Trait and density mediated indirect effects in a three trophic level aquatic system with *Asellus aquaticus* and *Aeshna*

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1. Abstract
Predation through TMII (Trait mediated indirect interaction) and DMII (Density mediated indirect interaction) has been recognised as an important factor in natural environments. These indirect effects suggest that predators can affect either the defensive traits of the prey or the density and therefore have an indirect effect on the amount of resources that can be consumed. Classic TMII is when predators change the preys’ antipredator behaviour which in turn affects food intake which ultimately affects resource densities. In this thesis I examine the TMII and the DMII in a three trophic level aquatic system consisting of Aeshna (predators), Asellus aquaticus (prey) and Alnus incana leaves (resource). The results showed that the DMII were stronger than the TMII when there were low levels of resources, but equal when there were high levels of resources. This suggests prey took higher foraging risks at low resource densities.

2. Introduction
The relationship between predators and prey is an important relationship that affects both the life of the prey and the predator (Hölker and Mehner, 2005). It has been shown that predators and prey influence each other in many ways such as changes in feeding behaviour and refuges seeking (Johansson, 2005). The effect that the predator and prey have on each other can be indirect or direct. In a three trophic level system (predator-prey-resources), the predator can affect the amount of resources by consuming the prey, thus giving an indirect effect on the resources by affecting the density of the prey. A direct effect is when the predator affects the density of the prey by consuming the prey (Luttb erg et al. 2003).

The ability to avoid being eaten is very important for prey and they avoid being eaten by changing important traits. Some of the changes in traits may be increased use of refuge, change in activity and changes in habitats (Škaloudová et al. 2007). The amount of resources can have an effect on the way that the prey reacts to predators and how they change their behaviour to the predator risk (Luttb erg et al. 2003). Trait mediated indirect interaction (TMII) and density mediated indirect interactions (DMII) has been recognized as effects that can be driving communities (Okuyama and Bolker, 2007).

2.1. Trait-mediated indirect interactions (TMII) and density-mediated indirect interactions (DMII)
TMII is the indirect effect where the predators induce a change in a consumer trait. The traits can be a change in the behaviour or development (Hölker and Mehner, 2005). One example of TMII is when a predator’s presence reduces the prey’s foraging activity and which causes the prey to consume less resource and that leads to an increase in resources (Luttb erg et al. 2003).

DMII on the other hand is an indirect effect where the density of the prey changed by the predators by direct consumption of the prey. Trophic cascades are an example of DMII. When the amount of predators increase that will lead to an increase in resources due to decrease in the amount of prey (Škaloudová et al. 2007). Some evidence seems to show that TMII and DMII are likely to operate at the same time in communities and it is therefore interesting to examine which one of these that is the most important (Okuyama and Bolker, 2007).
2.2. Predator and prey
The predators used in the experiment in my study are larvae of the order Odonata from the genus *Aeshna*. The larvae are predators that live in the water for many years before they start their adult life. *Aeshna* larvae are efficient predators and feed on smaller insects and other organisms and can even catch and feed on tadpoles and small fishes and commonly feed on conspecifics (Sandhall, 2000, Nilsson, 1997).

The prey used in my study is *Asellus aquaticus*, which is a freshwater isopod that can be found in many different types of environment. It is very common in ponds in Sweden (Johansson, 2005). These isopods feed on all sorts of plants and animal material (Adcock, 1982).

2.3. Main experiment

2.3.1. Focus in this report
In this report the focus is on the indirect effects that the predator, *Aeshna*, has on the break down of leaves by affecting the isopod prey, and thus affecting their ability to consume the resources. How fast the isopods break down the leaves depends on if there are any predators in their vicinity. It also depends on which part of the life cycle the *A. aquaticus* are in (Wojdak and Luttberg, 2005, Luttberg et al. 2003).

The *Aeshna* larvae can affect the isopods by feeding on them. This causes the rate of the leaf breakdown to slow down because the density of *A. aquaticus* decreases. The *Aeshna* larvae can also affect the isopods by a non-lethal effect. In this case, the isopods spend more time in refuges (because they hide from the predators) than foraging for food. Thus the rate of breakdown of leaves slows down.

My experiment was built as a three level food chain: the predator, *Aeshna* larvae; the detritus feeder, *A. aquaticus*; and the resources in the form of leaves from the alder tree, *Alnus incana*. Linear three-species chains are commonly used in experiments and they are easy to uphold in experimental environments because there are fewer parameters to control than in a natural environment (Wojdak and Luttberg, 2005, Luttberg et al. 2003).

2.3.2. Behavioural experiment
A behaviour experiment conducted by Johansson (2005), showed that larger *A. aquaticus* did not react behaviourally to the presence of the predator, *Enallagma cyathigerum* (Odonata), whereas *A. aquaticus* with a smaller size did react with anti-predatory behaviour. To see how the *A. aquaticus* would react to the *Aeshna* larvae in my experiment a behaviour experiment was conducted prior the main experiment. Another reason for a behaviour experiment was to determine which size class of the *A. aquaticus* that was going to be used during the experiment.

2.3.3. Main experiment
The focus in the main experiment was on the DMIIIs and the TMIIIs and which one of them that dominates at different resource levels. Two main hypotheses can be postulated concerning how DMIIIs and TMIIIs should vary with resource densities. The first theory predicts that when the resource levels increase TMII should decrease while DMII increase due to the fact that the prey might react less to the predators in the containers when there are more resources available for the prey. The second theory predicts that when the resource levels increase the TMII should increase and the DMII should decrease because the prey have a higher foraging success when there
are more resources present and so they do not have to risk their life to go out and search for food and thus reducing their risk for being faced with the risk of becoming prey (Wojdak and Luttberg, 2005, Luttberg et al. 2003). My experiment was meant to show which type of indirect effect that the prey in this experiment responds to and also to examine how the effect of the predators varies with different amounts of resources.

3. Material and method

3.1. Collecting of the leaves and animals
Alder leaves were used as the resources and they were collected on September 3, 2007 from trees around Lake Nydala in Umeå. They were dried for four days in a drying cupboard at +40°C. Each day all leaves were mixed twice so that air would come to all leaves and to speed up the drying process. After that the leaves were stored in dry conditions and in room temperature. To provide a refuge for the A. aquaticus rocks of the size of approximately 3 x 6 cm were collected. The rocks were washed several times in clean water before being used.

The experiment was run in 120 plastic containers (size 14 x 8.5 x 17 cm). Ninety containers had a non-transparent plastic cup with mesh bottom and 30 containers had no plastic cup. The plastic cups contained one Aeshna-predator during trials with non-lethal predators. Hence, the predators were not able to get access to the A. aquaticus but the design provided chemical cues from the predators.

The Aeshna larvae and the A. aquaticus were collected on September 5, 2007 from the Gimonäs pond also known as the Sofiehem pond in Umeå. The Aeshna larvae were held separately in three different size classes: small, medium and large, to prevent them from feeding on each other. They were kept in water with leaves and rocks and they were feed twice every week. The A. aquaticus were kept in containers with water and vegetation. More animals were collected through September to November and those were used as food for the Aeshna larvae or to replace dead larvae in the experiment. The Aeshna species that were used in the experiment were Aeshna grandis and A. juncea.

3.2. Behaviour experiment one and two
To estimate how the A. aquaticus would respond to the presence of Aeshna larvae in the containers and also to determine which size of the larvae that was going to be used in the main experiment a behaviour experiment was conducted. Sixty plastic containers (size 14 x 8.5 x 17 cm) were used for this experiment: 30 containers with predators and 30 containers without predators. A grid pattern was drawn in the bottom of each container. Each square of this grid was 3 x 3 cm and the containers were filled with water.

The containers were randomly placed in blocks of five. All the containers were connected to an air supply and the water was given air for 48 hours. In every other container the placement of the cup was altered. One Aeshna in medium size, 20-35 mm, and two A. aquaticus which the predators could feed on were put in the cups 24 hours before the behaviour experiment took place. Thereafter the air was shut off. The experiment started the on September 11, 2007 when one A. aquaticus, of medium size, 6-8 mm, was placed in the middle of each container and left for one hour so that they could get familiar with the new environment. After one hour, the position of the A. aquaticus in all the containers was checked according to the grid every fifteenth
minute for two hours. After a one hour break activity was estimated for another two hours.

The results of the experiment were then analyzed. The number of moves and the total distance of the moves were calculated. By counting every time the *A. aquaticus* moved in each container the total amount of times they moved was calculated. The total distance of movement was also calculated by summing up the distance moved by the *A. aquaticus*.

A t-test was conducted on both results to see if there were any significant differences between the containers with predators and the containers without predators in number of moves and distance moved.

A second behaviour experiment was conducted on 25 September, 2007 but this time *A. aquaticus* of a small size class, 3-5 mm, were used. Past experiments have shown that smaller *A. aquaticus* react more to presence of predators compared to larger ones (Johansson, 2005).

### 3.3. Main experiment

For the main experiment I used 120 plastic containers containing alder leaves. Forty containers were filled with 1 g of alder leaves, 40 containers were filled with 2.5 g of alder leaves and 40 containers were filled with 5 g of alder leaves (Table 1).

<table>
<thead>
<tr>
<th>Number of plastic containers</th>
<th>Resources (g)</th>
<th>Control</th>
<th>No predator</th>
<th>Non-lethal predator</th>
<th>Lethal predator</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Low levels</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2.5 Medium levels</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>5 High levels</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Water was added to the containers on October 3, 2007 to give the leaves the possibility to sink to the bottom. The leaves were also pressed down by hand two to three times each day to speed up this process. All the containers were connected to an air supply system.

To provide the *A. aquaticus* the same possibility for refuge use in the containers with 1 g and 2.5 g of resources as they had in the containers with 5 g of resources, artificial leaves were added. Blue silk ribbons were used as artificial leaves and they were cut to the same size as the leaves. Silk ribbons were added so that leaves and silk ribbons corresponded to the same number of levels as in the containers with 5 g of resources. The silk ribbons were cut in the length of 5-7 cm and put in water for 24 hours to reduce the amount of possible toxins and excess colour. Then the ribbons were put in hot water and then rinsed through with water. The process was repeated twice. Before being used, the ribbons were left in cold water again for 24 hours to make sure that as little as possible of potential toxins and excess colour were left in the ribbons. In the 40 containers that had 2.5 g of resources 20 silk ribbons pieces were put in the water. In the 40 containers that had 1 g of resources 30 silk ribbons pieces were put in the
water. In the containers that had 5 g of resources no silk ribbons were added. In addition six rocks were added in each container (Figure 1).

![Figure 1. View of plastic containers from above showing the position of the small rocks in the containers during the experiment conducted between October 18 and November 2, 2007.](image)

The experiments were done in a room where the climate could be controlled and all containers were randomly placed on four tables in the room. The temperature during the experiment was set to 15 °C but the temperature varied between 14.5 - 17 °C during the experimental period. One decilitre of water from the Gimonäs pond was added to each container to provide for microorganisms that could colonise the leaves. The water from the pond was filtered 3 times to eliminate any macroinvertebrates that could affect the outcome of the experiment.

Sixty *Aeshna* larvae were used for the experiment. Most of the larvae were of a small size; 15-24 mm and a few of them were of medium size 25-35 mm. The larvae were given an *A. aquaticus* one day before the experiment so that they had the same level of hunger. The experiment started on October 18, 2007.

Small *A. aquaticus* were selected in a size that ranged from 3 to 5 mm. Small *A. aquaticus* has been shown to react more to the presence of predators than larger *A. aquaticus*. This trend was shown in the behaviour experiment that was performed before this experiment and in a prior experiment by Johansson (2005).

Ten *A. aquaticus* were added to each container with no predators, non-lethal predators and lethal predators. No *A. aquaticus* or *Aeshna* larvae were added to the ten containers that were used as controls.

One time each week during the experiment the number of *A. aquaticus* was counted in the containers with lethal predators to be able to detect if the numbers got too small for an appropriate analysis. To replicate the disturbance that the counting caused in the containers with predators the water was disturbed and mixed in all the other containers each week at the same time as the counting was made. After 5 weeks the experiment was terminated and the *A. aquaticus* in all the containers were counted. The leaves were taken out of the water and faeces were scraped off them. Then the leaves were dried in 40 °C for 60 hours and left in room temperature for 48 hours. After that the leaves were weighed.

The *A. aquaticus* were put in ethanol and then they were dried in 40 °C for 60 hours and left in room temperature for 48 hours. After that they were weighed. The weight was compared to the weight of the *A. aquaticus* that were dried and weighed before
the experiment was started. The results from the weighing of the leaves were analyzed
in SYSTAT and MINI-TAB. ANOVA-tests were made to see if there was any
significant difference in the amount of leaves that were eaten during the experiment.
TMII and DMII were calculated using the amount of leaves remaining with the
formulas:

\[
TMII = \frac{\text{resources with caged predators}}{\text{average resources with no predator}} - 1
\]
\[
DMII = \frac{\text{resources with deadly predators}}{\text{average resources with caged predator}} - 1
\]

4. Results

4.1. Behaviour experiment number one with larger A. aquaticus

4.1.1. Movement
The mean for the total movement in containers with predators was 12.2 and 13.5 for
the containers without predators. The difference between them is not large, and a t-
test showed that there was no significant difference between containers with predators
and the containers without predators (t-value: 0.81, p-value: 0.42).

4.1.2. Distance
The A. aquaticus did not move longer distances in the containers with predators than
in the containers without predators. The total mean for the length of movement was
63.3 for the containers with predators present and 57.9 for containers without
predators present, and a t-test shows that there was no significant difference (t-value:
0.77, p-value: 0.45).

4.2. Behaviour experiment number two with smaller A. aquaticus

4.2.1 Movement
The total times the A. aquaticus moved during the second behaviour experiment was
11.8 in containers with predators and for the containers without predators the result
was 13.7. According to the t-test there was a trend for less movement in the containers
with predators but not a significant difference (t-value: 1.86, p-value: 0.08).

4.2.2. Distance
The mean distance of the total movement in the containers with predators was 59.1
and the mean distance of movement for the containers without predators was 58.5,
and a t-test showed that there was no significant difference between the predator
treatments (t-value: 0.14, p-value: 0.89).

4.3. Comparing behaviour experiment one and two with each other

4.3.1. Movement
The total number of movements in behaviour experiment one and two was combined
for further analyse. The containers with predators in the two experiments had a mean
number of moves of 11.5 and in the containers without predators the mean number of
moves was 13.2. The result of the t-test (t-value: 1.76, p-value: 0.08) suggests a trend
towards a difference in total movement between the containers with predators and the
containers without predators for the smaller *A. aquaticus*. They tend to move less in the containers with predators present.

### 4.3.2. Distance

The mean of the total distance of movement of the *A. aquaticus* in the containers with predators was 60.2 and 58.2 in containers without predators for the experiments when combined. A t-test showed that the difference was not significant (t-value: 0.67, p-value 0.51).

### 4.4. Which size of *A. aquaticus* to use in the main experiment?

Since the results from the behaviour experiment showed that smaller *A. aquaticus* had a tendency to react more to the presence of predators I decided to use the smaller *A. aquaticus* in the main experiment.

![Figure 2](image_url)

*Figure 2. The mean leaf biomass that has been eaten at different resource levels by the A. aquaticus during the experiment and the standard error, conducted between October 18 and November 2, 2007.*

### 4.5. Main experiment

The result of the mean weight of the amount of resources that was consumed by bacteria and other microscopic organisms during the experiment is presented as the control bars in Figure 2. The amount of resources consumed by the micro organisms was higher at high resource densities. The mean weight of the resources that was consumed during the experiment for the containers with 1 g of resources was 241.1 mg. The standard error was ± 10.2. The mean weight of the resources that was consumed in the containers with 2.5 g of resources was 960.2 mg, the standard error was ± 47.1 mg. The mean weight of the resources that was consumed in the containers with 5 g of resources was 1479.4 mg and the standard error was ± 55.5.

The mean weight from the containers that had no predators increased with higher resource densities (hatched bars in figure 2). In the containers with 1 g of resources 316.2 mg of the resources was eaten during the experiment and the standard error was ± 17.1. In the containers with 2.5 g of resources the amount of resources that were
eaten by the *A. aquaticus* was 1077.1 mg and the standard error was ± 73.9. In the containers with 5 g of resources the amount of resources that was eaten during the experiment was 1657.1 mg and the standard error was ± 61.7.

The mean biomass of the resources that was eaten during the experiment in the containers with non-lethal predators present is given as grey bars in Figure 2. In the containers with 1 g of resources the mean weight of the resources that was eaten during the experiment was 334.6 mg and the standard error for the amount of resources that had been eaten was ± 21.4. In the containers with 2.5 g of resources the mean weight of the resources that was eaten was 952.9 mg and the standard error was ± 57.5. In the containers with 5 g of resources the mean weight of resources eaten during the experiment was 1561.7 mg and the standard error was ± 37.8.

The black bars in Figure 2 show the mean biomass of the resources that was eaten during the experiment in the containers with lethal predators present. In the containers with 1 g of resources the mean weight of the resources that was eaten during the experiment was 260.5 mg and the standard error was ± 12.5. In the containers with 2.5 g of resources the mean weight of the resources that was eaten was 794.1 g and the standard error was ± 34.1. In the containers with 5 g of resources, the mean weight of resources eaten during the experiment was 1476.9 mg and the standard error was ± 40.1.

### 4.6. Statistics

To examine if the consuming of the resources had been affected by the amount of resources and if it had been affected by the presence of predators in the containers a two-way ANOVA-test was performed (excluding the controls). The ANOVA-test showed that the amount of resources that was eaten during the experiment differs with resource levels (*F*$_{2,81}$ = 847.42, *p*-value = 0.000001). In general more leaves were eaten at higher resource levels. The ANOVA also showed that the presence of the *Aeshna* larvae in the containers had a significant effect on the amount of leaves that was eaten (*F*$_{2,81}$ = 13.11, *p*-value = 0.00001). In the presence of predators a smaller amount of leaves was eaten while the highest amount of leaves that was eaten was in the containers with no predators present. The interaction between the presence of *Aeshna* larvae and the amount of resources showed no significant difference in the experiment according to the ANOVA (*F*$_{4,8}$ = 1.60, *p*-value = 0.18).

#### 4.6.1. 1 g of resources

An ANOVA-test showed that there was a significant difference in how much resources that had been eaten if there was a predator present or not in the containers with 1 g of resources (*F*$_{2,27}$ = 5.04, *p*-value = 0.01). To examine if there was a significant difference between the different predator treatments more ANOVA-tests were performed. There were no significant difference in the amount of resources that had been eaten at the end of the experiment between the containers with no predators and the containers with non-lethal predators (*F*$_{1,27}$ = 0.39, *p*-value = 0.53; Figure 2). In contrast there was a significant difference in the amount of resources that the *A. aquaticus* had eaten between the containers that had no predators and the containers that had lethal predators present (*F*$_{1,27}$ = 5.66, *p*-value = 0.02). The isopods in the containers that had no predators present had eaten more than in the containers with lethal predators (Figure 2). There was a significant difference between the containers that had non-lethal predators and the containers that had lethal predators present (*F*$_{1,27}$
In the containers that had non-lethal predators the *A. aquaticus* had eaten more than in the containers with lethal predators (Figure 2).

For the per capita results the t-tests showed that there was no significant difference in the results for the treatment between no predator and non-lethal predator (t-value: -0.43, p-value: 0.67). For the treatment with no predator and lethal predator there was a significant difference (t-value: -3.27, p-value: 0.01) and also for the treatment with non-lethal predator and lethal predator (t-value: -3.12, p-value: 0.01). According to these results the *A. aquaticus* in the treatments with lethal predators ate more than those in the other treatments.

### 4.6.2. 2.5 g of resources

According to the ANOVA-test the presence of predators had an effect on the amount of resources that had been eaten during the experiment in the containers that had 2.5 g of resources (F<sub>2,27</sub> = 6.37, p-value = 0.01). But there was no significant difference in the amount of resources that the *A. aquaticus* had eaten in the containers that had no predators and the containers that had non-lethal predators during the experiment (F<sub>1,27</sub> = 2.04, p-value = 0.16; Figure 2). There was a significant difference in the amount of resources that had been eaten between the containers that had no predators and the containers that had lethal predators. In the containers that had no predators more resources had been eaten during the experiment (F<sub>1,27</sub> = 12.59, p-value = 0.001; Figure 2). In the containers that had non-lethal predators more resources had been eaten compared to containers with lethal predators (F<sub>1,27</sub> = 4.49, p-value = 0.04; Figure 2).

For the per capita results there were significant differences in the results for some of the treatments. There was a significant difference for the treatment between no predator and non-lethal predator (t-value: 2.21, p-value: 0.05). For the treatment with no predator and lethal predator the results showed no significant difference (t-value: -1.76, p-value: 0.09) and for the treatment with non-lethal predator and lethal predator there was a significant difference (t-value: -3.95, p-value: 0.002).

### 4.6.3. 5 g of resources

According to the ANOVA-test the presence of predators had an effect on the amount of resources that had been eaten during the experiment in the containers that had 5 g of resources (F<sub>2,27</sub> = 3.44, p-value = 0.05). There was no significant difference in the amount of resources that had been eaten between containers that had no predators and the containers with non-lethal predators (F<sub>1,27</sub> = 1.69, p-value = 0.20; Figure 2). The *A. aquaticus* had eaten more in the containers with no predators compared to the containers with lethal predators (F<sub>1,27</sub> = 6.89, p-value = 0.05; Figure 2). There was no significant difference in the amount of resources that had been eaten by the *A. aquaticus* in the containers with non-lethal predator and the containers with lethal predators (F<sub>1,27</sub> = 1.75, p-value = 0.19; Figure 2). For the per capita results the t-tests showed that there was no significant difference in the results for the treatment with no predator and non-lethal predator (t-value: 0.75, p-value: 0.47). For the treatment with no predator and lethal predator there was a significant difference (t-value: -2.79, p-value: 0.02) and also for the treatment with non-lethal predator and lethal predator (t-value: -3.25, p-value: 0.01). According to these results the *A. aquaticus* in the treatments with lethal predators ate more than those in the other treatments.
4.7. TMII and DMII

TMII and the DMII are visualized in Figure 3. DMIIs are stronger when the levels of resources are low and declines at higher resources levels. The TMIIIs are low when the resources are low and stronger when the levels of resources increase. At 5 g resource levels there are almost no difference between DMIIs and TMIIIs. According to the ANOVA-test there was a significant difference ($F_{1,54} = 6.03$, p-value $= 0.02$) between TMII and DMII and there was also a significant difference in indirect effect depending on the resource levels ($F_{2,54} = 4.14$, p-value $= 0.02$). The interaction between the indirect effects and resources was also significant ($F_{2,5} = 4.80$, p-value $= 0.01$), suggesting that the strength of the indirect effects varied with resource levels.

4.8. Effects on $A. aquaticus$

4.8.1. No predator

In the end of the experiment the total number of $A. aquaticus$ that was left in the experiment was counted and the results showed that in the containers with 1 g of resources and no predators 79 % of $A. aquaticus$ had survived the experiment (Table 2). The mean weight for the $A. aquaticus$ before the experiment was 0.98 mg and 3.36 mg after the experiment. A t-test showed that there was a significant difference between the weight of the $A. aquaticus$ before and after the experiment (Table 2).

In the containers with 2.5 g of resources and no predators 77% of the $A. aquaticus$ survived until the end of the experiment (Table 2). The mean weight of the $A. aquaticus$ before the experiment was 0.98 mg and after the experiment it was 5.62 mg. A t-test showed that there was a significant difference between the weight for the $A. aquaticus$ before and after the experiment (Table 2).

In the containers with 5 g of resources and no predators the $A. aquaticus$ that were left at the end of the experiment was 80 % (Table 2). The mean weight for the $A.
aquaticus before the experiment was 0.98 mg and the mean weight in the end of the experiment for the A. aquaticus was 3.36 mg. A t-test that compared the weight between the A. aquaticus before and after the experiment showed that there was a significant difference in weight (Table 2).

Table 2. Results from the t-tests of weight increase and the survival rate of the A. aquaticus in each treatment.

<table>
<thead>
<tr>
<th>Resource (g)</th>
<th>No predator</th>
<th>Non-lethal predator</th>
<th>Lethal predator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight: t-value: 6.98 p-value: 1.45 x 10^{-65} Survival: 79 %</td>
<td>Weight: t-value: 10.02 p-value: 2.69 x 10^{-68} Survival: 81 %</td>
<td>Weight: t-value: 8.13 p-value: 1.87 x 10^{-66} Survival: 37 %</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>Weight: t-value: 11.47 p-value: 1.85 x 10^{-07} Survival: 77 %</td>
<td>Weight: t-value: 10.84 p-value: 2.20 x 10^{-07} Survival: 84 %</td>
<td>Weight: t-value: 4.11 p-value: 0.0026 Survival: 49 %</td>
</tr>
<tr>
<td>5</td>
<td>Weight: t-value: 13.28 p-value: 4.09 x 10^{-08} Survival: 80 %</td>
<td>Weight: t-value: 11.64 p-value: 1.59 x 10^{-07} Survival: 80 %</td>
<td>Weight: t-value: 8.86 p-value: 2.45 x 10^{-06} Survival: 44 %</td>
</tr>
</tbody>
</table>

4.8.2. Non-lethal predator
In the containers with 1 g of resources and non-lethal predator, the amount of A. aquaticus that was left in the end of the experiment was 81 % (Table 2). The mean weight of the A. aquaticus from before and after the experiment was 0.98 and 3.21 mg respectively, and a t-test showed that there was a significant increase in weight (Table 2).

In the containers with 2.5 g of resources and non-lethal predators the percent of A. aquaticus that was left was 84 % (Table 2). The mean weight of the A. aquaticus was 0.98 mg before the experiment and 5.01 mg after and a t-test showed that there was a significant increase in weight (Table 2).

In the containers with 5 g of resources and non-lethal predators 80 % of the A. aquaticus survived (Table 2). The mean weight for the A. aquaticus before the experiment was 0.98 mg and 5.62 mg after the experiment and this weight increase was significant (Table 2).

4.8.3. Lethal predator
In the containers with 1 g of resources and lethal predators the percent of A. aquaticus left in the end of the experiment was 37 % (Table 2). The mean weight in the A. aquaticus before the experiment was 0.98 mg and after the experiment it was 3.39 mg. A t-test showed that there was a significant difference between the weight in the A. aquaticus before the experiment and the A. aquaticus after the end of the experiment (Table 2).

In the containers with 2.5 g of resources and lethal predators the percent of A. aquaticus left after the experiment was 49 % (Table 2). The mean weight of the A. aquaticus before the experiment was 0.98 mg and after the experiment it was 4.90 mg and a t-test showed that there was a significant increase in weight (Table 2).
In the containers with 5 g of resources and lethal predators the percent of *A. aquaticus* left in the end of the experiment was 44% (Table 2). The mean weight of the *A. aquaticus* before and after the experiment was 0.98 mg and 4.43 mg respectively. A t-test showed that this increase in weight was significant (Table 2).

To examine if the amount of resources had an effect on the survival of *A. aquaticus* an ANOVA-test was performed on the number of individuals that survived. The ANOVA showed that resource levels did not affect survival ($F_{2,81} = 0.65$, p-value = 0.52). In contrast the presence of predators had an effect on the number of surviving *A. aquaticus* (ANOVA: $F_{2,81} = 61.39$, p-value=0.000001). The interaction between resources and predators had no significant effect on the amount of *A. aquaticus* that was left in the end of the experiment according to the ANOVA-test ($F_{4,8} = 0.62$, p=0.65).

4.9. The weight of the *A. aquaticus* between different treatments

4.9.1. 1 g of resources

4.9.1.1. No predator and non-lethal predator

When a t-test was performed on the results of the weight of the *A. aquaticus* between the treatments with no predators (25.73 mg) and the treatments with non-lethal predators (26.61 mg) the results showed no significant difference between the different treatments (t-value: -0.24, p-value: 0.81). This result is correlated with the ANOVA-test ($F_{1,27} = 0.39$, p-value=0.53) that showed that the *A. aquaticus* did not eat more resources when there were no predators present compared to when there were non-lethal predators present. When a t-test was conducted on the per capita amount of food eaten, the results showed that there was no significant difference between the different treatments (t-value: 0.39, p-value: 0.69).

4.9.1.2. No predator and lethal predator

In the treatments with no predators and lethal predators the mean weight of the *A. aquaticus* was 25.73 mg in the treatments with no predators and the mean weight was 12.51 mg for the *A. aquaticus* in the treatments with lethal predators and this difference was significant (t-value: 4.99, p-value: 9.29 x 10^{-05}). This correlated with the results from the experiment that showed that there were more resources eaten in the treatments with no predators than in the treatments with lethal predators according to the ANOVA-test ($F_{1,27} = 5.65$, p-value=0.02). The t-test on the per capita results the test showed that there were no difference between the different treatments (t-value: -0.07, p-value: 0.95).

4.9.1.3. Non-lethal predator and lethal predator

In the treatments with non-lethal predators and lethal predators the mean weight of the *A. aquaticus* was 26.61 mg in the treatments with non-lethal predators and the mean weight was 12.51 mg for the *A. aquaticus* in the treatments with lethal predators, and this difference was significant (t-value: 3.89, p-value: 0.0016). This correlates with the results from the experiment that showed that there were more resources eaten in the treatments with non-lethal predators then in the treatments with lethal predators according to the ANOVA-test ($F_{1,27} = 9.05$, p-value=0.005). The t-test on the per capita results showed that there was no difference between the different treatments (t-value: -0.53, p-value: 0.60).
4.9.2. 2.5 g of resources

4.9.2.1. No predator and non-lethal predator
In the treatments with no predators and non-lethal predators the mean weight of the \textit{A. aquaticus} was 42.86 mg and 42.41 mg respectively, and this difference was non-significant (t-value: 0.09, p-value: 0.92) This correlates with the results from the experiment that showed that there was no significant difference between the different treatments according to the ANOVA-test \((F_{1,27} = 2.03, p-value = 0.17)\). The t-test on the per capita results showed that there was no difference between the different treatments (t-value: 1.17, p-value: 0.25).

4.9.2.2. No predator and lethal predator
In the treatments with no predators and lethal predators the mean weight of the \textit{A. aquaticus} was 42.86 mg and 22.52 mg respectively, and this difference was significant (t-value: 5.08, p-value: \(7.83 \times 10^{-5}\)). This correlates with the results from the experiment that showed that there was more resources eaten in the treatments with no predators than in the treatments with lethal predators according to the ANOVA-test \((F_{1,27} = 12.58, p-value = 0.0014)\). The t-test on the per capita results showed that there was no difference between the different treatments (t-value: 0.70, p-value: 0.49).

4.9.2.3. Non-lethal predator and lethal predator
In the treatments with non-lethal predators and lethal predators the mean weight of the \textit{A. aquaticus} was 42.40 mg and 22.52 mg respectively. This difference was significant (t-value: 4.22, p-value: 0.0006). This correlates with the results from the experiment that showed that there was more resources eaten in the treatments with no predators than in the treatments with lethal predators according to the ANOVA-test \((F_{1,27} = 4.49, p-value = 0.004)\). The t-test on the per capita results showed that there was no difference between the different treatments (t-value: 0.11, p-value: 0.91).

4.9.3. 5 g of resources

4.9.3.1. No predator and non-lethal predator
In the treatments with no predators and non-lethal predators the mean weight of the \textit{A. aquaticus} was 44.80 mg and 42.03 mg respectively, and this difference was not significant (t-value: 0.49, p-value: 0.62). This correlates with the results from the experiment that showed that there was no significant difference in resource amounts eaten between the different treatments according to the ANOVA-test \((F_{1,27} = 1.69, p-value = 0.20)\). The t-test on the per capita results showed that there was no difference between the different treatments (t-value: 0.69, p-value: 0.49).

4.9.3.2. No predator and lethal predator
In the treatments with no predators and lethal predators the mean weight of the \textit{A. aquaticus} was 44.81 mg and 19.69 mg respectively. This difference was significant according to the t-test (t-value: 4.96, p-value: 0.0002). This correlates with the results from the experiment that showed that there was more resources eaten in the treatments with no predators than in the treatments with lethal predators according to the ANOVA-test \((F_{1,27} = 6.88, p-value= 0.01)\). The t-test on the per capita results showed that there was a difference between the different treatments (t-value: 2.34, p-value: 0.03).
4.9.3.3. Non-lethal predator and lethal predator
In the treatments with non-lethal predators and lethal predators the mean weight for the *A. aquaticus* was 42.03 mg and 19.69 mg respectively, and this difference was significant (t-value: 4.91, p-value: 0.0001). This result did also correlate with the results from the experiment that showed how much resources had been eaten according to the ANOVA-test (F_{1,27} = 4.49, p-value = 0.004). The t-test on the per capita results showed that there was no difference between the different treatments (t-value: 1.62, p-value: 0.12).

5. Discussion
How resource levels affect the relative strength TMII and DMII is an area in ecology that has been sparsely examined over the years. Those few studies that have been made have often shown conflicting results depending on which type of system that has been studied. For example Wodjak and Luttbeg (2005) showed that TMII decreased when the resources increased while DMII increased. In contrast the theoretical model by Luttbeg et al. (2003) predicts that TMII should increase and DMII decrease when the resource levels increased. My results showed that TMII was low when there were low levels of resources and increased somewhat when the levels of resources increased. The DMII was on the other hand high when the levels of resources were low and then decreased when the levels of resources increased (Figure 3). Hence, I found some support for the model predictions.

5.1. Behaviour experiment number one and two

5.1.1. Movement
The total movement of the *A. aquaticus* in the behaviour experiment showed that they did not move more in the containers without predators than with predators. This might imply that the *A. aquaticus* did not react to the presence of the *Aeshna* predators. But there was a trend showing that there was a significant difference between the containers without predators and the containers with predators. This tendency in a decrease of activity in the presence of the *Aeshna* predators in addition to the results from the experiment conducted by Johansson (2005), which showed a clear difference in the movement between the small and the larger *A. aquaticus*, suggest that the prey do respond to the predators. The gape limitation of the predators restrict the predators to choose the smaller prey (Gill and Hart, 1994), and thus giving the smaller prey more reasons to avoid the predators. This should result in a stronger activity effect in small prey. Even if there was no significant difference in the movement between the smaller and the larger *A. aquaticus* there was still a trend that showed that the smaller *A. aquaticus* moved less in the presence of predators than in the containers without predators, I therefore selected the smaller *A. aquaticus* for the main experiment.

5.2. Main experiment
The amount of leaves that were eaten during the experiment showed that the least eaten amount of leaves was in the containers with lethal predators and the most eaten amount was in the containers with no predators. This result shows that the *A. aquaticus* ate less in the presence of predators compared to in the containers with non-lethal predators and no predators at all. I suggest that this pattern is a result of anti-predator behaviour. The *A. aquaticus* simply spent more time avoiding the predators by being less active. Similar predator avoidance patterns with a corresponding
response on resource have been found by Malmqvist (1993), where the prey ate less in the presence of predators compared to when there were no predators present.

5.2.1. Weight of \textit{A. aquaticus} compared to animals before main experiment

In all three treatments, no predator, non-lethal predators and lethal predators and at all three resource levels, 1, 2.5 and 5 g, the results showed that all the \textit{A. aquaticus} put on weight during the experiment (Table 2). This shows that the \textit{A. aquaticus} in this treatment have all been eating during the experiment and this was also confirmed visually when the experiment ended.

5.2.2. Comparing different treatments

5.2.2.1. Controls

The fact that the micro organisms seemed to have consumed more resources than in some of the containers with no predators, non-lethal predators and lethal predators at different resource levels are hard to explain. One hypothesis could be that extra growth of micro organisms on the leaves, and that these micro organisms caused a strong breakdown of the leaves.

5.2.2.2. Treatment with no predator and non-lethal predator

5.2.2.2.1. Amount of leaves eaten

\textit{A. aquaticus} did not eat less in the presence of non-lethal predators compared to in the absence of predators. This suggests that they do not appear to change their antipredator behaviours. This pattern was the same for all three resource levels. This pattern is not the same as in the report from Luttbeg et al. (2003), where the treatments with non-lethal predators caused the prey to eat less than the prey in the treatments with no predators. One reason for the absence of an effect in my study could be that there was strong intra-specific competition between the \textit{A. aquaticus} in my experiment. Hence, the intraspecific interactions might have caused less foraging.

5.2.2.2.2. Weight of \textit{A. aquaticus}

There was no difference in the weight of the \textit{A. aquaticus} between the non-lethal and the absence of predators’ treatments. This result also supports the interpretation that the \textit{A. aquaticus} did not react to the presence of non-lethal predators compared to the containers with no predators in them. The \textit{A. aquaticus} do not seem to feel the presence of the non-lethal predators or they do not consider the predators as a strong threat. The same results were obviously independent of resource level: 1, 2.5 and 5 g of resources. When the per capita results were calculated there was no significant difference for all of the results.

5.2.2.3. Treatment with no predator and lethal predator

5.2.2.3.1. Amount of leaves eaten

When it came to the amount of leaves that had been eaten during the experiment there was a clear significant difference between the different treatments and this was evident for all three resource levels: the \textit{A. aquaticus} ate less in the presence of \textit{Aeshna} larvae. The results from Luttberg et al. (2003), also showed a decrease in the amount of resources that were eaten in the presence of a predator. This could be interpreted as if the \textit{A. aquaticus} do react to the presence of predators and this affects
the amount of leaves that are consumed. However, when I analysed per capita food consumption the results showed that the *A. aquaticus* had eaten more in the treatments with lethal predators than in the treatments with no predators. This suggests that even though the *A. aquaticus* did react to the presence of predators they consumed more food per capita.

### 5.2.2.3.2. Weight of *A. aquaticus*

There was a significant difference in the results when it came to the difference in the weight that the *A. aquaticus* had put on during the experiment in the different treatments according to the statistics. This gives more evidence to the fact that the *A. aquaticus* in the treatments reacted to the predators. The same results are shown in all the containers with the treatments of no predators and lethal predators. The per capita results showed that there were no significant differences for all of the results except for the levels with 5g of resources. There the *A. aquaticus* with no predators had put on more weight then the *A. aquaticus* with lethal predators suggesting that more food was consumed as an effect of “thinning”.

### 5.2.2.4. Treatment with non-lethal predator and lethal predator

#### 5.2.2.4.1. Amount of leaves eaten

When it came to the amount of leaves that were eaten during the treatment with non-lethal predators and lethal predators there was a significant difference in the amount that were eaten at the different levels of resources. The *A. aquaticus* did react to the presence of predators and ate less in the treatments with lethal predators compared to non-lethal predators. But there was not a significant difference in the amount of leaves that were eaten in the treatments with 5g of resources. This is hard to explain but one explanation could be that at high amounts of resources in the treatments with lethal predators the *A. aquaticus* do not need to take the predators into consideration. There is so much to eat for the *A. aquaticus* that even if they show anti-predator behaviour the amount of food eaten will be the same as without any anti-predator behaviour. In contrast Luttbeg et al. (2003), found no large difference between the two treatments. The per capita amount of leaves that were eaten also showed that there was a significant difference in all the resource levels. In the treatments with lethal predators the *A. aquaticus* had eaten more per individual than in the treatments with non-lethal predators. This might suggests a thinning effect.

#### 5.2.2.4.2. Weight of *A. aquaticus*

There was a significant difference when the weight of the *A. aquaticus* was measured between the different treatments and at all resource levels. The animals in the treatment with non-lethal predators did put on more weight then the *A. aquaticus* in the treatment with lethal predators. This suggests that the amount of leaves eaten is reflected in weight gain of the *A. aquaticus*. For the per capita results there were no significant differences in the amount of weight that they had put on.

### 5.2.3. TMII and DMII

In my study I found that the indirect effect of DMII was stronger than the TMII when there were fewer resources, and when the resource levels increased the DMII decreased and the TMII increased. At 5g of resources the DMII was about the same as the TMII. These results support the theoretical predictions given in Luttbeg et al. (2003). They predicted that DMII should be stronger compared to TMII when there
were low resource levels and that TMII should be stronger than the DMII when there were high levels of resources. In an empirical study Wojdak and Luttbeg (2005) found that TMII and the DMII were the opposite of my results and the model predictions.

My results show that when the resources are low the DMII is higher than the TMII and this implies that the *A. aquaticus* are taking more risks to forage and thus becoming more vulnerable to the predators (Luttbeg et al. 2003). When the resources are high there is little difference between TMII and DMII suggesting that *A. aquaticus* decreases its foraging effort. It seems as if the resource levels are high enough to gather enough amounts of resources without a high activity.

Because TMII and DMII in this type of experiments is measured for a short period of time I can not be sure that it will represent the long term situation under natural environmental conditions. Other major factors contribute to influence the DMII and TMII when there is a longer period in the experiment and in nature where more parameters can influence the TMII and the DMII than in a controlled experiment in a laboratory (Luttbeg et al. 2003, Gabrowski and Kimbro, 2005).

5.3. Possible improvements and some problems
One limitation with experiments conducted in the laboratory is that the effect that the predator has on the prey is likely to be overestimated because the prey or the predators can not leave the containers and emigrate (Malmqvist, 1993). My experiment was not a long-term experiment so the results could be different if the experiment was to be conducted during a longer time period. In nature there can be many different factors that can influence the *A. aquaticus* and the *Aeshna* larvae such as other predators and resources. It would be very interesting to be able to do a larger experiment and also do a field experiment to compare the results with results such as those of Wojdak and Luttbeg (2005).

In some of the plastic containers the *Aeshna* larvae died a natural death and that could have had an impact on the results even if the *Aeshna* was replaced as soon as possible, which reduced the possible impact. Other factors that might influence the results are which stage of the lifecycle that the prey and predators represent. Life cycle stage can for example be important for how much prey is consumed during the time of the experiment (Luttbeg et al. 2003 and Malmqvist, 1993).

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I would like to thank my supervisor Frank Johansson for giving me the help I needed for this report. I would also like to thank my parents, brothers, sister and my friends for always standing by me and supporting me. The outstanding support from Peter Holmblad has been very important for me during this time.

7. References


Luttbeg B., Rowe L. and Mangel M., 2003, Prey state and experimental design affects relative size of trait- and density mediated indirect effects, Ecology, 84 (5).


Sandhall Å., 2000, Trollsländor i Europa, Interpublishing.

