The behavioural trade-off of *Margaritifera margaritifera* with regard to feeding behaviour and kairomones

MSc-Thesis Aquatic Ecology and Water Quality Management, report no. 020/2012

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Acknowledgements

This report contains a thesis project for the second year course of the Master of Environment Sciences, from the Wageningen University in The Netherlands. It is a project made for the University of Bergen, Department of Aquatic Behavioural Ecology, Norway.

I would like to thank Professor P. Jakobsen and all other members at the Mussel research station in Austevoll for their feedback, advice and help during my thesis.

Finally I would like to thank Dr. R. Roijackers, and many other people for helping me prepare for this thesis.

Austevoll, Norway -September 2012

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Abstract

Margaritifera margaritifera, more commonly known as pearl river mussel, serves an important purpose in freshwater environments and functions as a flagship, indicator, keystone and an umbrella-species simultaneously. Today, however, river pearl mussels are amongst the most critically threatened bivalve molluscs in the world and during the last 100 years most mussel populations have declined by 90%. A fuller understanding of the behaviour of this species is necessary to increase survival in current and future conservation projects, and ultimately to help *M. margaritifera* populations recover. There is growing evidence for a theory that predicts a trade-off between resource acquisition and predation risk. It predicts that animals will reduce their activity when either environmental resources or predation risk is high. The behavioural trade-off for juvenile *M. margaritifera* was tested to determine whether predatorial kairomones affect feeding behaviour. Co-existed predators were collected from Oselven river in Western Norway and odour from caddisflies introduced to juvenile mussels caused most significant change in juvenile mussel activity and movement. The behavioral trade-off in relation to food availability and predation risk was found for *M. margaritifera* in this study. Results indicated that predators appear to significantly reduce mussel activity and movement in the short term. This result however did not seem to extrapolate to the long term, as predator odours did not appear to effect the mussel's choice for food in the second phase of the study. The short term trade-off in favour of food acquisition indicates that *M. margaritifera* apparent high energetic demand requires to disregard the predators presence in order to obtain food for long term survival. The results of this study show that mussels do have a low (energetic) cost avoidance reaction in the short term, but are not influenced by predator presence over longer temporal scales regarding habitat choice.

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1. Introduction

Margaritifera margaritifera, more commonly known as pearl river mussel, serves an important purpose in freshwater environments. The species functions as a flagship, indicator, keystone and an umbrella-species concepts simultaneously (Gum et al., 2011). Due to their important function in river ecology the pearl river mussel is thought to play a large part in particle processing, nutrient release and sediment mixing (see appendix 2 for more information). The survival of juvenile pearl river mussel in river substratum is also considered to be a potential indicator of stream-bed quality (Geist & Auerswald, 2007).

The survival of juvenile mussel is dependent on the suitability of the settlement location in the river sediment. The interstitial zone of the river bed is a subsurface water contained in pore spaces between the grains of rock and sediments (Buddensiek, 1995; McGraw-Hill, 2002). This zone offers a suitable habitat for mussel settlement due the provision of material in which complete burial is possible. The juvenile mussels stay buried for a period of at least five years (Geist & Auerswald, 2007; Hastie et al., 2000).

The juvenile phase of *M. margaritifera* is considered to be the most vulnerable and limiting for mussel recruitment. However, little is known about juvenile mussel behaviour and the potential predators during this stage (Cosgrove et al., 2007; Geist & Auerswald, 2007; Wilson et al., 2012).

Kairomones are chemicals emitted by predators and act as warning chemicals to potential prey, making them favourable to the receiver and not the emitter (Ruther et al., 2002). *M. margaritifera* has chemoreceptors among various sense organs and is able to gather information from i.e. kairomones to make an adaptive response to changes in its environment (Wilson et al., 2012). The few kairomone samples that have been partly characterized show that they consist of proteins and large non-peptides (Dodson et al., 1994).

There is growing evidence for a theory that predicts a trade-off between resource acquisition and predation risk (Anholt & Werner, 1998). Anholt & Werner (1995) describe the theory as "Adaptive foraging behaviour in the face of predation risk". It predicts that animals will reduce their activity when either environmental resources or predation risk is high.

For *M. margaritifera* the detection of predatorial kairomones could evoke a behavioural or physiological response causing the mussel to potentially reduce its activity. For juvenile mussels a high enough food intake is necessary to outgrow the predators size regime to reduce its vulnerability to predation (Werner & Hall, 1988). A behavioural response of decreased activity by juvenile mussels in the presence of a predator decreases probability of predation, but increases the risk of death by starvation (Anholt & Werner, 1998).

Recent research by (Wilson et al., 2012) has shown that four year old mussels, exposed to predatory crayfish odour (kairomones) have increased shell-closure time compared to mussels exposed to predator-free water. Wilson et al. (2012) described shell closure behaviour (shell-closing as an active behaviour) as a protective response to predators. The implications of shell closure is increased energy expenditure, reduced oxygen absorption, loss of feeding time and reduced ability to eject waste products within its closed environment (Wilson et al., 2012). Based on their research, Wilsons et al. (2012) suggested that the trade-off between feeding and predation risk might also apply to *Margaritifera* sp. species. If the impact of kairomones on mussel feeding activity is high, then one can expect that mussel culturing can be impacted.

M. margaritifera used to have a wide distribution, spreading from arctic and temperate zones of western Russia, Europe and the eastern United States (Geist & Auerswald, 2007). Today, however, the river pearl mussel is amongst the most critically threatened bivalve molluscs in the world (Machordom et al., 2003). During the last 100 years most mussel populations have declined by 90% (Geist et al., 2003). Excessive pearl fishing, habitat destruction due to water pollution, eutrophication, acidification, river engineering

and local decline of host fish populations have all contributed to the decline in river mussel populations (Geist et al., 2010b; Hastie et al., 2000).
Mussel longevity and their large reproductive ability may increase the likelihood for potential recovery of *M. margaritifera* populations (Geist et al., 2010b). Research into the behaviour of *M. margaritifera* is largely unexplored, therefore a fuller understanding of this species is necessary for proper conservation in current and future conservation projects in order to ultimately help *M. margaritifera* populations recover (Wilson et al., 2012).

The aim of the present research is to investigate the behavioral trade-off of 3-4 month old *Margaritifera margaritifera* in relation to feeding behaviour and predation risk. This study will provide insight into the effect of kairomones from co-existed predators on mussel feeding behaviour in Oselven River, Western Norway.

Main research question:
What is the behavioural trade-off for *Margaritifera margaritifera* for feeding, when exposed to kairomones?

2. Methods

2.1 Study species and study area

Juvenile pearl river mussel (n=600) were collected from the Oselven river in Western Norway during the summer of 2011 (see Figure). Collection was limited to juvenile mussels which were in their parasitical stage and attached to the gills of their Atlantic salmon (*Salmo salar*) hosts. Live salmon host fish were trapped/collected and transported to a mussel rearing station on the island of Austevoll (Western Norway). Host fish were infected by juvenile mussel (see appendix 2) and, after infection, kept in the rearing station for 11-12 months until juvenile mussels reached a body length of 0.4-0.5 mm (Taeubert et al., 2010). Upon reaching this body length, juvenile mussels start to decyst and detach from their hosts. The detached juvenile mussels were collected and kept in a 'feeding' box within a concrete room under controlled temperature levels. The juveniles were fed a standardized nutrient mixture and standardized filtered mud at two day intervals (see appendix I for mussel-feed details).

The river Oselven was also used to collect three co-existing predators, namely

- Leeches (*Glossiphonia complanata*)
- Caddisflies (Family - Limnephilidea)
- Flatworms (Class - Turbellaria)

These predators were selected from the Oselven river because they are known to co-exist and predate upon juvenile mussels (Kent, 2000; Siddall & Burreson, 1998; Wiggins, 2004).

Leeches (2a) can be found in terrestrial and aquatic ecosystems, however, most are found in freshwater environments where they play an important role as predators of (micro)invertebrates (Siddall & Burreson, 1998; Sket & Trontelj, 2008). Larval caddisflies (Figure 2b) can be found in freshwater streams involved in leaf breakdown, however they are also known to be opportunistic feeders, with examples of individuals found feeding on black fly larvae, invertebrates and dead fish (Landeiro et al., 2012; Wiggins, 2004). Flatworms are free-living carnivorous worms usually found in freshwater environments that have been observed to predate on juvenile mussels (Figure c) (Barnhart, 2006; Kent, 2000; Zimmerman et al., 2003).

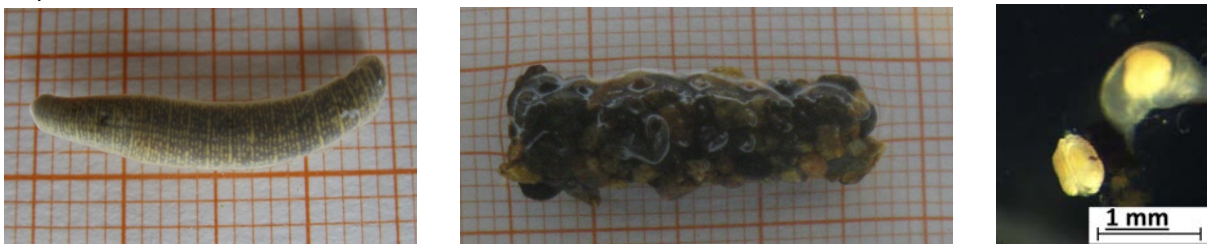

Figure 2a: Adult leech (*Glossiphonia complanata*) ca. 4 cm. in length. 2b: Case of larval caddisfly consisting of small rocks and natural debris (family: Limnephilidea), ca. 4.3 cm. in length . 2c: predated juvenile pearl river mussels observed within the body of a flatworm (magnified x250)

Caddisfly and leech samples were collected from the Oselven river (60°11'41.84"N, 5°28'21.78"E). Flatworm samples were collected simultaneously with the juvenile mussels. In this research only the aquatic larval stage of caddisflies are considered. All water used for this study was purified lake water collected from lake Kvemavannet, treated on-site at the mussel rearing station (60° 5'47.82"N, 5°14'25.47"E, see Figure). Water was collected directly from the final stage of treatment, the UV-filter, in order to avoid potential contamination from kairomones in the water pipelines.

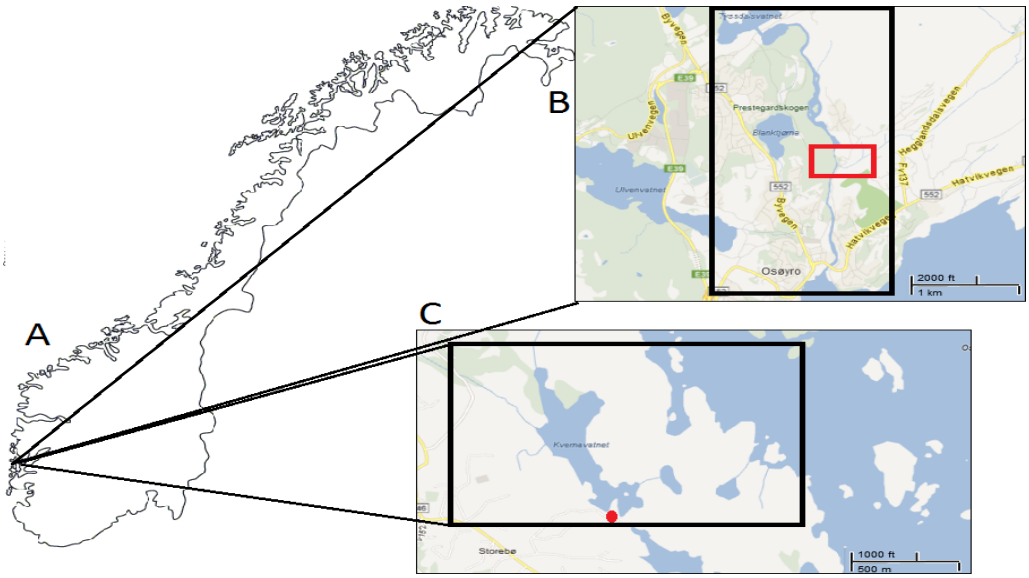

Figure 1A: The location of the Oselven river and the mussel rearing station in western Norway [1]. Figure 1B: indicates the location of the Oselven river, while the red box indicates the collection area of the co-existed predators [2]. Figure 1C: Location of mussel rearing station indicated with red dot, with Kvemavannet lake displayed [2].

2.2 Data collection and preparation

Data collection consisted of two phases, namely predator selection followed by behavioural trade-off (see Figure 1). The first phase was to determine which co-existed predator would provoke the greatest startle response from the mussels. For the purpose of this research, a startle response is defined as either a significant difference in the mussel's foot movement (activity) and/or overall body displacement (movement). Movement and activity responses were chosen as startle responses because these would provide the most pronounced behaviour in the mussels. The second phase of data collection used the phase 1 selected predator with greatest influence on mussel activity and movement. Both experiments will be used to establish a possible behavioural trade-off. For each experiment, a single individual mussel was only used once to avoid potential habitation to the stimuli.

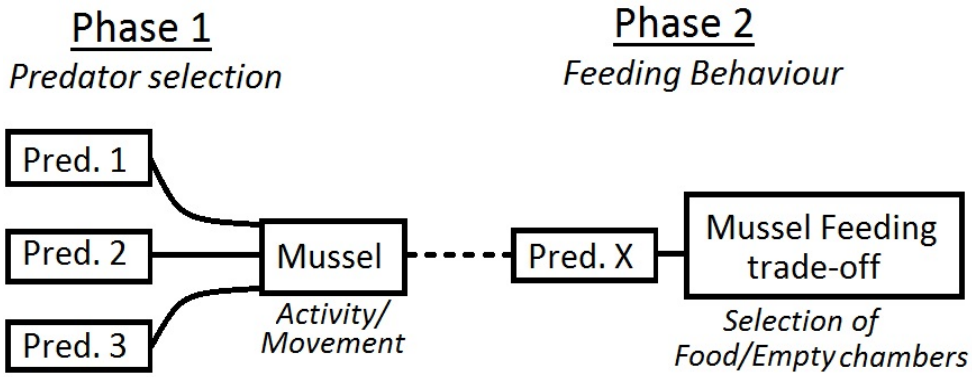

Figure 1: The two research phases conducted to study the behavioural trade-off in mussels. Phase 1 selected the predators odour that provided the most significant increase in mussel activity/movement. In phase 2 the selected predator was used to determine the degree of influence on mussel feeding behaviour.

Phase 1 - Predator Selection

Phase 1 involved selecting the co-existed predator which had most influence on the activity or movement of mussel larvae (experimental setup displayed in Figure 2). The predators, i.e. flatworms (n=100), caddisflies (n=9), and leeches (n=18) were sampled at a single event. After collection, all sampled predators were placed in their respective closed plastic containers. A control container

(containing no predator) with identical environmental conditions was also tested. All containers (predator n=3, control n=1) were filled with 0.5 litres water.
Microscopic photographic evidence of juvenile mussel movement and activity was collected using an adapted microscope (magnified 250x) with a camera mounting. Software NIS-Elements (V. 441.1032.3200.090723) was used to capture the cameras images. The microscope was installed in a concrete room maintained at a constant 17°C at the time of experimentation for a constant environment to eliminate environmental variations.
For microscopic analyses, mussel juveniles were extracted from their feeding boxes by means of a 100 µm filter and transferred to a small plastic petri dish (ca. 3 cm diameter). A pipette was used to extract an additional 3 ml from a predator-containing container using a 100 µm filter, which was added to the mussel-containing petri dish. The mussel was allowed 1 minute to 'acclimatise' to its new surroundings before data recording was initiated. After data collection for each group, photographs were analysed for degree of startle response (juvenile mussel activity and movement). Activity was determined by the visibility of the mussel's foot, and movement was determined by degree of body displacement, i.e. change in body position or location. Prior to returning the mussel juveniles to different feeding boxes, measurements of longitudinal shell-length were taken to determine the potential influence of shell length on startle response behaviour.
Photographic evidence of mussel startle response behaviour was made for 10 minutes at 10 second intervals. This was done for all four containers in a randomised order and 20 trials were repeated for each container (total trials n=80). Statistical analyses were conducted to determine the predator with the greatest (significant) influence on startle response behaviour, further information on the statistical analysis is discussed in 2.3.

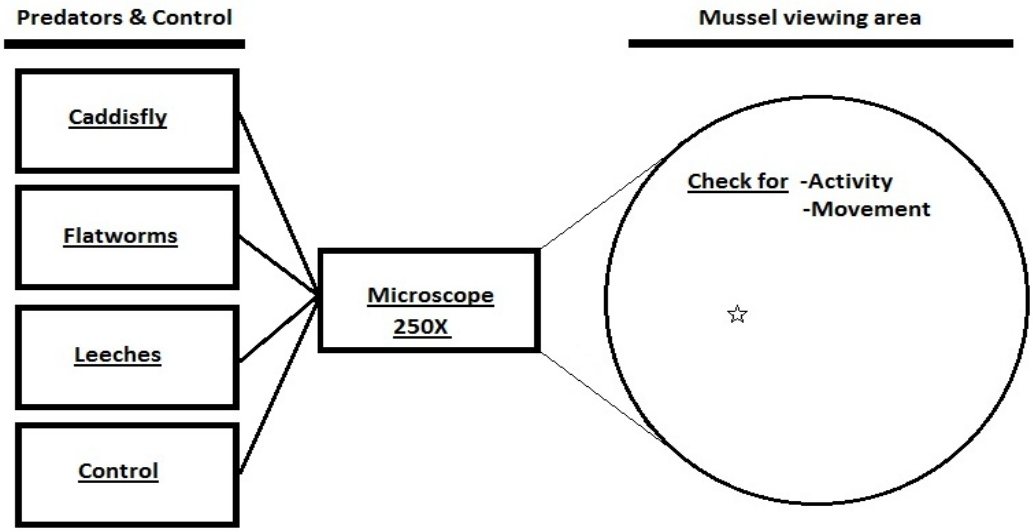

Figure 2 The setup of phase 1 experiments. Water was taken from a filter from a randomly selected unit of "predator and control". The water was inserted into a small petri dish that contains a mussel and placed under a microscope at a 250x magnification in the mussel viewing area. At every 10 second interval, for the duration of 10 minutes, the mussel was checked for activity and movement. The data gathered from this experiment determine what predator has the most significant effect on the mussel, using the control as a benchmark.

Phase 2 - Feeding Trade-Off

During phase 2, the phase 1 selected predator were transferred to modified petri dishes, hereafter referred to as T-dishes. Pre-constructed T-dishes (n=40) were used for continued experimental analyses (see Figure 3) where variation of water inflow and food availability were used as variables. Three peristaltic pumps where used, each equipped with 12 nozzles. The water inflow was approx. 3.7 ml/hr per nozzle and was defined as either 'C' (Control), containing UV-treated water, or 'P' (Predator),

containing predator odorized water. For P-water phase 1 selected predators were used (n=10) that were housed and contained in 5 litres of water to ensure the uniform concentration of kairomones. For T-dishes which contained food patches, standardised mussel food was used as feed. The experimental design enabled the testing of all possible pairwise comparisons for the different water types (C or P) and the availability of feed on food patches.

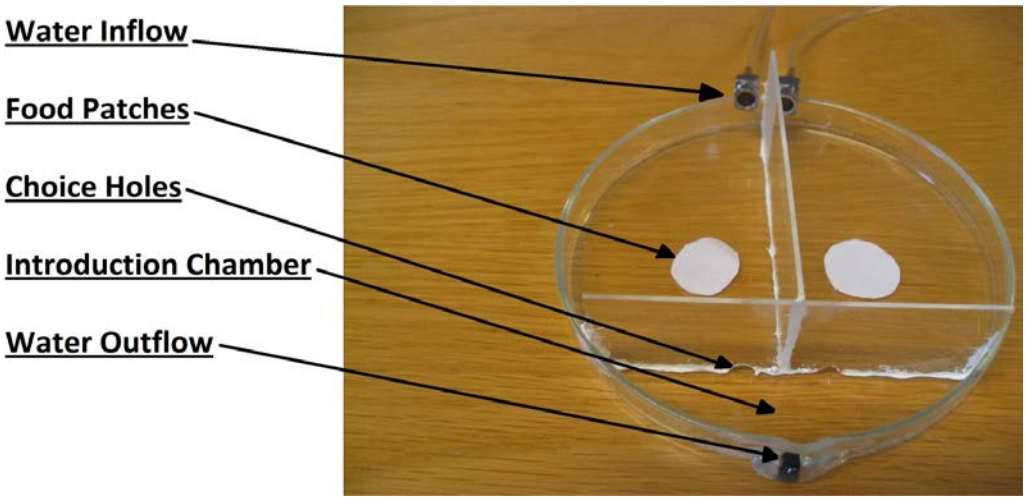

Figure 3 shows T-dish as a modified petri dish designed to function as a choice experiment for mussels. The experiment was set up with chambers where food patch was present or absent combined with either predator-odorized and/or control/UV-treated water. Mussels were placed in the introduction chamber where preferences for a particular chamber could be recorded.

In the T-dish, either C or P type water enters a single chamber and flows through (see Figure 3). Glass panels were sealed to prevent either water types from mixing and contaminating other chambers. The water would flow over the potential food patch and through a 'choice hole'. The water types do not blend until the 'introduction chamber' is reached, where water from both chambers are mixed. For each T-dish, a single mussel juvenile was placed within the introduction chamber and exposed to both C and/or P water streams. After the principal exposure to both water types, the mussel juveniles were allowed four days to migrate to a preferred location, which was either in 'clean' and/or predator odorized water.

Each four-day experiment used 36 T-dishes with three peristaltic pumps, to equalize the amount of water being distributed over all phase 2 T-dishes. The tubing used for the peristaltic pumps were all of equal thickness, further ensuring equal distribution of water. An example of the phase 2 experimental design for four T-dishes is displayed in Figure 4.

Standardised mussel feed was used for feed in the T-dish, however the recipe was corrected for the reduced height of the T-dish water column (see appendix I). The recipe correction was necessary due to the fact that lower sedimentation rates if the same food concentration was used in a water column with reduced height.

All phase 2 pairwise comparisons tested for this experimental design are displayed in table 1. Two control groups were used to verify the influence of water odour and food quality variables during the experiment, and one experimental group which was used to establish the potential trade-off. The used variables were:

- Water odour: Control (UV-treated) OR Predator inhabited water
- Food Availability: Standardised food (high energy availability for mussel) OR No food (no energy availability for mussel)

Table 1 Food control uses only control water inflow to test for mussel food preference, the experiment was run twice using total 72 T-dishes. Predator control tests predator odorized water influence on mussel through food availability combined with either 'C' and 'P' water. The predator control experiment was run once, using 36 T-dishes. Trade-off experiment tests the behavioural trade-off by combining food availability and predator odorized water. Trade-off experiment was run once, using 36 T-dishes. All experimental setups were randomized using A and B settings to prevent preference to a single chamber.

Table 1 The three setups used in the phase 2 experiment with variables food availability and water odour inflow ('C' or 'P'). Experiments were randomized using settings A and B, with the slash symbol indicating the division between the left and right chambers in the T-dishes. Food control tests and verifies food chamber preference. Predator control tests the effect of predatory odour on mussel startle response behaviour. Trade-off experiment tests the behavioural trade-off of food availability and predation risk.

	A		B	
Experiment	Water odour	Food availability	Water odour	Food availability
Food control	**Control**/Control	**Yes**/No	**Control**/Control	**No**/Yes
Predator control	**Predator**/Control	**Yes**/Yes	**Control**/Predator	**Yes**/Yes
Trade off exp	**Predator**/Control	**Yes**/No	**Control**/Predator	**No**/Yes

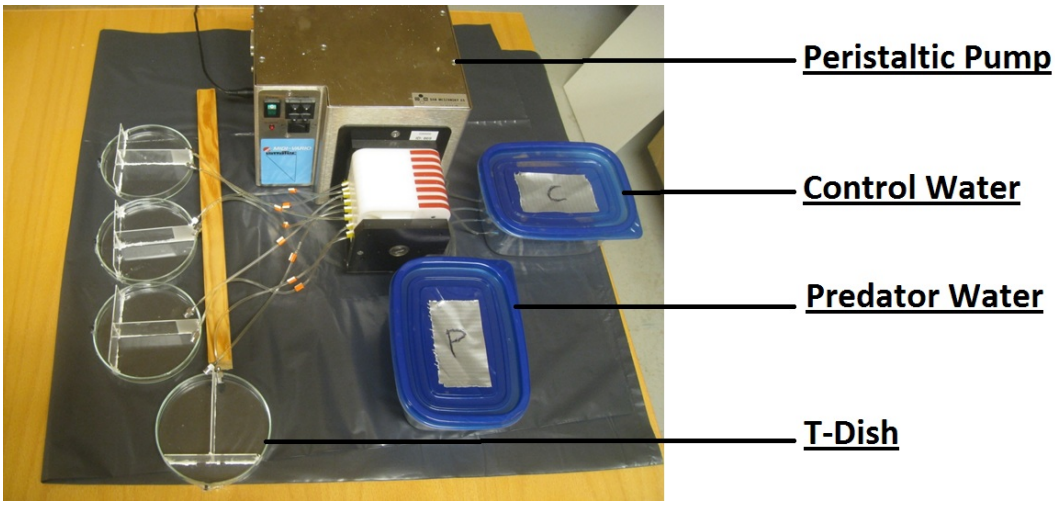

Figure 4 An example of the phase 2 experimental setup. Water is extracted from two (blue) boxes (labeled P and C) by a peristaltic pump. In this image the box marked with 'C' denotes the box with UV-treated water, and 'P' marks the box containing predators. The peristaltic pump equalizes the amount of water flowing to the T-dishes, using tubes of equal thickness.

2.3 Data analysis

SPSS (V. 16.0) was used to perform all statistical analyses in this project.

Phase 1

Independent samples t-test was used to determine whether the mussel body size had significant influence on mussel activity and movement. Binary logistic regression was used to determine whether predator odour influenced mussel activity and movement over time. Post Hoc one-way ANOVA (Tukey HSD) was used to determine which predator had the greatest significant influence on mussel activity and movement.

Phase 2

The chi-square test was used to establish a correlation between the variables. The mussels were categorized into 3 groups: small (<0.8 mm), medium (≥ 0.8mm - ≤ 1mm), large (>1mm).

3. Results

Phase 1 results

Difference in body size had significant influence on mussel movement (p=0.000), but no significant influence on activity (p=542). Predator odour had significant influence on movement (p=0.019) and on activity (p=0.000) over time.

Caddisflies showed significant difference for activity in relation to control subjects (p=0.028; Table 2). No significant differences were found for leech (p=0.558) or flatworm (p=0.648). There were significant differences for movement for caddisflies (p=0.000) and flatworms (0.001) in relation to control subjects(Table 3). No significant differences were found for leeches (p=0.490). Because only the Caddisfly significantly influenced both activity and movement of the mussel, it was selected as the predator for phase II of the project.

Table 2 one-way ANOVA comparing mussel activity in control situation to mussels introduced to either of 3 predators.

Compared to	Predator Type	Significance
Control	Flatworm	0.648
Control	Caddisfly	0.028
Control	Leech	0.558

Table 3 one-way ANOVA comparing mussel movement in control situation to mussels introduced to either of 3 predators.

Compared to	Predator Type	Significance
Control	Flatworm	0.001
Control	Caddisfly	0.000
Control	Leech	0.490

Phase 2 results

The mussels that stayed in the introduction chamber during the whole experiment and did not choose for either of the two food chambers were excluded from further analyses.

Results from Food Control in indicate that from 43% of usable trials (n=31), mussels selected the food chamber 87% of the time (n=27). For Predator Control, both chambers contained food. From usable trials, 53% of the mussels selected the food chamber with predator odour (n=9), and 47% selected the food chamber without predator odour(n=8). Results from Trade-Off show that mussels choose food 100% of the time (n=14).

Table 4 Results from phase 2 are displayed in the 3 setups: Food Control, Predator Control and Trade-Off. Results for each setup are displayed in the corresponding rows.

	Food Chamber	Intro Chamber	Other	Total Trials
Food Control	37.5%	57.0%	5.5%	72
Predator Control	25.0%	52.8%	22.2%	36
Trade-Off	38.9%	61.1%	0.0%	36

Chi-square test indicates that mussel size does not have a significant influence on mussel food choice (p=0.850). Table 5 displays the size distribution for the mussels in respect to the chosen chamber.

Table 5 size distribution between mussels staying in the Food, Introduction and Other chamber.

	Small	Medium	Large	Total mussels
Food Chamber	8%	80%	12%	50
Introduction Chamber	7%	77%	16%	82
Other Chamber	8%	67%	25%	12

4. Discussion

The behavioural trade-off between food availability and predation risk was studied for *Margaritifera margaritifera*. Phase 1 results showed that caddisfly odour significantly influenced both mussel activity and movement, while impact of flatworms was restricted to movement, and leeches showed no significant effect on either. Phase 2 results showed mussels selecting food chambers in similar amounts, regardless of the presence of a predator.

With the results, a relationship was found for predatory odour, where exposure to odours from co-existing predators appeared to significantly reduce short term mussel activity and movement. No long term (four days) trade-off relations were found during this study as predator odours did not appear to effect the mussels choice for food in the second phase of the study.

The short term trade-off in favour of food acquisition indicates that *M. margaritifera's* apparent high energetic demand requires it to disregard the predators presence in order to obtain food for long term survival. These findings are supported by Huntingford et al. (1988), where feeding intensity in 0-1 year old Atlantic salmon (*Salmo solar*) showed differences in feeding behaviour in individuals with lower modal growth (LMG) and upper modal growth (UMG) when presented with a predator. Initially LMG and UMG salmon showed the same reaction to predators when feeding motivation was similar. However, when feeding motivation differed, risk taking to feed in the presence of a predator increased in LMG salmon, indicating that differences observed in foraging can be ascribed to difference in feeding motivation. Taking higher risks to feed indicated that a lack of growth poses a higher risk of mortality (starvation) than the threat of predation. Thus, a critical amount of growth is needed for the salmon to survive its first winter, causing the predator to be increasingly disregarded by LMG salmon. Research by Buddensiek (1995) showed that, like the Atlantic salmon, survival of juvenile mussel's first winter is also largely dependent on body-size. This indicates that a critical size-threshold exists for juvenile mussels in order to increase their survival chances, which is to be obtained by ensuring sufficient energetic intake. It is possible that juvenile mussels, in order to ward off starvation in the winter, must disregard predators to optimise their chances of ensuring sufficient energetic intake.

Rapid growth can also improve short term survival chances because of increased difficulty to kill and consume animals with larger body size. Rapid growth furthermore provides increased storage of energy, increasing disproportionately with body size, lowering the risk of starvation in periods of food shortage, such as during the winter (Metcalfe & Monaghan, 2003). Results of my study also show that mussels do have a low (energetic) cost avoidance reaction in the short term, but are not influenced by predator presence over longer temporal scales regarding habitat choice.

Results from phase 1 regarding predator selection, indicate that predatorial odours do significantly affect juvenile mussel activity and movement. A time effect on the startle response was also observed, indicating that the predatorial odour significantly influenced mussel activity and movement for the duration of the 10 minute experiment. These results coincide with Anholt & Werner (1995), which states that mussel activity was reduced in the presence of predators. The most pronounced impact on both movement and activity on mussels was from the caddisfly odour, therefore this predator was used for continued experiments in phase 2; feeding behaviour. The phase 1 experiment also showed that mussels which were subjected to flatworm odour, had significantly reduced their movements. Leeches showed to have no impact on either activity or movement; however this does not exclude the possibility that flatworms and leeches might influence mussel activity/movement if tested over a longer time period. The flatworm is a known predator to mussels(Barnhart, 2006; Zimmerman et al., 2003), therefore it is likely that this predator will affect mussel behaviour during similar studies .

Results from phase 2, regarding feeding behaviour, indicate that food is a positive stimulus for mussels and influences mussel behavioural decisions. The present study showed that the introduction of predator odour into the mussel's direct environment did not cause change in mussel distribution. This

indicates that despite the potential presence of a predator, the mussel will not alter its position if it may negatively influence its food acquisition. It is also possible that juvenile mussel select the food chamber not for its nutrients, but for sheltering purposes. However, this may be unlikely as juvenile choice of food chamber was consistent for all experiments, regardless of the presence of predatorial odour, indicating that it was not used as shelter. Furthermore, Juvenile mussels have high energetic demand, which is likely to have influenced their chamber choice decision.

As mentioned previously, no long term effects of predator odour on mussel behaviour were found during this study. However, mussels exposed to predatorial odour over short time periods did indicate a change in behaviour. Studies on zebra mussels have shown that the aggregation of mussels increased in the presence of predators. Aggregation is a common anti-predator defense in bivalves as it reduces accessibility to predators and increases difficulty of capturing individuals from aggregation centers (Kobak et al., 2010). While mussels also tend to aggregate under natural conditions, a greater proportion of blue mussels (*Mytilus edulis*) have been shown to aggregate at greater speed when under threat of predation, compared to control conditions (Côté & Jelnikar, 1999). Future studies should aim to include the effects of long term exposure of predator presence on *M. margaritifera* to determine whether predators similarly affect mussel group dynamics and aggregation.

Similarly, the influence of caddisfly on mussel behavior was determined based on a short term exposure (10 minutes), whereas experiments conducted during phase 2 were based on a long term exposure (four days). This difference in predator exposure time may have influenced the results. A study conducted by Turner (1997) showed contrasts between short and long-term effects of predation risk on freshwater snails (*Lepomis gibbosus*). Snails initially responded strongly to predator presence and its associated predation risk in the short term, however in the long term similar patterns of habitat use were observed. In contrast, phytoplankton have been shown to increase their movement when predatory kairomones are detected (Harvey & Menden-Deuer, 2012). Turners study suggested that snails balance predation risk against foraging gains when selecting habitats. While it is true that vulnerability of starvation is extreme for juvenile mussels compared to freshwater snails, it is possible that a similar balance might also exist for mussels.

Wilson et. al (2012) also found clear evidence that bivalves (*M. margaritifera*) are sensitive to changes in predation risk and change their behaviour accordingly. During my study *M. margaritifera* populations were used that had not necessarily co-existed with its introduced predator (*Austropotamobius pallipes*). Additionally, juvenile mussels used by Wilson et al. (2012) were already capable of filterfeeding, whereas species in my research were younger and still dependent on their foot to gain nutrients from the environment. This means that filterfeeders can remain sessile, while the younger mussels must move to feed, causing potential differences in comparing movement data.

During my study the length of mussels was also considered, and larger mussels showed significantly more movement than smaller mussels. However, the larger mussel's higher movement rate did not relate to an increase in trade-off choice for food during the phase 2 experiments. These results, however, might be due to increased movement capabilities and/or higher food requirements by larger mussels. The relation of mussel bodysize to movement was retested in phase 2, yet no correlation was found during these experiments. Results showed that larger and more active mussels had a higher chance of finding a choice hole in the T-dish. However, no difference in size distribution was found between mussels staying in the introduction chamber, and mussels entering a choice hole making it unlikely that phase 2 results are size-conditional. The phase 2 experiments were conducted with a relatively small sample size (n=144) and may therefore have influenced our findings. The time period between phase 1 and phase 2 experiments consisted of 14 days, in which mussels were likely to increase in body size due to natural body growth. Measurements of body growth between the experimental phases were not recorded during this study and its influence on the results was not determined. Studies covering a longer time period may reconsider this and include body size growth into their analyses.

Kvemavannet lake water, treated on-site at the mussel rearing station was used during the experiment as control water. Water was collected directly after the final UV-treatment stage rather than building taps, to avoid potential predatorial kairomone contamination from animals living in the facilities water pipes. It is, however, not possible to verify a complete absence of kairomones contained in the collected samples of lake water. All lake water used during experiment for predator and control experiments were used in identical amounts, ensuring equal treatment and potential effects of the lake water. Influence of kairomones could have an upper concentration limit in the water, possibly explaining phase 2 results where treatment with caddiefly odour had no effect. However, short term experiments in phase 1 showed that caddisfly odour had a behavioural effect on mussels, strongly suggesting that this is not the case.

Another possible external influence on mussel choice was that behavior of captive reared mussels may differ from mussels inhabitating natural environments. The use of reared mussels could have caused increased boldness in mussel choice during phase 2 experiments. Wilson et al. (2012) stated that captive rearing is likely to cause difference in boldness of vertebrates when compared individuals raised in the wild. However, all mussels in the rearing stations were housed and treated under equal and constant environmental conditions. Similar experiments using wild mussels could determine whether a difference exists between natural and captive counterparts, and their implications for cultivation and conservation.

Other variables of mussels such as mass and age of juvenile individuals most likely varied throughout the experiment, however these were not recorded during this study. It is therefore possible that this may have influenced the behavioural trade-off decisions as was observed in barnacles (*Balanus glandula*) where mass had an effect on hiding time (Dill & Gillett, 1991). All mussels used for experimentation were from the same spawning year (2012), however weight was not measured due to lack of equipment. It is recommended that future studies, similar to this attempt, incorporate this variable to determine its influence on mussel trade off decision.

To further ensure equal treatment in phase 2, randomization of the experimental setup was done within the three setups (Food Control, Predator Control and Trade-Off). However, randomization was not done between the three setups, potentially allowing a time effect on mussel choice.

Limitations to phase 2 was availability of larval caddisfly specimen. The seasonality of larval caddisflies coupled with weather conditions and access to study site limited availability of the predators, also limiting the number of potential experiments.

For future experiments it is recommended to reduce the number of mussels disregarded from results. Mussel result is disregarded if it remains in the introduction chamber during phase 2. By increasing the experimentation length beyond the initial four days could help reduce the omission of data. Also, modification of the T-dish design, by reducing the size of the introduction chamber could actively stimulate mussels to choose between choice chambers (containing food or no food), which would positively influence the data collection efficiency.

For phase 1, a possible limitation is the one minute acclimatization period to predator odour that was used during this study. The set amount of time may have caused different responses from individual mussels as the impact of predator odour on individuals is likely to vary.

Results provided by this research gave insight into stressors of juvenile mussels. Identification of potential stressors is an important factor to the endangered pearl river mussel during its most vulnerable and critical juvenile stage (Geist & Auerswald, 2007). Further research into mussel behaviour is recommended to aid in the survival of critically endangered *M. margaritifera* populations (Geist et al., 2003).

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Figures

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Appendixes

1 Mussel feeding

The mussels are kept in plastic 'feeding' boxes aprox. 17 x 11.5 cm. The boxes used are also used for human food storage, ensuring that no harmful chemicals have been used that could potentially harm the juvenile mussels.

Feeding of the mussels is as follows. Boxed water, including the mussels are filtered leaving behind only the mussels in the filter. The mussels are then reintroduced to the box where mussel feed (in water solution) is added (see contents below). Once the feed has been added, aprox. 100 ml. of mud is also introduced (depending on the thickness of the mud, this amount may vary). The mud ensures that the mussels excrement binds to the mud, instead of decreasing its own water quality.

Contents of juvenile mussel feed in 10 liters of water:

- 1 ml. calcium
- 250 µl Shellfish Diet 1800 - (Produced by: Reed Mariculture)
- 2 ml. of following mixture:
  - 50ml water
  - 2 mosquito larvae
  - 1ml. defrosted nano algae (Nanno 3600 - Produced by: Reed Mariculture)
  - a pinch of *Spirulina* - (produced by: Bio-Lifes Green Products)

Feed formula for Juvenile mussels has been determined in order to attain a correct concentration at a 7 cm. water column, the height of the feeding boxes. For the mussel feed correction of the phase II the water column in the T-dishes is reduced to 0.5 cm. Due to less sedimentation at 0.5 cm, the recipe is concentrated 14 times (7: 0.5 =14).

Standardization of mud:

Watery mud rich in detritus is collected from shallow streams. It passes through a filter of 30 µm to equalize the sediment. The equalized mud is then stored in an aerated container to keep the water saturated with oxygen to increase survival chances of aerobic organisms, on which the mussels feed (Skinner et al., 2003).

2 *Margaritifera margaritifera* Life Cycle and Ecology

Margartifera matures between 10-15 years and have a maximum life span of 190-200+ years (Geist, 2010a; Philipp & Abele, 2010; Skinner et al., 2003). Growing to a maximum size of 15 cm, the mussel's life span appears to depend on its growth rate. Faster growing populations in Spain tend to have a shorter life span, approximately 35 years, compared to cooler Scandinavian populations that can exceed the age of 200 years (Geist, 2010a).

The river mussel has a complex life cycle due to its dispersive parasitic larval (glochidia) stage (see Figure 5). During the lifespan of the pearl river mussel, a female produces up to 200 million glochidia (Buddensiek, 1995). The glochidia, resembling tiny mussels, are released by the female in a highly synchronized event, releasing between 1 and 4 million glochidia over 1-2 days in mid-summer (Skinner et al., 2003; Taeubert et al., 2010). At a length of approximately 0.07 mm glochidia keep their shells fully opened, until a stimulus from a host fish such as mucus, or gill tissue induces a characteristic "snapping" behaviour. Research by Young and Williams (1984) noted that glochidia's shell had not been observed to reopen once it had "snapped (closed)", its shel The glochidia that are ingested by their salmonid host fish have an increased "snapping" rate, causing them attach and become parasites on its gills. The gills of host fish are parasitized where, for approximately 12 months, glochidia encyst to allow growth into juvenile stage. After approximately 12 months of growth the mussels have grown to 0.4-0.5 mm length (Taeubert et al., 2010; Young & Willems, 1984). Upon reaching this length, juveniles will detach from the host fish gills and settle on the riverbed to continue growth to adult life forms (Treasurer et al., 2006). The success rate of glochidia reaching the life stage of a juvenile is estimated at fewer than 10 for every 1 million produced by a single female.

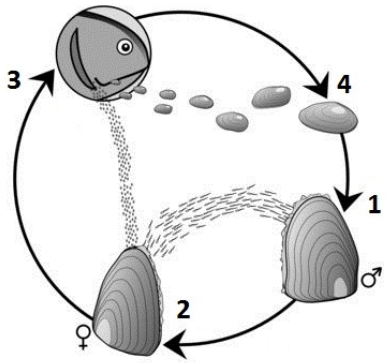

Figure 5 Reproductive cycle of *Margaritifera margaritifera.* It displays the 4 main stages of reproduction. First stage displays fertilization of female mussel by male mussels, followed by second larval (glochidial) stage. The mussels attach and grow on the gills of the host fish, until they reach their juvenile stage. The juvenile mussels decyst from the gills, and drop into the riverbed to continue growth into adulthood (Treasurer et al., 2006). (Lindgren, 2011) -edited with MS paint by D. Ophof.

M. margaritifera can be found partly buried in the riverbed in fast flowing, oligotrophic and unpolluted streams filtering minute organic particles on which they feed. By filtering the water with its syphons (see Figure 6), it is possible that large amounts *M. margaritifera* could have clarified river water to the benefit of other species (Skinner et al., 2003). With other bivalve species (*Anodonta, Unio, Lampsilis*) it has been shown that individual mussels can filter 20 -70 litres of water daily, depending on body size. *Margaritifera* species may also develop pearls when epithelial mantle cells are introduced to the connective tissue of the mantle. Epithelial mantle cells are introduced to a deeper layer of the mussels mantle by small particles or organisms that enter the mussel while the shell is opened for feeding. An outer epithelium is formed around the cells, referred to as a pearl-sac, where mother-of-pearl is deposited layer by layer from which the pearl is formed (Ziuganov et al., 1994).

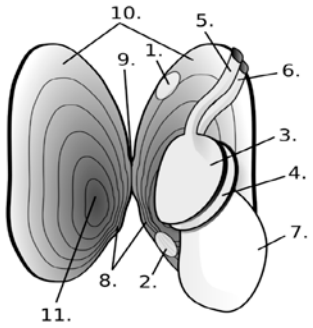

Figure 6 Schematic depiction of *Margaritifera margaritifera* anatomy. 1. posterior adductor 2. anterior adductor 3. outer gill demibranch 4. inner gill demibranch 5. excurrent siphon 6. incurrent siphon 7. foot 8. teeth 9. hinge 10. mantle 11 umbo (thickest part of the shell) (Lindgren, 2007).