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Different anti-predator defenses induced by roach and signal crayfish in the invader zebra mussel

David Cayón Díaz

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Biology Education Centre and Limnology, Uppsala University
Supervisors: Philipp Hirsch and Richard Svanbäck

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Abstract

Invasive species are successful colonizers in a new ecosystem due to their plasticity to face the new conditions. Anti-predator defenses are a common mechanism developed by invasive species in presence of new predators in the new ecosystem.

The invasive mollusc zebra mussel (*Dreissena polymorpha*, Pallas) has colonized most of European freshwater systems. We studied the anti-predator defenses developed by zebra mussel in presence of two new predators: signal crayfish (*Pacifastacus leniusculus*), and the fish roach (*Rutilus rutilus*). Their feeding mechanisms are different. Signal crayfish detach zebra mussel from substrata and then crush the shell whereas roach detach the mussel and then swallow. We carried out a 5 weeks experiment in which the zebra mussel individuals were exposed to control conditions, signal crayfish predator, roach predator and both predators at the same time. Then we analyzed differences in parameters associated to the development of anti-predator defenses such as shell strength, shape, attachment strength, growth, aggregation, distance travelled and selection of dark attachment sites.

We found that zebra mussel individuals exposed to signal crayfish predator developed stronger shells, a more rotund shell shape, and grew less. These results suggest that zebra mussels in presence of signal crayfish invest the energy in hardening their shell, instead of growing, in order to avoid being crushed. On the other hand zebra mussel individuals exposed to roach developed more elongated shell shapes and grew more. These results suggest that zebra mussel individuals in presence of roach invest energy in growing or lengthening their shape in order to avoid being swallowed. However, zebra mussel individuals exposed to both predators at the same time did not differ from zebra mussels in control conditions. This result suggests that zebra mussels do not prioritize for any predator when exposed to both. Zebra mussel individuals exposed to crayfish, roach or both predators at the same time increased the attachment strength compared to control conditions. This result suggests that zebra mussels in presence of a high density of predator cues invest more energy in attachment to avoid being detached. On the other hand we did not find differences in aggregation, distance travelled and selection of dark attachment sites.

We conclude that zebra mussels acquire specific anti-predator defenses depending on which predator is exposed. This ability might be one of the reasons of their invasion success.

Introduction

The introduction of invasive species in a new ecosystem is currently a striking topic in evolutionary ecology. Invasive species present exceptional characteristics, which favor their success in new ecosystems. In addition, lack of natural predators in the new ecosystem facilitates their colonization. However, some native species in the new ecosystem may change their diet in order to feed on the invader. The presence of new predators in the new ecosystem may induce defenses in the invader. Inducible anti-predator defenses are common in different organisms like plants (Karban and Myers 1989), algae (Cronin and Hay 1996), gastropods (Trussell 1996), amphibians (McCollum and Buskirk 1996), fishes (Bronmark and Pettersson 1994) or birds (Dumbacher *et al*. 1992) in both terrestrial and aquatic ecosystems (Leonard *et al*. 1999). These induced anti-predator defenses are associated to the phenomenon of phenotypic plasticity. Invasions also trigger biological processes in the new ecosystem affecting native species (Hirsch *et al*. submitted manuscript). Native species might adapt in presence of the invader in order to avoid extinction (defensive adaptation) or in order to profit from a new resource facilitated by the invader (offensive adaptation) (Hirsch *et al*. submitted manuscript).

In the last 200 years a lot of invasion episodes have occurred in freshwater systems, mainly favored by humans (Lee and Bell 1999). Especially invasive invertebrate species have colonized freshwater systems in North America, Europe and Asia (Lee 1999). One of the most dangerous is the invasive mollusc zebra mussel (*Dreissena polymorpha*, Pallas). Originating from the Ponto-Caspian area, the zebra mussel has invaded most of European and North American freshwater systems (MacIsaac 1996). In Sweden the first record of zebra mussel was in the 1920ies in Lake Mälaren (Grandin *et al*. 2006) and nowadays it exists in several lakes within the Upplands region.

Previous studies found behavioral anti-predator defenses developed by zebra mussels, induced by different predators (Kobak 2006; Nadaffi *et al*. 2007; Kobak and Kakareko 2009; Kobak and Kakareko 2010). Zebra mussels notice the presence of a potential predator by means of waterborne cues that the predator releases (Kobak and Kakareko 2009). These cues released by the predators are called kairomones and are common in diverse organisms such as crustaceans (Pijanowska and Kowalczewski 1997) or fish (Gliwicz 2005). However, these previous studies cited above are of short time duration and only address some of the induced anti-predator defenses that the zebra mussels can develop. Morphological induced defenses such as increase in shell strength, changes in shell shape or changes in growing cannot be addressed in short duration experiments. Therefore we aim to study all the induced defenses that zebra mussels can develop.

Our study analyzes the different anti-predator defenses developed by zebra mussel in a new environment in presence of two different predators, signal crayfish (*Pacifastacus leniusculus*) and roach (*Rutilus rutilus*). They are two of the most likely predators of zebra mussel in Swedish lakes (Nagelkerke and Sibbing 1996; Schreiber *et*

al. 1998; Nystrom 2005). The invasive signal crayfish use the maxillipeds to detach the mussel from substrata and then use the mandibles to crush the shell in order to obtain the flesh (Schreiber *et al*. 1998). On the other hand roach try to detach the mussel from the substrate for a couple of seconds and if succeed swallow it and then use the pharyngeal teeth to crush the shell (Nagelkerke and Sibbing 1996). According to the different feeding mechanism of signal crayfish and roach, zebra mussels develop different defenses depending on which predator they have to defend from (Kobak and Kakareko 2009).

Zebra mussels exposed to signal crayfish must avoid being crushed. Marine snails (*Nucella lamellosa*) may adopt rotund shapes and thicker shells in order to defend against a shell crushing crab predator (Bourdeau 2009). In a similar way freshwater snails (*Physa sp.*) may develop rotund shells to avoid being crushed by fish predators (DeWitt *et al*. 2000). Blue mussel (*Mytilus edulis)*, a marine bivalve which is eaten by crabs in the same way as zebra mussel, develop thicker shells in habitats with high predator density (Leonard *et al*. 1999). A recent study shows that zebra mussels under high mortality levels increase the shell strength (Czarnoleski *et al*. 2006). Indeed a previous study found that signal crayfish rejected more often hard mussel shells as they could not crush them (Schreiber *et al*. 1998). On the other hand zebra mussel exposed to roach must avoid being swallowed. Large mussels are less often consumed by roach predators because the relation between energy costs and benefits is less optimal than in smaller mussels (Prejs *et al*. 1990).

Furthermore, zebra mussels exposed to both predators must avoid being detached from the substrate. A marine bivalve (*Perna viridis*) produced stronger byssal threads and detached less often from the substrate in presence of potential predators (Cheung *et al*. 2004). *Mytilus sp*. increases attachment strength and aggregation in presence of sea stars and crab predators (Reimer and Tedengren 1997). Previous studies show that zebra mussels increase attachment strength and aggregation in presence of roach (Kobak and Kakareko 2009; Kobak and Kakareko 2010). There are no studies indicating that signal crayfish induce increased attachment strength for zebra mussels. However, zebra mussels exposed to signal crayfish also have to avoid being detached from the substrate.

Other behavioral response to the presence of predators is the decrease in the distance travelled. A moving mussel is more noticeable for predators and easy prey as they are dislodged from the substrate. Previous research indicates that small zebra mussels exposed to roach decrease their upward movement (Kobak and Kakareko 2009).

In addition, zebra mussels also respond to light conditions. Recent studies show that the illumination of substrates affects the attachment site selection of zebra mussels (Kobak 2006; Kobak and Nowacki 2007). They prefer dark attachment sites over illuminated ones. This behavior can be explained as in dark conditions zebra mussels are more sheltered from visual predators like fish, while in light conditions zebra mussels are more noticeable to predators.

In our study we also analyze the anti-predator defenses developed by zebra mussels in a combined treatment with both predators. In many Swedish Lakes zebra

mussels co-exist with both signal crayfish and roach (Nagelkerke and Sibbing 1996; Nystrom 2005). Therefore they have to defend from both predators at the same time. However the anti-predator defenses adopted by zebra mussel against one of the predators might be adverse to defend against the other predator. Indeed more anti-predator defenses developed involve higher energy cost. In a similar study with two predators, marine snails *(Nucella lamellosa)* prioritized defenses against the most dangerous predator (Bourdeau 2009). Zebra mussels might prioritize which one to acquire when both predators are present or try to adopt intermediate defenses for both predators (Matsuda *et al*. 1996).

We carried out a laboratory experiment to analyze all the parameters that zebra mussel could change, such as shell strength, shape, growth, attachment strength, aggregation, distance travelled and selection of dark attachment sites in response to signal crayfish, roach and both predators simultaneously (combined treatment). We hypothesized that the zebra mussel will increase shell strength, attachment strength and will acquire rotund shells in order to defend against signal crayfish. On the other hand we hypothesized that the zebra mussel will increase attachment strength, growth, and will acquire elongated shapes in order to defend against roach. We also hypothesized increase in aggregation, decrease in distance travelled and selection of dark attachment sites in the treatments containing any or both of the predators as they are common behavioral defenses against predators. Finally, in the combined treatment we hypothesized that the zebra mussel will acquire intermediate traits to defend against both predators, as there are no previous studies showing that one of the predators is more dangerous than the other one.

Material and Methods

Study site and sampling

We carried out the study at Lake Erken (59°51’ N, 18°35’ E) situated in the southeastern part of Sweden. We chose Lake Erken because the invasive zebra mussel and the predators signal crayfish and the fish roach cohabit there. The zebra mussel invaded Lake Erken in 1975 in unknown ways. Signal crayfish was humanly introduced at Lake Erken during the period of 1966-1969 (Schreiber *et al*. 1998). Finally roach is a dominant fish in Lake Erken, especially in littoral zones (Naddafi *et al*. 2007). Within the lake we selected a site with an even distribution of both predators for the study. Thereby we avoid beforehand traits developed by the zebra mussel individuals against the more densely represented predator. We collected the zebra mussel individuals by carefully removing them from stones by hand, in a 20 m^2 rocky littoral area at approximately 1 meter depth, less than 24 hours before start of every experiment. The mean size of mussels was approximately 10.84 mm length, 5.55 mm width, 5.41 mm height, and the mean age was around one year old. Before the experiment started the mussels were placed in a container with small holes, attached to a foot bridge by a rope and under

water of the lake all the time. Extra mussels for feeding the predators remained in the container while the experiment was running.

We caught the predators at the same site as the zebra mussels. A previous study shows that signal crayfish and roach individuals at that site actually feed on zebra mussels (unpublished data). Thereby they are potential predators for the zebra mussel individuals collected for the experiment. For the crayfish catch we used crayfish traps with dead fish as bait. At least one day before running the experiment the crayfish individuals were acclimated in a similar container to the one used for zebra mussels. We placed inside the container small zebra mussels in order to serve as food for crayfish. For the roach catch we used ordinary fishing rods with unbarbed hooks baited with worms. Before running the experiment the roach individuals were kept for 3 days in a keeping net tied to a foot bridge and fed with small mussels.

Experimental set up

The experiment took place in a lab next to the lake (Fig.1). We conducted the experiment in 28 (4 treatments × 7 replicates per treatment) 96-l tanks. The tanks were supplied with water directly pumped from the lake (Fig.2). The water flow was 5.71 ml/s. Every tank has an inlet water hose running 24 hours per day. Thereby the water of the tanks was totally renewed every 4 hours, 40 minutes. Once the water level of the tank reached a certain height the surplus water left the tank by the outlet pipe. Considering that the filtration rate of a zebra mussel individual of the size used in the experiment is approx 0.01 ml/s (Lei 1993), the water renewal was fast enough to avoid oxygen and food depletion. The water in the tanks started running 2 days before the beginning of the experiment. The water temperature was stabilized between 17-18ºC using thermostats. This is the highest temperature usually reach in the littoral zone where we collected the mussels. High temperatures, if not too high, generally favor the growth of zebra mussels (Spidle *et al*. 1995). Thereby this is the optimal growth temperature for mussels at that site.

Fig.1. Lab at Lake Erken. Preview of the position of the tanks in the experiment.

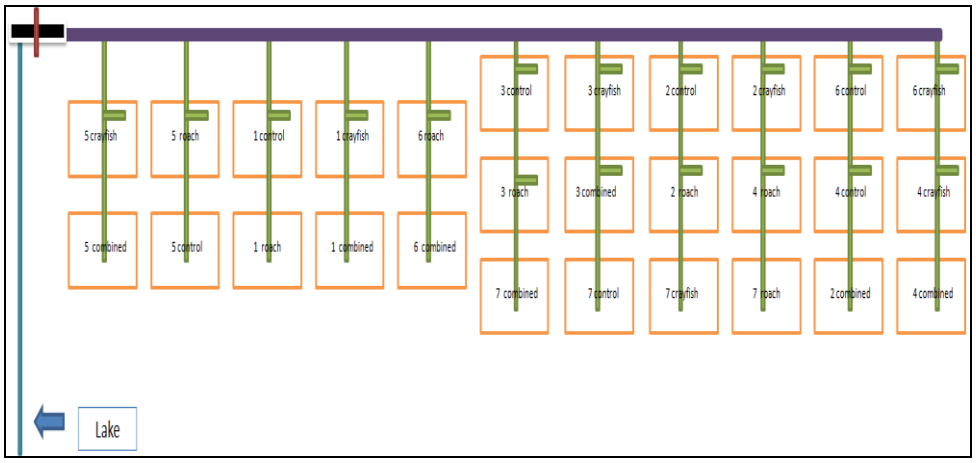

Fig.2. Design of the water circuit in the experiment. The water was pumped directly from the lake. A general tap regulated the inlet of water. Every tank had its own valve to regulate the water flow.

We used 4 different treatments: Control treatment, crayfish treatment (1 crayfish predator; mean weight: 33.65 g, SD: 3.50 g), roach treatment (1 roach predator; mean weight: 59.86 g, SD: 24.00 g) and combined treatment (1 crayfish; mean weight: 39.52 g, SD: 8.35 g and 1 roach predator; mean weight: 66.77 g, SD: 22.60 g). We fed the predators in the tanks every 3 days with zebra mussels the same size as the initial size mussels used in the experiment. If one predator died it was immediately replaced by a similar size predator.

Before set the mussels in the tanks we measured their original size in 3 dimensions (length, width and height) using a digital vernier caliper (±0.01 mm). At every tank we placed 16 mussels on 4 tiles of unglazed chime square (30 × 30 cm) (Fig.3). We selected small mussels with a proper size to grow fast. The mussels were first cleaned and then placed in the middle of the 4 tiles square equidistantly from each

other. The tiles were placed in the tanks 2 days before start of the experiment in order to create a biofilm layer and thereby make the substrate more suitable for the mussels (Kavouras and Maki 2003). We placed 2 white and 2 black tiles per tank in order to take into account the effect of light versus dark conditions. In different tanks we rotated the position of the white and black tiles in order to avoid the influence of the ambient light on the selection of the attachment sites.

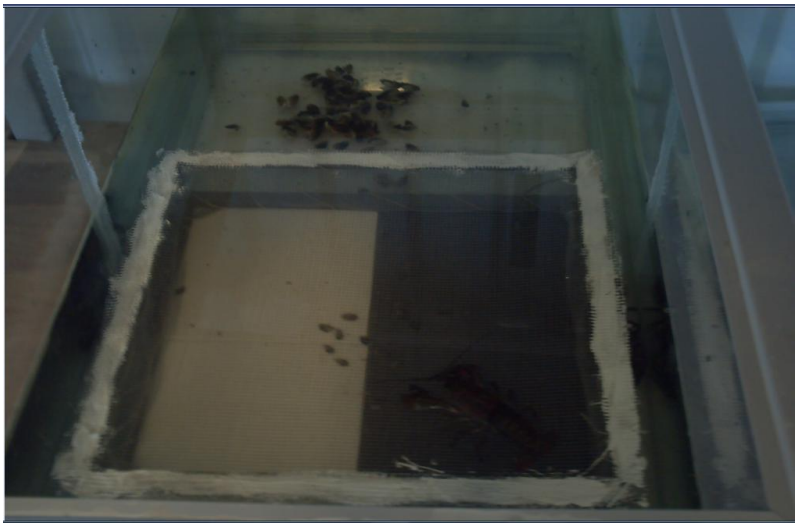

Fig.3. Test-run experiment. The mussels are placed in the middle of the 4 tiles square (30 × 30 cm). They are protected from predators (crayfish on the picture) by means of a cage 32.5 × 32.5 × 10 cm).

The mussels were protected from predators by means of a cage (32.5 × 32.5 × 10 cm) covered by a 3 mm mesh size net (Fig.3). Every tank, even the control replicates, presented a cage covering the 4 tiles square and subsequently the mussels. We also constructed a shelter for predators which consisted of a 15 cm long 8 cm diameter pipe with a line tied to it floating in the water. The crayfish individuals sheltered inside the pipe while the roach individuals sheltered behind the line floating in the water as it simulated the shape of a macrophyte. The upper part of the tanks was covered by a pair of lids in order to avoid predator escapisms. Once everything was ready we run the experiment for 5 weeks (14 June 2010-19 July 2010). Light followed the natural light-dark rhythm as we did the experiment in a lab room with windows.

Laboratory analyses

After 5 weeks we stopped the experiment. First we measured the fraction of mussels aggregated as the number of individuals touching each other divided by the number of total individuals (16). Then we counted the number of individuals attached to white or black tiles respectively. Later on for the analyses we used the fraction of mussels attached to black tiles as the number of mussels attached to black tiles divided by the total number of mussels in the tank (16). Afterwards we measured the distance travelled by every mussel as the distance from their original position to the final position at the end of the experiment using a ruler (±0.1 mm). We did not know which mussel belonged to which original position, thus we used the center of the 4 tiles square as original position. Then we carefully detached the mussels from the tiles using a scalpel and measured their attachment strength counting the number of byssal threads produced on the underside of the mussel (Clarke 1999). We counted the threads through a dissection microscope 45X.

Once we collected the mussels, we brought them to the Limnology Department, Uppsala University, in order to be analyzed. The time of transport was around 1 hour. The mussels were frozen in a – 20ºC freezer for 24 hours. After that time we defrosted the mussels and measured their size in the 3 dimensions (length, width and height) using a digital vernier caliper (±0.01 mm). Thereby we calculated the mussel growth comparing the initial size at the beginning of the experiment with the size reached at the end of the experiment. We unified the 3 dimensions growth in one single value, mean growth, in order to simplify later on the analysis. Then we scanned the shells using a copying machine scanner (Ricoh Afficio MP) in order to analyze differences in the shape. The copying machine scanner sent the pictures to a connected computer. The pictures were analyzed using Shape v.1.3., a package of informatics programs for evaluating biological contour shapes based on elliptic Fourier descriptors. Fourier analysis captures the outline of the shell and thus the shape. The program generates shape characteristics as principal components (PC1 to PC 50) to analyze shell shape variation (Iwata 2006). PC1 is the principal component which explains a higher percentage of the variation between shell shapes, followed by PC2, PC3 and so on, until PC50 which explains the lowest percentage of the variation. However, sometimes the whole variation is explained with a few principal components, so the program only calculates these principal components. The program allows you visualize the variations explained by each principal component (Fig.4). In our analysis PC1 explained 31.8% of the variation, while PC1 and PC2 together explained the 50.7%. Indeed PC1 and PC2 explain very well the differences between rotund versus elongated shell shape (Fig.4).

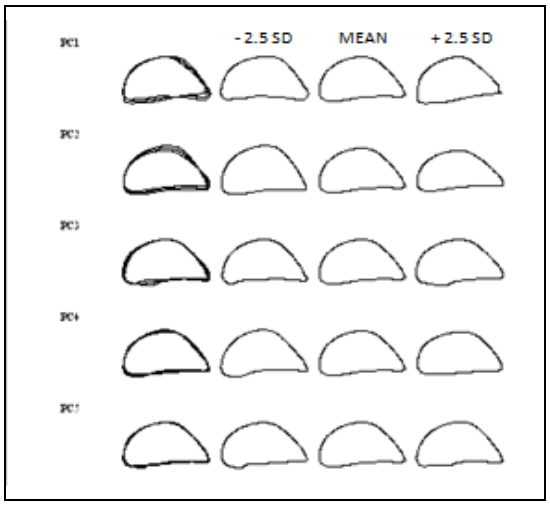

Fig.4. Shape differences generated as principal components by the informatics program Shape v.1.3. In the first column the different shapes overlapped. The second column depicts the more extreme negative value in PC (- 2.5 SD), the third column the mean value and the fourth column the more extreme positive value (+ 2.5 SD).

We used a force gauge (Mark-10^{R} motorized test stand ESM 3000) in order to measure the shell strength of the mussels. The force gauge bears a sensor (SSM 10 ±50 N, accuracy ±0.15) fixed to a needle. The force gauge goes down until the needle breaks the mussel shell. The sensor measures the force needed to break the shells and the force value is transferred to the computer through an informatics program (Rs Input.exe). We placed the weakest part of the shell perpendicular to the needle. A previous study shows that this is the part where signal crayfish crush the zebra mussel shells (Schreiber *et al*. 1998).

Statistical analyses

We analyzed shell strength and shape using one-way ANCOVA. However for attachment strength, mean growth, fraction of mussels aggregated, distance travelled and fraction of mussels attached to black tiles we used one-way ANOVA as the size of the mussels did not influence such parameters. In all of the analyses the parameter to study was the dependent variable whilst "Treatment" was the main fixed factor. For the shape we did two ANCOVA analyses, one with PC1 and the other one with PC2 as dependent variable. The reason is that PC1 and PC2 are the two variables which explain better the differences between rotund versus elongated shape (Fig.4). We used for the analyses the mean value of every tank in all the parameters. The reason is that the value of every individual depends on the tank which it belongs as the conditions of every tank, even within the same treatment, might be slightly different. We took as covariate the size of the mussels calculated for every individual as a PC value of the three size dimensions (length, width and height). The PC had an eigenvalue of 2.33 and explained 77.72 % of the total variance in the three size dimensions. The reason to use size as covariate is that during a 5 weeks experiment some mussels might grow more than

others and might have an influence in the values of some parameters such as shell strength or shape. Thereby we corrected the values including size as covariate. We used Wilks'Lambda statistical test and then Tukey HSD test as a post-hoc procedure. We log-transformed the data when they violated the assumptions of normality and homogeneity of variance (checked with Kolmogorov-Smirnov and Levene tests, respectively).

Results

The shell strength was slightly significantly different between treatments (ANCOVA: $F_{3, 24}$=2.99, p=0.05). We found significant differences in the post-hoc test between crayfish and control treatment (ANCOVA: $F_{3, 24}$=2.99, p=0.02) and between crayfish and roach treatment (ANCOVA: $F_{3, 24}$=2.99, p=0.01). Mussel shells in crayfish treatment (mean shell strength=0.31 N) were significantly stronger than mussel shells in control (mean shell strength=0.26 N) and roach treatment (mean shell strength=0.25 N) (Fig.5).

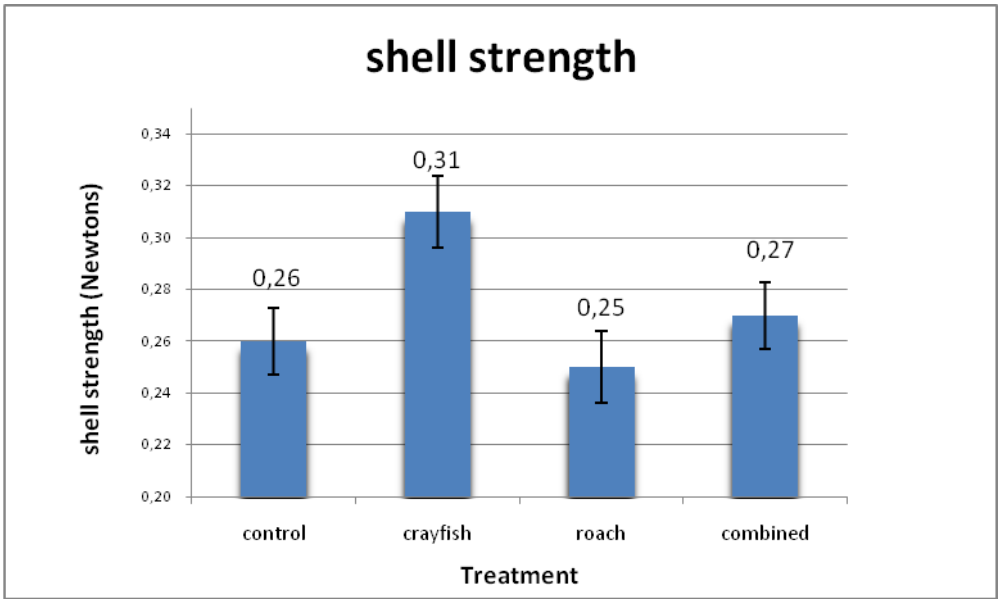

Fig.5. Mussel shell strength in the different treatments. The "x" axis represents the treatment and the "y" axis respresents the shell strength (Newtons). The shell strength values are corrected with PC of size as covariate. The error bars show standard deviations of means. Each point is the mean of 7 tanks with 16 mussels per tank.

The shape, characterized as PC1 and PC2, was not significantly different between treatments. For PC1 (ANCOVA: $F_{3, 24}$=2.17, p=0.12) and for PC2 (ANCOVA: $F_{3, 24}$=1.50, p=0.24). However, in the post-hoc test we found significant differences between crayfish and roach treatment. For PC1 (ANCOVA: $F_{3, 24}$=2.17, p=0.03) and for

PC2 (ANCOVA: $F_{3, 24}$=1.50, p<0.05). There were also significant differences for PC1 between crayfish and combined treatment. (ANCOVA: $F_{3, 24}$=2.17, p=0.04). Mussel shells in roach treatment were more elongated while mussel shells in crayfish treatment were more rotund (Fig.6 and Fig.7).

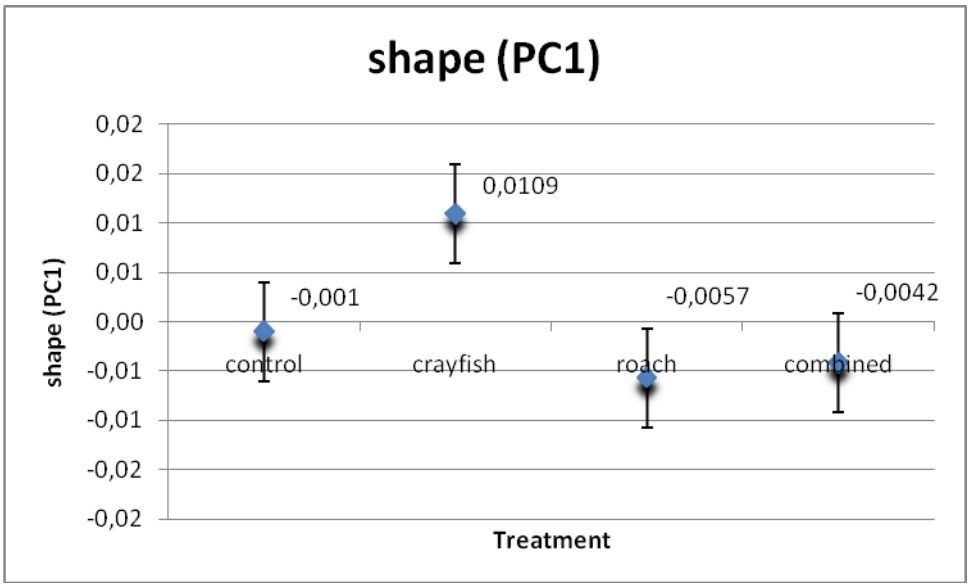

Fig.6. Differences in values of PC1 (shape) in the different treatments. The "x" axis represents the treatment and the "y" axis respresents the shape (PC1).The PC1 values are corrected with PC of size as covariate. The error bars show standard deviations of means. Each point is the mean of 7 tanks with 16 mussels per tank.

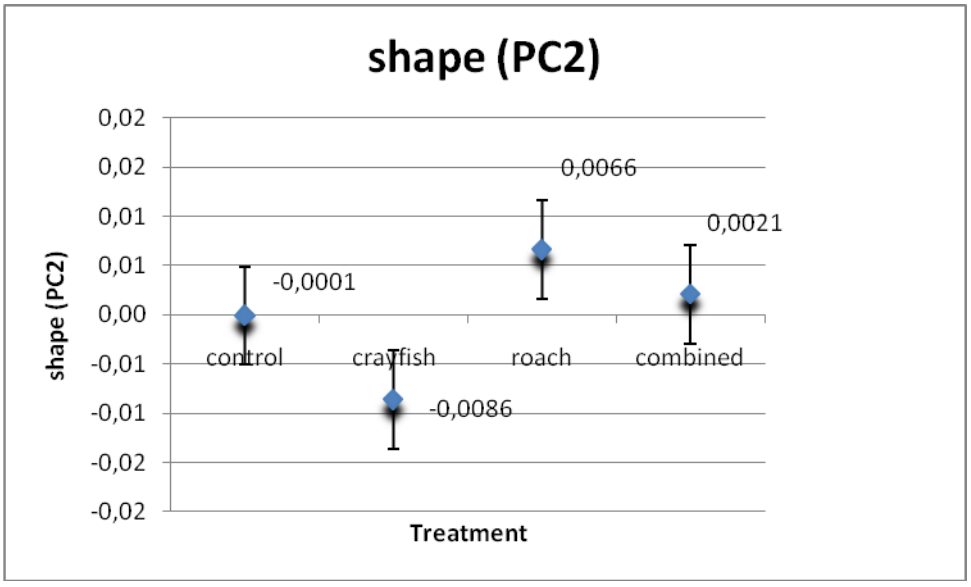

Fig.7. Differences in values of PC2 (shape) in the different treatments. The "x" axis represents the treatment and the "y" axis respresents the shape (PC2). The PC2 values are corrected with PC of size as covariate. The error bars show standard deviations of means. Each point is the mean of 7 tanks with 16 mussels per tank.

The attachment strength was significantly different between treatments (ANOVA: $F_{3, 24}$= 6.31, p=0.01). In the post-hoc test we found significant differences between control treatment and the rest of treatments; control versus crayfish treatment (ANOVA: $F_{3, 24}$= 6.31, p=0.01), control versus roach treatment (ANOVA: $F_{3, 24}$= 6.31, p=0.01) and control versus combined treatment (ANOVA: $F_{3, 24}$= 6.31, p=0.01). The attachment strength in treatments with predators was significantly higher than control conditions (Fig.8).

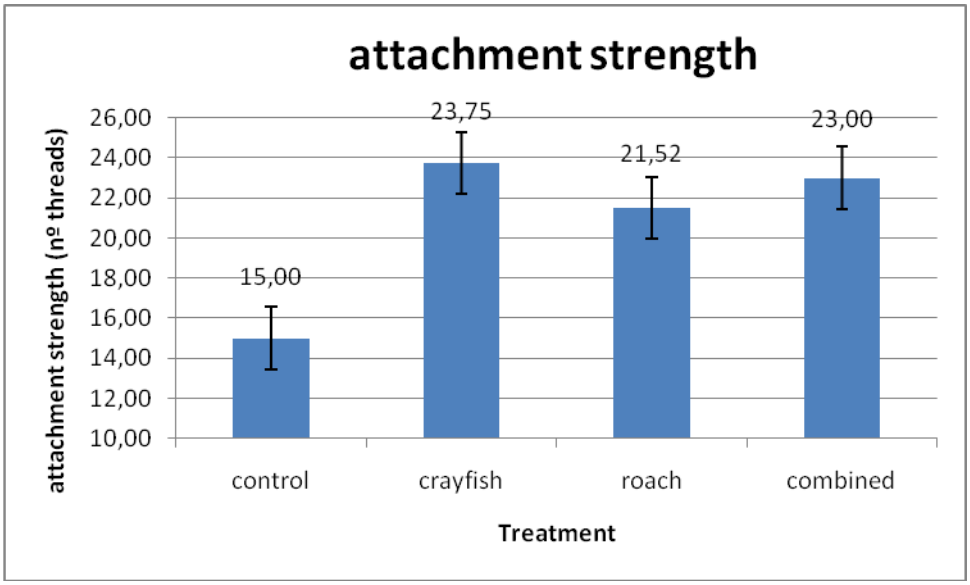

Fig.8. Difference in attachment strength between treatments. The "x" axis represents the treatment and the "y" axis respresents the attachment strength (number of threads). The error bars show standard deviations of means. Each point is the mean of 7 tanks with 16 mussels per tank.

The mean growth was significantly different between treatments (ANOVA: $F_{3, 24}$=5.76, p=0.03). In the post-hoc test we found significant differences between crayfish treatment and the rest of treatments; crayfish versus control (ANOVA: $F_{3, 24}$=5.76, p=0.05), crayfish versus roach treatment (ANOVA: $F_{3, 24}$=5.76, p=0.01) and crayfish versus combined treatment (ANOVA: $F_{3, 24}$ =5.76, p=0.01). The mussels in crayfish treatment grew significantly less than mussels in the rest of the treatments (Fig.9).

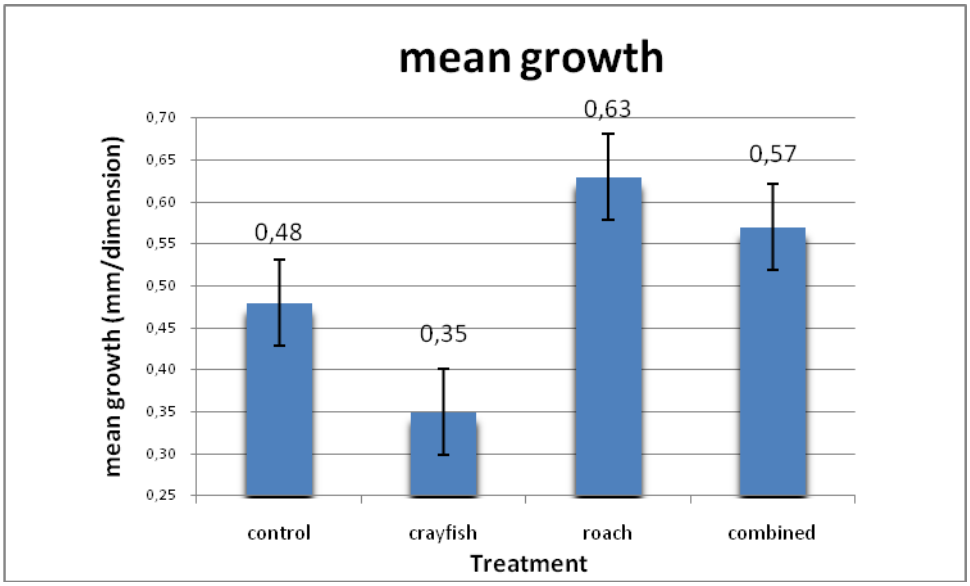

Fig.9. Difference in mean growth between treatments. The "x" axis represents the treatment and the "y" axis respresents the mean growth (mm/dimension). The error bars show standard deviations of means. Each point is the mean of 7 tanks with 16 mussels per tank.

The fraction of mussels aggregated was not significantly different between treatments (ANOVA: $F_{3, 24}$=1.74, p=0.19). In the post-hoc test we did not find significant differences between treatments. However, the fraction of mussels aggregated was a bit higher in roach treatment (fraction=0.52) and combined treatment (fraction=0.45) than in control (fraction=0.35) and crayfish treatment (fraction=0.32) (Fig.10).

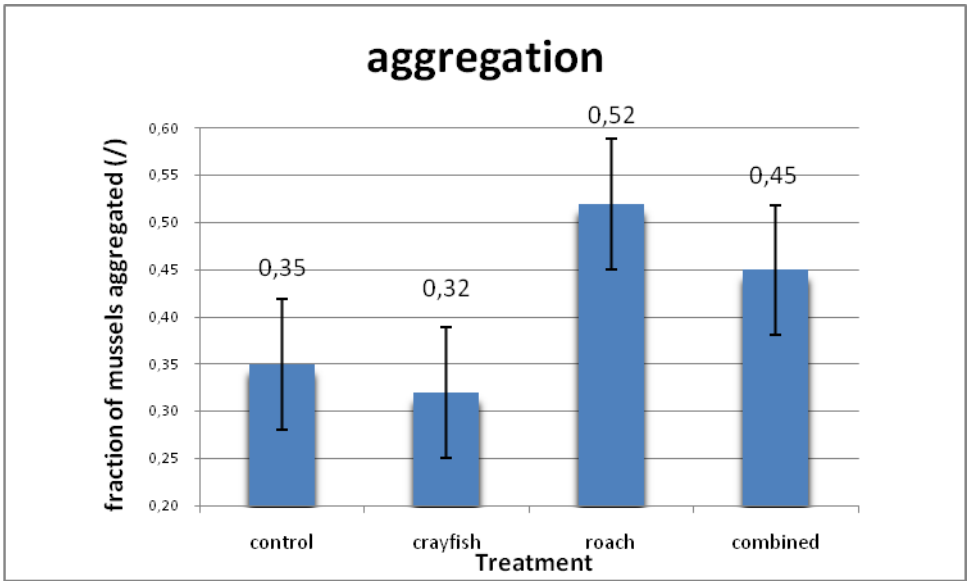

Fig.10. Difference in the fraction of mussels aggregated between treatments. The error bars show standard deviations of means. The "x" axis represents the treatment and the "y" axis respresents the fraction of mussels aggregated. Each point is the mean of 7 tanks with 16 mussels per tank.

The distance travelled by mussels was not significantly different between treatments (ANOVA: $F_{3, 24}$=1.75, p=0.18). However, in the post-hoc test we found slightly significant differences between control and roach treatment (ANOVA: $F_{3, 24}$=1.75, p=0.05). The distance travelled by zebra mussels in roach treatment was a bit shorter than in the rest of the treatments (Fig.11).

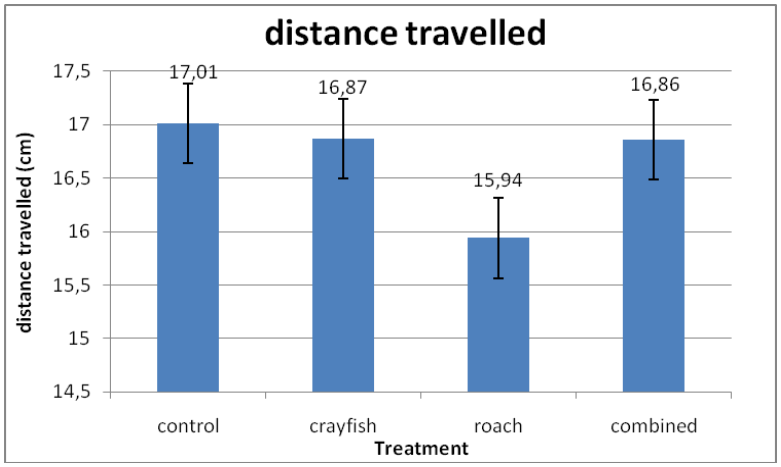

Fig.11. Difference in mussel distance travelled between treatments. The "x" axis represents the treatment and the "y" axis respresents the distance travelled (cm). The error bars show standard deviations of means. Each point is the mean of 7 tanks with 16 mussels per tank.

We did not find differences in the selection of attachment sites (Fig.12). The fraction of mussels attached in black tiles was not significantly different between treatments (ANOVA: $F_{3, 24}$=0.12, p=0.95).

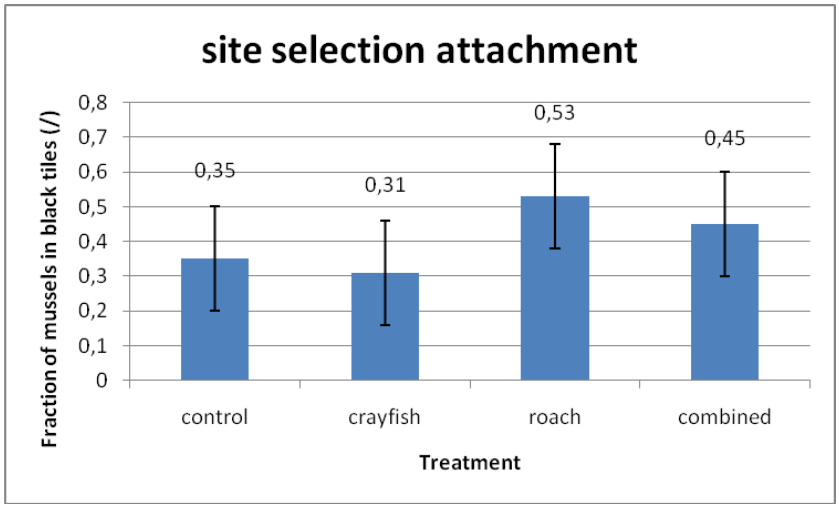

Fig.12. Difference in the fraction of mussels attached in black tiles between treatments. The "x" axis represents the treatment and the "y" axis respresents the fraction of mussels in black tiles. The error bars show standard deviations of means. Each point is the mean of 7 tanks with 16 mussels per tank.

Discussion

Zebra mussels exposed to signal crayfish predator alone increased shell strength (Fig.5) and adopted a more rotund shell shape (Fig.6 and Fig.7). Similar experiments showed an increase in shell strength in marine snails (Bourdeau 2009) and blue mussels (Leonard *et al*. 1999) induced by different crab predators. Furthermore zebra mussels in lakes with high population mortality have been assumed to increase shell strength (Czarnoleski *et al*. 2006). The feeding mechanism of signal crayfish induces zebra mussels to increase the shell strength and acquire a more rotund shell shape. Signal crayfish prey on zebra mussels by crushing the shell in order to obtain the flesh. Hard mussel shells are more likely rejected by signal crayfish as they cannot crush them or because the handling time and energy cost are higher (Schreiber *et al*. 1998). Indeed rotund shells disperse better the crushing forces applied by a predator which is why they are more resistant to be crushed (DeWitt *et al*. 2000). Thereby increasing shell strength and acquiring a more rotund shell shape mussels might avoid being eaten by signal crayfish.

Zebra mussels exposed to roach adopted a more elongated shell shape (Fig.6 and Fig.7) and grew more (Fig.9). Roach feed on rather small mussels (Nagelkerke and Sibbing 1996) while they reject large mussels (Prejs *et al*. 1990). The reason is that the balance between energy gained and energy costs is less positive in large than in small mussels. A recent short duration experiment showed that large mussels (> 17 mm mean shell length) did not respond to roach as they did not consider roach as a threat (Kobak and Kakareko 2009). In addition the feeding mechanism of roach is more efficient for small mussels than large mussels. At certain a size mussels cannot be swallowed by roach (Nagelkerke and Sibbing 1996). Therefore, by growing more and acquiring elongated shell shapes zebra mussels might avoid being ingested by roach.

Zebra mussels exposed to both predators increased the attachment strength (Fig.8). Similar short duration experiments indicate that after 6 days exposed to roach zebra mussels increased attachment strength and aggregation (Kobak and Kakareko 2009; Kobak and Kakareko 2010). Regarding signal crayfish, there are no previous studies that indicate an increase in the attachment strength of zebra mussels induced by this predator. However, the first step in the attacking mode of both predators consists on detaching the mussel from the substrate. Therefore, we could expect that the presence of signal crayfish might induce the increase in the attachment strength of the mussels. The results of the experiment show that zebra mussels increase the number of byssal threads produced in order to avoid being detached either by roach or by signal crayfish. On the other hand signal crayfish and roach did not induce an increase in zebra mussel aggregation although in presence of roach the fraction of mussels aggregated was a bit higher (Fig.10). Kobak and Kakareko indicate that the aggregation of zebra mussels increased in presence of roach predator while it did not increase in presence of crayfish (*Orconectes limosus*) with a similar feeding mechanism as signal crayfish (Kobak and Kakareko 2009). Thereby based on these results increased aggregation might be a more efficient defensive mechanism against roach predator than against signal crayfish.

However, there is no clear evidence of this fact. The aggregation results of our experiment are inconclusive.

The exposure to roach predator decreased the distance travelled by zebra mussels (Fig.11). A moving mussel is more vulnerable to detection and detachment. However, a similar short duration experiment did not find differences in distance travelled in zebra mussels exposed to roach neither crayfish predators (Kobak and Kakareko 2009). In our experiment we noticed that many mussels already reached the edges of the cage 1 hour after start of the experiment and most of them kept this position during the whole experiment and where attached there. The mussel distance travelled was limited by the dimensions of the cage (32.5 × 32.5 × 10 cm). Maybe if the mussels would have had more distance to travel the results could have been different.

Furthermore the exposure to predators did not affect the selection of attachment sites in zebra mussels (Fig.12). A recent experiment indicates that zebra mussels prefer darker attachment sites than illuminated ones (Kobak and Nowacki 2007). In illuminated sites it is more likely that predators will find zebra mussel preys. Thereby we expected that the presence of predator cues would induce the mussels select to more often select dark attachment sites. However, we noticed that the vast majority of the mussels, whether in tanks exposed to predators or not, they selected attachment sites opposite to the windows of the lab. Thereby we argue that they responded to a more powerful threat, the entrance of sunlight only from one side of the lab, selecting the farthest attachment sites to this entrance of sunlight. Probably the unconsidered effect of the entrance of sunlight only from one side of the lab concealed the effect of predators in the attachment site selection. The experiment should have taken place in a room with an even distribution of light services.

Finally zebra mussels in the combined treatment did not develop specific defenses against any of both predators. A prey exposed to two predators with different feeding mechanisms might adopt traits to defend against both predators simultaneously (Bourdeau 2009). Zebra mussels exposed to roach and signal crayfish simultaneously might invest part of their energy in the development of traits against roach and part of their energy in the development of traits against signal crayfish. In addition the development of specific traits against one of the predators (e.g.: rotund shell shape to defend from signal crayfish) may trigger a trade-off against the other predator (roach). On the other hand zebra mussels exposed to signal crayfish invest all energy in traits against signal crayfish while zebra mussels exposed to roach invest all energy in traits against roach. This fact might indicate that none of both predators is more dangerous than the other, so zebra mussels need to defend from both simultaneously. On the other hand most of the parameters analyzed for zebra mussels in the combined treatment did not differ from zebra mussels in the control treatment. Therefore, we cannot claim that zebra mussels in the combined treatment acquired intermediate traits. However, the combined treatment contained the same biomass of both predators while the control treatment consisted also of lake water, with the same density of both predators. The reason why there were no significant differences for most parameters between control and crayfish treatment is because the proportion of cues from both predators was even in both treatments. Therefore, the zebra mussels might not prioritize for any predator

when exposed to the same proportion of both predators. However, we found that the attachment strength in the combined treatment was significantly higher than in the control treatment. Increasing their attachment strength is an efficient technique for zebra mussels against being detached by roach and signal crayfish, consequently without trade-offs against any of both predators. Therefore, in the combined treatment with a higher amount of predator cues from both predators than in control treatment, although the same proportion, zebra mussels invested more energy in increasing their shell strength.

We conclude that zebra mussels respond to signal crayfish and roach presence by developing specific anti-predator defenses. Zebra mussels might decide which anti-predator defenses to develop depending on which predator they need to defend. This good decision in the anti-predator defenses developed might be one of the reasons of the invasion success of zebra mussels. In theory these anti-predator defenses developed by zebra mussels should increase their chances of survival. However, no study has approached this issue yet. These and other issues such as the underlying mechanism in the acquisition of these induced anti-predator defenses or the threshold level of predator cues to which zebra mussels respond, should be addressed in further studies.

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