged vegetation consisting of stoneworts (*Chara spp.*), and (3) to find out what size of *Asellus* used in the experiments that would be the most profitable for perch to feed upon.

3 Materials and Methods

3.1 Study organisms and ecology

Eurasian perch (*Perca fluviatilis*) is a common fish species in most lakes in northern Europe. It is an omnivorous predator which goes through several habitat and diet shifts in its early life.
Perch spawn in spring on fallen branches and in vegetation in the littoral zone. After hatching, perch larvae move to the pelagic habitat where they live on small zooplankton. 1-5 weeks later at a size of 10-30 mm the perch shifts back to the littoral zone where large zooplankton and benthic macroinvertebrates eventually becomes the most important food source (Byström and Garcia-Berthou 1999, Byström et al. 2003, Cech et al. 2009). When the perch reaches a length of about 15 cm it switches to a piscivorous diet consisting of young-of-the-year (YOY) fry of most fish species including small perch, but macroinvertebrates will continue to make up an important part of the diet the rest of the perch’s life (Jacobsen et al. 2002, Edeline et al 2008).

The freshwater isopod Asellus aquaticus is a widespread species in lakes, ponds and in slow-flowing rivers in Eurasia (Verovnik et al. 2005). It lives in various vegetated habitats including reed belts, where it feeds on decaying leaves, and macrophyte beds, where periphyton make up the bulk of the diet (Arakelova 2001).

3.2 Fish, isopods and substrate
YOY perch were brought to the laboratory from a fish hatchery (Östgös AB, Söderköping, Sweden) in May 2009. The juveniles were hatched from eggs collected from Lake Hjälmaren, and fry were reared on dry feed before delivery. At the research facility, the juveniles were placed in four transparent plastic tanks (65 × 45 × 50 cm length × depth × height), with 16 individuals in each tank. The fish were kept in dechlorinated tap water (18°C), which was aerated, and a water cleaning device was also put in each tank. The light regime was natural daylight since there were large windows close to the fish tanks. Every week the filtering device and the tanks were cleaned from visible growth and some of the water was exchanged for new. The fish were feed commercial fish food (brand and size) but mostly frozen chironomids until a few weeks prior to the experiments when live A. aquaticus were introduced as part of the diet.

Isopods (A. aquaticus) were collected on two occasions in Lake Tåkern in the autumn of 2009. Substrate in the form of decaying reed (leaves and stems) and living stoneworts for the selectivity experiment was also collected at these two occasions.

3.3 Handling time experiment
In this laboratory experiment I wanted to find out which size of A. aquaticus used in the prey selection experiment that would be the most profitable for the perch to eat, in terms of mass of prey consumed per unit of time. Because of the difficulties associated with measuring relevant search times in an artificial environment (i.e. mimicking complex macrophyte habitats and at the same time see the predator at all times, and estimating the density of prey numbers and sizes in the real habitat) (Persson and Greenberg 1990), I only measured handling time, which should be the same in any habitat.

I used 14 perch in the size range 9-10.5 cm as predators and three size classes of dark colored isopods (5.7 ± 0.4 mm (mean ± SD, n = 42), 7.0 ± 0.4 mm, n = 35, and 9.0 ± 0.6 mm, n = 18) as prey. The isopods in each size class were not measured before the experiment, only visually assessed that they were approximately of the same size. A number (n) of them were photographed and afterwards measured using the software Image J 1.42q (NIH 2004). The experimental trials were conducted in two aquaria (70 × 35 × 38 cm length × depth × height) which were filled halfway with dechlorinated tap water (20°C). Light sources consisted of two florescent tubes placed 30 cm above each aquaria. Each aquarium was divided in half by a vertically sliding Plexiglas door which was attached to a thin line so that it could be pulled...
up while the observer was hidden behind a blind (Figure 1.). The bottom of the aquaria were covered with light-colored sand and a 5 cm high Plexiglas piece in the middle that made it impossible for the isopods to leave the arena. The use of dark colored isopods on a light colored background should have made it easy for the perch to spot the prey.

Before each trial the perch were starved for approximately 18-22 h. At the beginnings of a trial two perches were randomly chosen and placed in the aquarium on one side of the divider. The reason for using two perch in each trial was that preliminary studies showed that single perch were reluctant to feed. Then ten isopods (only five in the biggest size, due to a shortage of large *Asellus*) all of the same size, were released on the other side, which was used as the predation arena. The same thing was done in the other aquaria so that I could start a second trail as soon as the first had ended. To help record the foraging behavior of the perch, a video camera (Panasonic xx) placed on a tripod in front of the aquarium was used. I started the trials by putting on the video camera and then went behind a paper partition in front of the experimental arena so that I wouldn’t disturb the perch. The divider between predator and the predation arena was then pulled up and the feeding could begin. Through small holes in the paper partition I then observed the perch feeding on the isopods and ended when all had been eaten, which took less than 30 min in all trials.

![Aquarium setup](image)

*Figure 1. Aquarium used in the handling time experiment. The aquarium was divided by a vertically sliding, transparent Plexiglas-door, separating the experimental arena from the other half, in which the fish were left to acclimatize before the experiment.*

Every perch was used four times, one time per *Asellus* size and a final session. The purpose of the last session was to exclude the possibility that the perch had learned to handle the prey faster in the end of the experiment compared to in the beginning. If that had happened the recorded handling times wouldn’t depend solely on prey size. Prey handling time (i.e. the time from the beginning of an attack until mastication movements had ceased) was determined with the help of a stop watch while looking at the recorded trials on a computer. I only measured handling time on the two first captured isopods by every perch, so that hunger
levels wouldn’t have an influence. When mean handling times was calculated for each prey size, the mean of each perch’s two captures was used as independent replicates.

To find out the connection between *Asellus* length and weight, 21 individuals in the size range 3-9 mm were selected and dried in 55° C for 24 h before being weighed. The length-weight data was used to estimate the relationship between *Asellus* length and mass, by means of a simple linear regression; \( R^2 = 0.98, p < 0.000, n = 21 \). From this relationship the three size classes of prey was transformed to the correspondent mass. To find out which size class of *Asellus* was the most efficient to feed on for perch of the size used in the experiment, mean handling time was divided by prey mass.

### 3.4 Prey selection experiment

In this laboratory experiment, which was divided in two trials, I wanted to find out if perch feed selectively on *A. aquaticus* regarding pigmentation and size and if this selectivity differs between the two macrophyte habitats.

This experiment took place in a greenhouse 4-30 November 2009. Water temperature was kept constant at 18° C and the setup was illuminated by natural daylight, which provided light between approximately 08:00-16:00.

24 transparent plastic aquaria (65 × 45 × 50 cm length × depth × height) were evenly distributed in four independent blocks, each consisting of six aquaria (Figure 2). All aquaria in a block were half filled with dechlorinated tap water (18° C), and aerated. Within a block the aquaria were connected by silicone tubing, in a circular system (Figure 2.), making the water to slowly flow through all tanks. This arrangement was used to even out any differences in water quality and chemical ques from fish, which could otherwise have affected *Asellus* behavior and survival. To prevent isopods from moving between the aquaria, through the tubes, filters consisting of pieces of fine meshed net were attached on one end of the tubes. On the bottom of the aquaria 2 L of sand was spread out in a thin layer, three-treatments with black sand (Rådasand, Blästersilikat 0.2-1.5 mm) and three with light colored sand (Rådasand, Specialsand 0.8-1.5 mm). The treatments were randomly distributed in each block. In aquaria with dark sand 2.5 L of decaying reed substrate was added to mimic the bottom substrate of the reed habitat, and in tanks with light sand live stoneworts (*Chara tomentosa L.* ) (≈100 g dry weight) was added to mimic the stonewort habitat.
Figure 2. To the left you can see the setup of the prey selection experiment. The four blocks, each containing six aquaria connected by silicone tubing in a circular system. To the right you can see an illustration of one block. In each block three aquaria contained light sand and living Chara tomentosa, mimicking the stonewort habitat, while the other three contained black sand and decaying reed substrate, mimicking the bottom in the reed habitat. The blue arrows show the movement of the water through the aquaria, driven by an electric pump.

A few days before the start of the first experimental trial a mixture of 700 ± 50 (Chara and reed) isopods (including the complete pigmentation and size range) was added to each aquarium. The density corresponds to 2400 ind/m², which is relevant for natural conditions (Hargeby et al. 2004). As predators 32 perch (size range 8.5–11 cm) were used. The fishes were evenly distributed among eight aquaria, four with reed habitat and four with stonewort habitat. In the end all four blocks, consisting of six aquaria per block, had two aquaria each with four perch per aquarium, one in reed and one in stoneworts. In each block two of the four aquaria without perch were assigned to be control aquaria, one from each habitat. The remaining two aquaria were left alone until the second trial of this experiment. The perch were then left for a week to forage before the results were read.

After a week the experiment was terminated, and the 32 perch were transferred to two holding aquaria where they were fed daily with frozen chironomids. In each block, the two aquaria in which the perch had been incubated, and the two corresponding reference aquaria, were sampled for remaining Asellus. Two samples, covering totally 14 % of the bottom area in an aquarium, were taken in all control and perch aquaria. To take a sample a metal cylinder (16 cm φ) was pushed through the bottom substrate and then all the isopods and plant material in that cylinder were collected by means of a 0.2 mm mesh hand net. The isopods were then picked out and photographed in a transparent Petri dish with water-, and placed on mm-grid to allow for measurements of prey size from the photographs. Each photograph also included a Kodak (Rochester, NY) neutral grey card (18 % reflection) and a white card (90 % reflection) to allow for calibration of any difference in light conditions between the images. After photographing, the isopods were put back in the aquarium they came from.

In the second trial the 2×4 = 8 aquaria which were unused in the first trial were stocked with four perch per tank. As in the first trial they were left alone to feed for a week and then transferred to the holding tanks. Samples were taken in the same manner as before; with the exception that in five aquaria stocked with perch more than two samples were needed, to
reach a minimum limit of 25 isopods, which was regarded required for statistical analyses. The isopods were photographed and put back in the aquaria.

### 3.4.1 Size and pigmentation

The images of the isopods were analyzed for reflectance and size using the software Image J 1.42 q (NIH 2004). The whole dorsal area of the isopod was used to measure pigmentation and the mean reflectance value was used as a measurement of pigmentation of each animal. The scale of pigmentation in the software ranged from zero (white, 100 % reflection) to 255 (black, 100 % saturation). The isopods were photographed in daylight spread out over a number of days, resulting in different light intensities between the images. This problem was corrected by using the measured mean reflectance from the two standardized Kodak cards, which correspond to 18 % resp. 90 % reflection, the measured means for the isopods on that image were converted to a reflectance number between 0-100 percent. This was done for every image so that it would be possible to compare them in a correct way.

### 3.5 Statistics

Selectivity was tested with two-way ANOVAs using the median size and reflectance of *Asellus*, in every fish and control aquaria. Difference in perch handling time between the three size classes of prey were tested with a one-way ANOVA. All statistics was performed with Statistica version 9 (Statsoft Inc.).

### 4 Results

#### 4.1 Handling time experiment

Perch handling time for the three prey sizes were 2.8 ± 0.9 s (mean ± SD, n = 16) for 5.7 mm *Asellus*, 2.1 ± 1.0 s, n = 13, for 7.0 mm, and 2.8 ± 1.4 s, n = 8 for 9.0 mm. A one-way ANOVA showed that there were no significant differences in handling time between the three size classes (F(2) = 2.27, p = 0.12). Furthermore, mean handling time in the first two sessions (5.7 mm = 2.7 s, resp. 7.0 mm = 2.3 s) did not differ significantly from the last session (2.9 s resp. 1.7 s); (t(14) = -0.41, p = 0.69 resp. t(11) = 1.20, p = 0.26, t-test). This shows that perch handling time was not affected by perch learning to handle prey, during the course of this experiment. The corresponding weight for the three prey sizes were (R² = 0.98, p < 0.000), 1.14 mg (5.7 mm), 2.10 mg (7.0 mm) and 4.42 mg (9.0 mm). Intake rate was calculated for perch around 10 cm in length. By dividing prey weight with handling time you get the most profitable *Asellus* size for perch around 10 cm in length to eat, provided that search times for all three sizes were the same. As can be seen in Figure 3 the largest size of prey is the most profitable with 1.6 mg (prey mass)/s (handling time), while the smallest size is the least profitable with 0.4mg/s.
Figure 3. Intake rate of prey (Asellus aquaticus) expressed as mass intake during handling of prey by perch feeding on three size classes. Numerals denote independent replicates (number of perch that fed on at least two Asellus in a trial) for each prey size.

4.2 Prey selection experiment

4.2.1 Number of prey eaten
In both trials there were significantly less isopods left in the fish aquaria than in the control aquaria ($F_{(1)} = 34.78; p < 0.000$ and $F_{(1)} = 15.29; p = 0.002$), showing that the perch had fed on them (Table 1.). There was also a significant interaction between fish and habitat, which means that the number of Asellus eaten by the perch depended on the habitat. In the first trial there was a tendency that perch had eaten more in the Chara habitat, but in the second trial no trend was detected. From the time of sampling after the first trial to the time of sampling after the second trial, a number of Asellus had died in the control aquaria. That explains the difference in mean values between the controls in the first and the second trial (Figure 4, 5 and 6.). The overall results were similar in both trials.

Table 1. Results of two-way ANOVAs on number of Asellus found after the first and the second trial as depending variable, and fish presence/absence and vegetation as factors.

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Figure 4. Density of Asellus (mean ± SD) found in the four treatments (Reed Fish, Chara Fish, Reed Control, and Chara Control) after the first (A) and the second (B) trial of the prey selection experiment.

4.2.2 Size (length)
Isopods were smaller in the fish aquaria than in the control aquaria (p = 0.053 and p < 0.000). Perch seemed to feed on larger Asellus when given a choice. There was also a significant interaction between fish and habitat in the second trial (Table 2.), which means that the size preferences by the perch depended on the habitat.

Table 2. Results of 2-way ANOVAs with isopod length as depending variable, and fish presence/absence and vegetation as factors.

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Figure 5. Size (mean + SD) in Asellus sampled in the four treatments (Reed Fish, Chara Fish, Reed Control, and Chara Control) after the first (A) and the second (B) trial of the prey selection experiment.

4.2.3 Pigmentation (reflection)
Isopods in the control tanks were significantly darker than in fish tanks in both trials (p = 0.001 and p = 0.004), (Table 2.), showing that the perch preferred to feed on Asellus with a darker pigmentation in both habitats.

Table 3. Result of 2-way ANOVAs with isopod reflectance as depending variable, and fish presence/absence and vegetation as factors.

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Figure 6. Pigmentation (mean + SD) in Asellus sampled in the four treatments (Reed Fish, Chara Fish, Reed Control, and Chara Control) after the first (A) and the second (B) trial of the prey selection experiment. The Y-axis is showing pigmentation as standardized reflectance in percent where zero is (black, 100 % saturation) and 100 is (white, 100 % reflection).

5 Discussion

5.1 Size
Perch starts to feed on macroinvertebrates at a size around 6 cm in length (Persson 1983, Hargeby et al. 2004). If I had used perch that small I might have seen a connection between increasing prey size and longer handling time. Perch in the size range used in this experiment (9-10.5 cm) however, fed on all sizes of Asellus (5.7-9.0 mm) at equal rates. The prey handling time did thus not increase with increasing size of the prey, indicating that perch used in the experiments were not gape limited when feeding on Asellus. Hence, the largest Asellus were the most energy efficient size for the perch to feed upon, and therefore would probably be the preferred choice in the prey selection experiment.

In agreement with this expectation, isopods were significantly larger in the controls compared to the fish treatments (Figure 5). How can this then explain the observed size difference in A. aquaticus between the reed and the stonewort habitats? Well, maybe predation from fish is more intense in the stoneworts than in the reed. A study conducted in the middle of the summer in Lake Tåkern seems to supports this theory. The authors found that fish (of which perch constituted 26 %) were common in the stonewort habitat, while nearly absent in the reed habitat. At the same time they found much higher numbers of invertebrate predators in the reed habitat (Wagner and Hansson 1998). Invertebrate predators are less efficient in handling large prey (thereby preferring smaller prey) and if they are the main predators in the reed it would explain why the typical reed isopods grow to a larger size. This distribution of predators is probably very similar in Lake Krankesjön, since the same species of fish and predatory invertebrates are shared between the two lakes (Wagner and Hansson 1998, Hargeby et al. 2005). Alternate predation regimes likely strengthen divergent selection between habitats over that occurring from resource difference alone (Vamosi and Schluter 2002).
5.2 Pigmentation
The reflectance of the isopods in the fish tanks was significantly higher than in the control tanks, showing that the perch had eaten a higher proportion of dark individuals, in both habitats. In the Chara habitat, selection was according to what could be expected from background matching, but in the reed habitat selection was quite the opposite. Several other laboratory studies have shown that predation efficiency of fish feeding on isopods depends on prey pigmentation in relation to background color (Merilaita 2001, Hargeby et al. 2005). Why did the perch select differently in the reed aquaria? One possible explanation is that the range of isopod phenotypes differed in both body size and pigmentation. The dark colored isopods had a larger mean body size than the light colored ones (E. M. L. Andersson pers. observ.). Maybe the size of an isopod was a more important factor than pigmentation, for prey detection by the perch, resulting in similar preferences in both habitats.

5.3 Evolutionary questions
Research regarding fish predation can help to answer evolutionary questions concerning natural selection. Divergent selection driven by alternate predatory regimes and resource difference in the two habitats can lead to assortative mating within each of the two Asellus populations. In a study by (Hargeby and Erlandsson 2006) assortative mating as a possible prezygotic reproductive barrier between Chara and reed isopods was investigated. They found that mating was size-assortative when Chara isopods were experimentally mixed with isopods from an adjacent reed site with large-size individuals, suggesting a partial prezygotic reproductive barrier. This means that isopods migrating from the reed habitat to the Chara habitat and vice versa are less likely to mate successfully, and over time this might lead to a point when these two populations have diverged so much that they have become two new species. An example where adaptation to alternative suits of predators is believed to have played an important role in promoting diversification and speciation is the threespine stickleback species (Gasterousteus aculeatus complex) from southwestern British Columbia, Canada (Vamosi 2003). Sympatric stickleback species are ecologically and morphologically highly differentiated. In every pair, one species (the limnetic) forages on zooplankton in the open water, whereas the other species (the benthic) forages on invertebrates in the littoral zone (Rundle et al. 2003).

5.4 Conclusion
Perch selected larger individuals and individuals with a darker pigmentation. A selective pressure like that would be likely to produce a population of small, lightly colored isopods, just like the one in the Chara habitat. It is therefore likely that predation by visually hunting fish is an important force of natural selection behind the habitat-specific adaptations seen in Asellus populations in the novel Chara habitat.

At the same time the results indicate that fish predation is less important in the reed habitat, because if fish predation was as intense in the reed as in the Chara the typical reed Asellus would be a small dark isopod instead of a large dark one.

6 Acknowledgements
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7 References

Verovnik, R., B. Sket, and P. Trontelj. 2005. The colonization of Europe by the freshwater crustacean *Asellus aquaticus* (Crustacea: Isopoda) proceeded from ancient refugia and was directed by habitat connectivity. Molecular Ecology 14: 4355-4369