Is signal crayfish (*Pacifastacus leniusculus*) able to biologically control the invasive zebra mussel (*Dreissena polymorpha*)?

Enclosure/exclosure experiments in Lake Erken, Sweden

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Photo front page: Zebra mussel from www.lakescientist.com 2015-06-02

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ABSTRACT

The aim of this study was to collect new knowledge about the importance of signal crayfish predation on zebra mussels in Lake Erken. Possibly with implications on biological control of the invasive species in Swedish lakes.
I performed a field experiment with enclosures (including signal crayfish) and exclosures (without signal crayfish) during 11 summer weeks in Lake Erken.
My results showed that signal crayfish can significantly reduce the abundance of zebra mussels.
I conclude that signal crayfish is able to affect a population of zebra mussel, but further research is needed to know by which mechanism: do signal crayfish exert an effective predation pressure on zebra mussels or have the invasive species already evolved an effective avoidance behavior towards signal crayfish?

INTRODUCTION

Schreiber et al. (1998) showed in laboratory experiments that signal crayfish (*Pacifastacus leniusculus)* is able to prey on zebra mussel [*Dreissena polymorpha*, (Pallas)]. They also showed that when mussels were offered alive (but not attached to a stone), a size-preference for small mussels was evident. Stanczykowska and Lewandowski (1992) stated that densities of mussels in Polish lakes were determined primarily by the mortality of planktonic veligers (larvae) during settlement and in the postveliger stage.

I hypothesized that signal crayfish can significantly reduce the abundance of adult zebra mussels in Lake Erken, through predation on settled postveligers.

Invasive species threaten biodiversity and ecosystem services, can have significant negative effects on human health and the economy. In December 2014 about 350 species in Sweden were considered invasive (Naturvårdsverket 2014). The zebra mussel is one of them. See the effects of zebra mussels in Table 1.

Sweden has at international, European and national scale committed to control, eradicate and prevent the introduction of invasive alien species (IAS) that threaten native ecosystems, habitats or species. In January 2016 we may meet requirements on management measures to control the populations of zebra mussels and requirements for monitoring them (Naturvårdsverket 2014).

Live organisms can be used as a biological action method to control invasive species and is generally regarded as less harmful to the environment than chemical or mechanical methods. The invasive species can be reduced to an acceptable level and the spread to new areas can be limited (Josefsson, 1999).

In Lake Erken, signal crayfish was introduced in 1966-1969 to compensate after the crayfish plague (Josefsson 1999). It originates from California, America. Signal crayfish are omnivores feeding on a wide range of food items such as detritus, macrophytes, invertebrates, vertebrates (e.g. fish) and each other (Guan and Wiles 1998). According to Nyström (1999) signal crayfish prefer snails as prey but snail shell thickness and size may influence the snail choice (Nyström and Perez 1998).

I hypothesized that signal crayfish would have an indirect positive effect on periphyton in my field experiment (a higher concentration of chlorophyll *a*).

The zebra mussel is a semi-sessile, filter-feeding bivalve. The reproductive mode is unusual for freshwater bivalves and very successful. A female mussel can produce up to more than 1 million eggs during one spawning event (Sprung, 1992). The eggs get fertilized in the water column and develop into planktonic veliger larvae. The veliger grow and develop into the postveliger stage and sink to settle on a substrate (Sprung 1992). Most mussels prefer textured and hard substrates (Marsden and Lansky 2000). During settlement up to 99% of the postveligers die. Several probable causes: suitable substrate is not encountered, predation by fish larvae and small fish, filtering of adult zebra mussels, bacterial infection, shortage of food (Sprung 1992).

Its origin area is the Black and Caspian Seas. The veligers can spread through ballast water/livewells. Postveligers/ juveniles/adults can attach to ship hulls/recreational boats/fishing tools/ aquatic plants or spread by birds. Since Pallas found the zebra mussel 1768 in the Caspian Sea (Arwidsson 1926), it has spread to great parts of Europe and to The Great Lakes water system in the United States and Canada. The zebra mussel was introduced in the Swedish lake Mälaren in the late 1920s (Arwidsson 1926) and is now reproductive in Lake Mälaren, Lake Hjälmaren and Lake Erken (Melanie Josefsson 1999). Since 2014 the zebra mussel is also believed to be reproductive in Lake Roxen and Lake Glan (Svensson & Lundberg 2014).

Signal crayfish and zebra mussels now co-exist in Lake Erken.

The aim of this study was to collect new knowledge about the importance of signal crayfish predation on zebra mussels in Lake Erken.

I performed a field experiment with enclosures (including signal crayfish) and exclosures (without signal crayfish). It showed that signal crayfish can significantly reduce the abundance of zebra mussels.

Table 1. Characteristics of zebra mussels (*Dreissena polymorpha*), problems and positive effects they possibly cause.

High filtration capacity (e.g. MacIsaac 1996)	
Possible problems	**Possible positive effects**
- Change energy and nutrient flows, changes to phosphorus cycling - Change sedimentation-conditions and the existing benthos (Josefsson, 1999) - Changes the food web dynamics: competes with other plankton-eating organisms, both snails and mussels. And exerts top-down control on primary producers.	- Decreases the risk of algal blooms in fresh water - Is an important food component for some fish, crayfish and some birds.
Bioaccumulation of toxic substances from water (Secor et al. 1993)	
Possible problems	**Possible positive effects**
Danger to those who eat animals that eat the zebra mussels	Clearing toxic substances out of the water
Grows on/in every possible substrate	
Possible problems	**Possible positive effects**
- Social stress on other (domestic) organisms changes foraging behavior, distribution and habitat. Decline, extinction (Josefsson, 1999) - Negative economic impacts (fouling of water intake pipes, ship hulls, navigational constructions, cages of aquaculture and reduced angling catches) - Affects supplies of drinking water and cooling water systems of power plants (Birnbaum 2011) - Sharp shells that covers lake bottoms and shores: lacerations to	- Crushed shells could be used as a fertilizer and poultry feed

walkers on shell-filled shores and bathers in waters with shells covering the bottom (Wallentinus & Werner 2008)	

MATERIAL AND METHOD

Study site

In the summer of 2005 I performed an enclosure/exclosure experiment in Lake Erken: enclosures with signal crayfish, exclosures without signal crayfish. Lake Erken is located in Norrtälje, Sweden (59°50'9.8"N, 18°37'53.6"E) and is a naturally meso-eutrophic and dimictic lake with a surface area of approximately 24 km^2 and a mean depth of approximately 9 m. The max depth is 20 m. Water retention time is 7 years. Dominating soil in the area is morain, rich in lime. That gives the lake a high pH value and a high buffering ability: pH 8.2 (+-0.2) and mean alkalinity 0.9 mmol/L $CaCO_3$ (SD=0.06).

At Lake Erken, the University of Uppsala, runs a laboratory which is a part of the SITES laboratories. The institution of Ecology and Genetics were kind to let me work at their field station.

Signal crayfish (*Pacifastacus leniusculus*) is one of the dominant predators of macroinvertebrates in Lake Erken and is able to prey on zebra mussels, *Dreissena polymorpha* (Schreiber et al. 1998).

Several gastropods and bivalves live in Lake Erken. Among them are the thickshelled grazer River Nerite (*Theodoxus fluviatilis).* The gastropod is eaten by several crayfish species and moves slowly (Abdallah 2015). The common Bithynia (*Bithynia tentaculata)* is both a grazer and a filter feeder (Tashiro 1982). Since the zebra mussel invaded Lake Erken in mid-1975 it has spread rapidly and now have become a dominant molluscan species. Adult zebra mussels are readily found, attached to and aggregated on stones at the bottom.

Experimental design

The study was conducted with ten identical cages (fig. 1), which were constructed by me on site. Outer measures for the wooden frame: 102.5 cm*47.5 cm*102.5 cm. Each cage were surrounded by a metal net with a 15*15 mm^2 mesh that permitted water flow but retained/excluded large crayfish and eventual predatory birds. The cages had plastic construction foil at the bottom to prevent mixing from the lake bottom and to prevent the crayfish to dig their way out. See Appendix for material specifications. Five of the cages were used as enclosures (with crayfish) and five were used as exclosures (without crayfish).

Both the five enclosures and the five exclosures contained four wavy concrete roof tiles each (total area of the four tiles: 1,31 m^2) and a white glazed tile (10*10 cm^2). See Fig. 2. In addition, the five enclosures contained five signal crayfish each. PVC tubes were put into every cage, but were excluded from the results since the periphyton growth was destroyed in the transport to the laboratory.

The cages were placed in a north-south direction in the south west corner of the bay of Kallvik (fig. 3). Enclosures and exclosures were placed alternately at the lake bottom along the shore line and in the littoral zone of Lake Erken (fig. 4).

Fig. 1. The ten cages: five used as enclosures (with signal crayfish) and five were used as exclosures (without signal crayfish).

Fig. 2. Wavy concrete tiles, stacked in four, which were used as signal crayfish living cavities and settling substrata for zebra mussel veligers. The glazed white square tiles were used as periphyton substrata.

Fig. 3. The bay of Kallvik (Lake Erken), were the ten cages were placed along the south west shore line.

Fig. 4. The ten enclosures and exclosures submerged, anchored at the bottom and marked with buoys.

The lake bottom consisted of rocks, boulders and pebbles and was gently sloping. Water depth for the bottom of the cages was generally 75 – 102 cm, the outer water depth at the location was 94.0 - 116 cm. The enclosures and exclosures were secured in the lake bottom with reinforcing bars.

Enteromorpha, occasional perfoliate pondweed (*Potamogeton perfoliatus*) and reeds (*Phragmites australis*) grew in the proximity of the cages. Deciduous trees were overhanging the water.

The signal crayfishes used in this experiment were collected by me from the bay of Kallvik. The initial mean carapace length was measured to 46,7 mm (range 35,5–58,0 mm). They were retained in lake water before released into the enclosures on 10–12 June 2005. The areal density of about 4,76 crayfish/m^2 was within the range of natural densities for lake Erken 2005 (personal comment Tommy Odelström).

When the cages were retrieved out of the lake on 29–31 August 2005 (+11 weeks and four days), all crayfish from one of the enclosures had escaped: the results from this cage were excluded. One of the exclosures were also excluded from the results. In one of the enclosures one of the crayfish were missing and in another enclosure one crayfish had migrated in. These cages was included in the results. In three of the exclosures, a total of four crayfish had migrated in. These cages were also included in the results.

Data analysis

When retrieved from the cages, the crayfish were measured and growth was calculated.

All visible macroinvertebrates on the roof tiles were sampled, counted and measured to the nearest mm with a calliper. Zebra mussles were measured on the long axis. Snails found in the cages were determined to be the river nerite (*Theodoxus fluviatilis*) and the common Bithynia (*Bithynia tentaculata*).

Abundances of the different macroinvertebrates on concrete tiles (number of animals/m^2) were calculated for eight cages (four enclosures, four exclosures). To test for differences between the abundances in enclosures and exclosures, I used a paired t-test of *D. polymorpha* and *T. fluviatilis* (Perry et al. 2000). Because of the low number of animals, I used a Mann-Whitney U-test for the test of abundance of *B. tentaculata* in eclosures/exclosures.

The proportions of the different macroinvertebrates were calculated from percent of abundance. The size range of zebra mussels was determined for the two treatments. The size structure was tested with Mann-Whitney U-test. Histograms with normal distribution curves were made. The proportional decreases of zebra mussels between treatments were calculated.

The glazed tiles were individually placed in freezer bags, cooled in a cooler bag with freezer packs and kept dark and cool in a freezer at the Erken laboratory. They were transported to a laboratory in Lund where the periphyton was removed from the tiles with a scalpel and toothbrush for analysis of chl. *a* concentration (µg/m^2) according to Ekologisk metodik – Enkla metoder för ekologisk beskrivning insamling och analys: en sammanställning, Lunds universitet, 1977.

Since the glazed tile in one enclosure lay underneath the concrete tiles during the field experiment, the chlorophyll *a* value from that cage was excluded from the analysis (*t*-test: $n_{en} = 3$, $n_{ex} = 4$).

All statistical analysis were performed in SPSS.

RESULTS

Abundance effects on macroinvertebrates

The abundances of macroinvertebrates on concrete tiles were significantly lower in enclosures with signal crayfish than in exclosures (fig. 5). *D. polymorpha* (*t*-test: p=0.018, n_{en}=4, n_{ex}=4). *T. fluviatilis* (*t*-test: p=0.000, n_{en}=4, n_{ex}=4). *B. tentaculata* (Mann-Whitney U test: p=0.038, n_{en}=4, n_{ex}=4).

The proportions of macroinvertebrates (fig. 6) demonstrate that in cages with signal crayfish (a), *D. polymorpha* dominated. In cages without crayfish (b), *T. fluviatilis* were the dominating species.

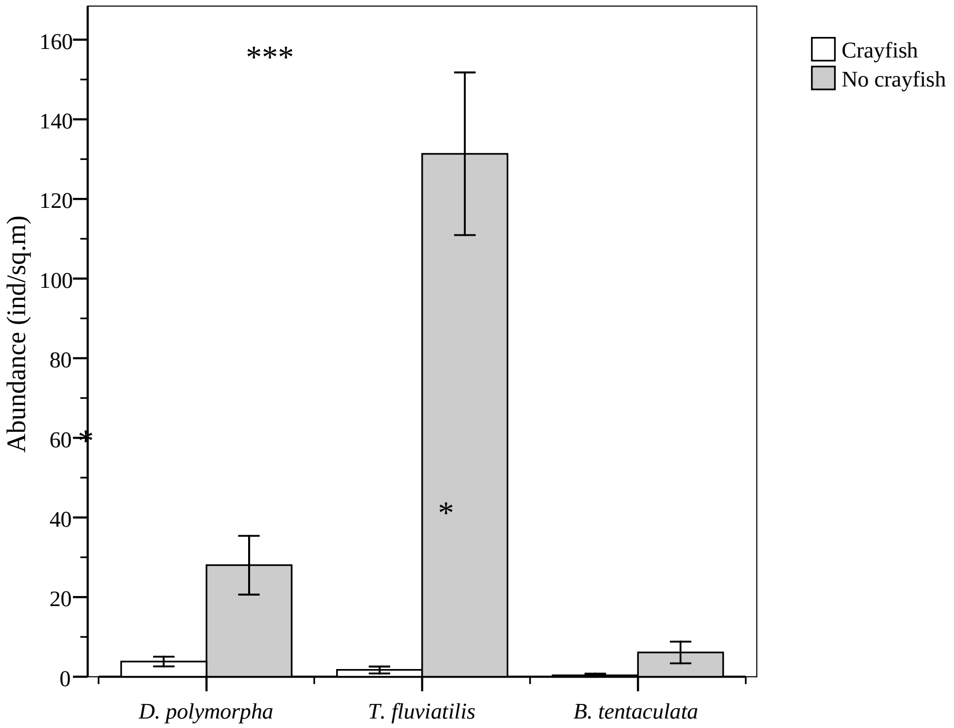

Fig. 5 Number of zebra mussels (*Dreissena polymorpha)*, river nerites (*Theodoxus fluviatilis)* and common bithynias (*Bithynia tentaculata)* per square meter of concrete tiles in four cages with signal crayfish *(Pacifastacus leniusculus)* and in four cages without crayfish. Asterisks indicate significant differences (* $p < 0.05$, *** $p < 0.001$). *T. fluviatilis* were absent in one enclosure and *B. tentaculata* were absent in three enclosures.

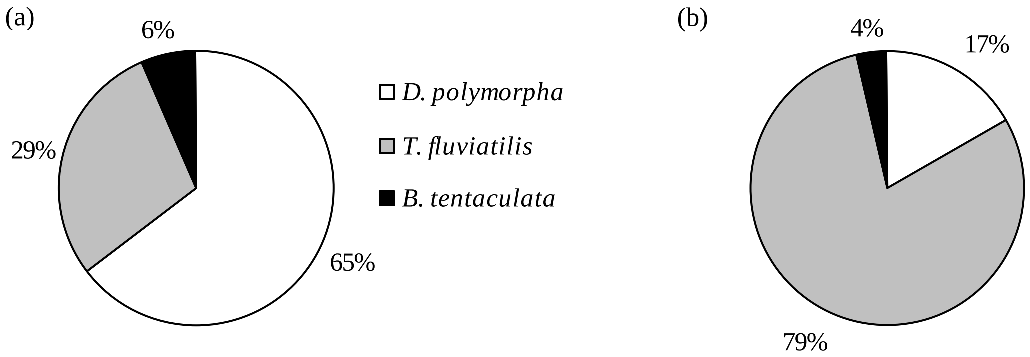

Fig. 6 Proportions of macroinvertebrates, in percent of abundance on concrete tiles, from (a) four cages with signal crayfish and (b) four cages without crayfish.

Size effects on zebra mussels

The size range of zebra mussels on concrete tiles were in cages with crayfish 4.92-17.21 mm, average size: 9,93 mm and median size 9,60 mm. In cages without crayfish, the size range of zebra mussels on concrete tiles were 3.8-17.32 mm, average size: 11,44 mm and median size 11,62 mm. The size structure showed a trend for a difference between treatments, but didn't significantly differ (Mann-Whitney U test: p = 0.069). See figure 7 for histograms with normal distribution curves.

The proportional decreases in number of zebra mussels between treatments are shown in table 2.

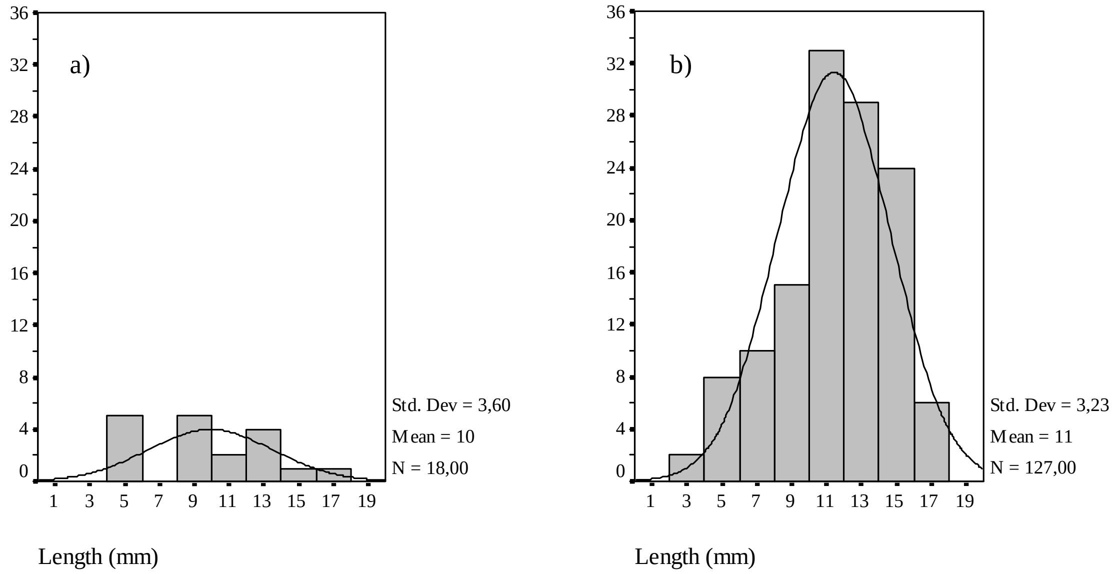

Fig. 7 Histograms with normal distribution curves for a) cages with signal crayfish and b) cages without crayfish. The size structure of zebra mussels on concrete tiles didn't significantly differ between treatments. Note the difference in number of mussels between the two treatments ($n_{en} = 18$, $n_{ex} = 127$).

Table 2 Proportional decrease in number of zebra mussels on concrete tiles between treatments.

Length (mm)	**N:r in exclosures**	**N:r in enclosures**	**Decrease (%)**	
2-4	2	0	100	Juveniles 50
4-6	8	5	38	
6-8	10	0	100	Adults 89
8-10	15	5	67	
10-12	33	2	94	
12-14	29	4	86	
14-16	24	1	96	
16-18	6	1	83	
Total	127	18	86	

Effects on periphyton

The chlorophyll *a* concentrations on the glazed ceramic tiles were significantly higher in cages with signal crayfish than in cages without (*t*-test: p = 0.000, $n_{en} = 3$, $n_{ex} = 4$). Fig. 8.

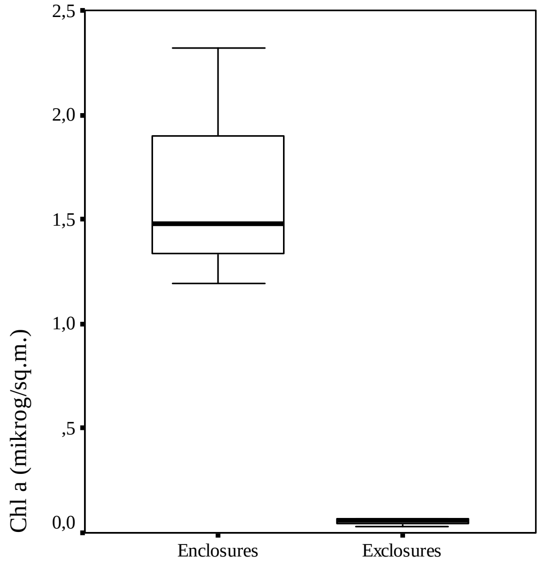

Fig. 8. The chlorophyll *a* concentrations (µg/m^2) were significantly higher in cages with signal crayfish than in cages without crayfish ($n_{en} = 3$, $n_{ex} = 4$). *T*-test: p = 0.000

DISCUSSION

My results showed that signal crayfish significantly reduced the abundance of zebra mussels on hard substrata in the field experiment in Lake Erken (fig. 5). This agrees with the work of Schreiber et al. (1998). The abundances of *B. tentaculata* and *T. fluviatilis* were also significantly reduced (fig. 5).

Even though it is evident that signal crayfish affect zebra mussels, one cannot be certain by what mechanism: Through predation on settled postveligers/juveniles/ adults? Through avoidance behavior from the mussels? Or through a combination of the both?

The proportions of macro invertebrates (fig. 6) show that *T. fluviatilis* either is distinctly susceptible to signal crayfish predation or show effective predator avoidance behaviour. Since it is considered being a slow grazer, the predation effect is most likely. *D. polymorpha*, in contrast, is either not as susceptible or show not as effective predator avoidance behaviour.

Since signal crayfish and zebra mussels have co-existed in Lake Erken for some years, the signal crayfish is not a naïve predator and the mussel is not a naïve prey. Have the zebra mussel evolved a necessary avoidance behavior and response for chemical cues from signal crayfish? Earlier research has shown that the mere presence of predators can produce strong behaviourally transmitted indirect effects in freshwater benthic food chains (Lima 1998). The zebra mussels are capable of releasing old byssus threads and forming new ones (Birnbaum 2011). Young-of-the-year zebra mussels can move using their retractable foot, but stop moving and siphoning when disturbed (Serrouya et al. 1995). It would be interesting to film feeding events in situ, to document an eventual avoidance behavior.

Schreiber et al. (1998) noted that 14 of the 54 signal crayfish in their aquaria experiment did not feed on mussels at all. Due to the cost/benefit ratio, they argued that only small mussels might be a reasonable prey, if they appeared in high abundance. Carlsson & Strayer (2009) showed that fish, within species, with longer exposure to zebra mussels consumed many more zebra mussels than fish with shorter or no previous experience with zebra mussels (naïve). It would be interesting to study if mortality of zebra mussels increases over time, after the signal crayfish have further adapted. If the net benefit is high, it is a possible adaptation: high abundance of zebra mussels (low cost foraging) in combination with learning of handling (lower handling time) could result in increased mortality of zebra mussels. Since zebra mussels are able to increase visibility in waters, the signal crayfish can benefit from the ability to forage by sight.

Schreiber et al. (1998) showed in aquaria experiments with signal crayfish that when mussels were offered alive (but not attached to a stone), a size-preference for small mussels (5.0-9.9 mm) was evident. My data didn't support a size-preference for small mussels, the size structure didn't significantly differ (fig. 7). But it showed a trend for a difference between treatments. Without crayfish, the zebra mussel average size was 11,44 mm and the median size was 11,62 mm. With signal crayfish, the average size was 9,93 mm and median size was 9,60 mm (although the smallest mussel was 1,36 mm longer than in the exclosures). Did the mussels in the enclosures detect the chemical cues of signal crayfish and allocate their energy to other purposes than growth?

In agreement with my hypothesis, I found that the chlorophyll *a* concentrations on the glazed ceramic tiles were significantly higher in cages with signal crayfish than in cages without (fig. 8). Since the abundances of the two grazers *Bithynia* and *T. fluviatilis* also significantly were reduced in enclosures, the result strengthens earlier research (Nyström 1999). Signal crayfish seems to have had an indirect positive effect on periphyton in the enclosures.

Karatayev et al. (1997) stated that roach larger than 180 mm is the most prominent consumer of zebra mussels in European and North American freshwater. Since the enclosures and exclosures were coated by a small sized mesh, I assumed that roaches would not affect the zebra mussels in my experiment. If so the case, both the treatments should be equally effected.
Small crayfish are likely to have been able to walk in and out of both treatments. I have assumed that the effect is spread evenly and thus haven't affected the results.

Conclusions

We need increased knowledge of how zebra mussels act in Swedish lakes to make safer risk assessments and predictions and thus counteract further spread and establishment. My study have increased the knowledge of interactions between signal crayfish and zebra mussels. The data will hopefully aid in the work to control the populations of zebra mussels and minimize the impact on biodiversity in the future.

Do I advise further introduction of signal crayfish into Swedish waters? Not if noble crayfish is present! But that is a whole other story…

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APPENDIX

Material specifications

Cages

Untreated batten from coniferous tree (5*5 mm)
Nils Ahlgren AB Mini angle iron 2.0 mm (2*40*40*20 mm)
Grunda plastic-coated wire (1.40 mm*60 m)
Bostik wood glue 700
Plastic construction foil: Icopal Akvaden Aldringsbestandig 4162/89 04/03 Norfolier.N.
Outer measures for the wooden frame: 102.5*47.5*102.5 cm.
Inner lower wooden frame (4(3,7H*2,5B*197L))
Net bottom (97,4*97,4 cm)

Wavy concrete roof tiles

41,5-42 cm*33 cm (waves excluded)
Living cavities; 3.5 (half) 3.75 (whole) 4,75 (half)

PVC tubes

SIS STF PP 75*2,7 T6 Wavin 07:28