Morphological analyses of modifications in signal crayfish mandible in relation to feeding habits

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ABSTRACT

The presence of zebra mussel, an invasive species, in lakes may change feeding habits of signal crayfish, another invasive species. In order to feed more efficiently on new prey, crayfish modify their morphology in the evolution. The study tries to associate differences in the mandibles shape and structure in signal crayfish with the presence or absence of zebra mussel. Crayfish individuals were sampled in Lake Erken at four different sites with different zebra mussel densities. The incisor ridges in crayfish mandibles were analyzed. Two morphological analysis methods (elliptic Fourier analysis and outline length) were used to assess the differences in crayfish mandibles. The results show significant differences in mandibles with sites. Indeed the sex of individual show significant differences in mandibles regardless the site of the lake. The abundance of zebra mussel in different lake sites affects the morphology of signal crayfish mandibles.

INTRODUCTION

Invasive species can rapidly colonize and adapt to a new environment. The negative effect on native species and economical losses is motive of concern and many studies are being carried out nowadays. However there are more fields of study in this issue which require more research. The effect of the presence of an invasive species in the adaptation of another invasive species when they are predator and prey is the focus of this study. This is an interesting issue scarcely studied.

The predator-prey is one of the most studied interactions in ecology. Predators feed on prey and both population densities are dependent on each other. The predator-prey interaction can lead to adaptations in predators and preys. Predators can adapt their morphology and behaviour to be more efficient hunters, while prey adapt to avoid predation. In our study we focus on these adaptations, in cases when both species are invasive.

One example on invasive species interactions occurs between round goby and zebra mussel. Round goby Neogobius melanostomus utilize a broad range of foods, but prefer zebra mussel Dreissena polymorpha (Ghedotti, 1995). Both are invasive species and come from the same region. Sometimes, however, two invasive species from different regions co-occur in the same habitat. For example, zebra mussels originating from the Ponto-Caspian region may co-occur with signal crayfish that originates from western United States. The different origin of both species and the coincidental presence in the same habitats allows the study of co-evolution between two invasive species. Both species have to adapt to the new circumstances to assure its survival.

Signal crayfish is an invasive species found in most Swedish freshwater ecosystems. They were introduced in most Swedish lakes in the 70’s to replace the decay of native crayfish. Lake Erken was not an exception and in 1966-1969, signal crayfish was introduced with the same purpose (Schreiber et al. 1998). Unfortunately signal crayfish carry a fungal disease exacerbating the stocks of native individuals leading to its extinction.

The zebra mussel is another invasive species that invaded Lake Erken in 1975 in unknown ways. The rapid dispersal of these mussels make them good colonizers and they outcompete native benthic species. Crayfish are omnivorous and can adapt their feeding habits in the presence of a new food resource (Moloney, 1993). Mussels are a common prey for crayfish in general.
and especially for signal crayfish (Schreiber et al. 1998). Zebra mussel is an easy food source for crayfish since they are easy accessible and not mobile. Moreover the mussel flesh is very nutritive with a high content of proteins and easy to digest. In order to crush mussel shells and have access to the flesh, crayfish requires the most efficient mouth-parts. An inefficient mandible will hamper access to the resource. Thereby crayfish are forced to adapt in order to improve its foraging success. Thus, the presence of zebra mussel in lakes can influence morphological adaptations in crayfish mandibles. A previous study claims that crayfish adapt its incisor ridge structures regarding food sources (Harlioglu, 2000). Thereby we carried out the study on different shapes of incisor ridge mandibles from a number of sites of Lake Erken with differences in zebra mussel densities. Crayfish individuals were caught and mandibles were removed and analyzed. At first sight we appreciated different shapes in incisor ridge mandibles. The two extremer shapes we considered were saw-like shape or dentate and blade-like shape or toothless (Harlioglu, 2000).

We hypothesize that crayfish change its diet specialization in presence of zebra mussels adapting its incisor ridge mandible to a more saw-like shape. Consequently in sites with more zebra mussel densities the incisor ridge in crayfish mandibles would be more saw-like or dentate. Crayfish crush zebra mussel shells (Piesik, 1974) and saw-like shape seems to ease crayfish to it in order to obtain the flesh. Conversely, according to our hypothesis crayfish in sites with lower zebra mussel densities would present more blade-like incisor ridge. A low abundance or absence of zebra mussel makes crayfish focus on different food sources like algae, macrophytes and detritus. Blade-like shape is more efficient for cutting these food sources. Secondly we hypothesize to find differences between sexes. Males are more aggressive competitors and displace females to worse habitats for feeding. Thereby males will specialize to feed on zebra mussels, having more saw-like shapes in order to crush shells. Females usually are subordinated in feeding habits to the larger males (Holdich, 2002) hence they would mostly feed on detrital food and vegetation instead of zebra mussels. Thereby females should present a more blade-like shape in mandibles.

Finally we hypothesize differences in relation to the crayfish size. Small juvenile crayfish feed on small food like zooplankton, macrophytes and detritus (Loya-Javellana, 1997) so they need a blade-like mandible to cut food. Conversely big crayfish feed on bigger organisms mostly animals requiring a more specific mandible. Hence we suppose that big adult crayfish possess saw-like mandibles, considering that they feed more in zebra mussel. Thereby we expect that this research on different mandible morphologies in crayfish with different zebra mussel densities will give an insight in crayfish adaptations in presence of zebra mussel.

MATERIAL AND METHODS

We carried out the study at Lake Erken (59°25’ N, 18°15’ E) (Fig 1) in Sweden during July 2008. The lake is located 70 kilometres north of Stockholm. Lake Erken was chosen because of the uneven distribution of zebra mussels. Previous studies have shown substantial differences in the density of zebra mussel in different parts of the lake (Naddafi et al. 2007). Four sites in different parts of the lake and with differences in zebra mussel density were chosen to carry out the study. The places were separated up to two kilometres from one another. In decreasing order of zebra mussel density the sites were site 7 (9213 zebra mussel/m²), site 5 (1320 zebra mussel/m²), site 3
(386 zebra mussel/m²) and site 6 (93 zebra mussel/m²) respectively (density data from an unpublished zebra mussel survey). Sites 3 and 5 are located in Northeast part of the lake whereas 6 and 7 are located in the Southwest part. The benthic zone was the area of study where zebra mussel habit attached to the rocks and the presence of crayfish is more frequent. Furthermore in site 3 the pelagic zone was analyzed too.

Crayfish were caught with crayfish traps. Fish meat of cyprinids was used in the traps to attract crayfish. Ten traps were situated every site in total. Five traps were put in the littoral zone 10 metres from the shore and roughly at 1.5 meter depth, and another 5 traps in the pelagic zone between 50-100 metres from the shore. After 12-14 hours the traps were retrieved and crayfish were frozen waiting for being analyzed (Fig 2). In the fish lab crayfish were defrosted and the mandibles removed. Afterwards carapace length was measured and crayfish were classified in sites and sexes. Pictures of the mandibles were taking using a mounted camera (Fig 3). The molars were placed in front of the camera objective with a millimetre scale next to the mandibles. For the analyses we used the pictures of 172 crayfish from the different sites of study. Sex distribution was almost even. The pictures were used to compare the differences between mandible incisor ridges using two different methods. The incisor ridge, present in all pictures, was the best conserved part of the mandibles and the aim of study. Individuals in moultng phase were excluded due to they regain teeth structure by moultng (Harlioglu, 2000) and while moultning the shape could be unfinished and different to the definitive.

The first method was the elliptic Fourier analysis. Using the informatics program Magic Xtreme Photo Designer 6 the contours of the mandible incisor ridges were drawn (Fig 4). The base and the upper sides of the ridges in the pictures were straightened to achieve a more uniform shape of the base of the mandibles. The base of the mandibles do not contain any analyzed information i.e. not blade or saw-like shape. Small shape changes in the base of the mandible however, were affecting the analyses of the incisor ridge structure and therefore the unification of the mandible base.
Figure 2. Picture of a caught crayfish.

Figure 3. Picture of a pair of incisor ridges in crayfish mandibles. The left one has broken the basal portion of the mandible.

Figure 4. Contour picture of an incisor ridge mandible. Made with Magic Xtreme Photo Designer.

The contours 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 mandible and thus the shape. The shape program generates shape characteristics as principal components (PC1 to PC5) (Fig 5) to analyze shape variation among sites and sex in crayfish.

Figure 5. Incisor ridge mandible shapes based in principal components. The first column are all the different shapes overlapped. The second column depicts the more extreme negative value in PC (-2 stdev), the third column the mean value and the fourth column the more extreme positive value (+2 stdev).
PC1 and PC4 were chosen to display the result due to PC1 represents the general outline shape whereby PC4 depicts the teeth shape. Two double axis graphs represent PC1 and PC4. The variables to study are sites and sexes. In the first two graphs (Fig 6 and Fig 7) the clusters are sites 3, 5, 6 and 7 respectively. The second two graphs (Fig 8 and Fig 9) show differences between males and females. In addition we plotted PC1 results with the length of crayfish (Fig 10) using crayfish carapace length. In order to test statistically the relevance and dependence of each factor we did a Multivariate Analysis of Variance (MANOVA) with PC1 to PC5 as dependent variables. The factors site, sex and site-sex interactions were tested using Wilks’Lambda statistical test. The p value was used to test the significance of each factor. The site-sex interaction depicts the dependence of the factors.

A second method was done to confirm the elliptic Fourier analysis results. The method is based on the analysis of incisor ridge outline length using the program ImageJ. It consists of measure the length of the outline and correlate with the grade of teeth in the incisor ridge. We consider the longer outline the more teeth in crayfish. The length measurement was corrected comparing with the theoretical semicircle length regarding its radius. The theoretical result was subtracted to the outline length result to obtain the value of interest. The purpose was to obtain a positive value. As in the first method the results were grouped in sex and sites.

RESULTS AND DISCUSSION

Sites

Principal component (Fig 6) and outline length results (Fig 7) show that crayfish mandibles show differences in relation to zebra mussel densities in site. Indeed MANOVA confirm the results showing significant differences between sites (p = 0.0001). Results in PC1 and PC4 in sites with high zebra mussel densities show a more stumped and blade-like shape in mandibles against our assumptions (Fig 6, site 7). Otherwise sites with low densities of zebra mussel show the more saw-like shape (Fig 6, site 6 and site 3). Outline length results show the same trend with less teeth in sites with more zebra mussel (Fig 7).

Conversely to our hypothesis blade-like shape could be more advantageous to feed on zebra mussel shells than saw-like shape. An old study claims that mandibles with a blade-like shape are used to scrape food, which could be an advantageous tool to gain access to the flesh (Bouchard, 1977). Up to now there is little research and information available related to which shape of crayfish mandibles is better for feeding on zebra mussel. Further research should be done in order to clarify the more efficient mandible shape in order to feed on zebra mussels.
Figure 6. Principal components (PC1 and PC4) results in relation to the sites where crayfish were caught. The “x” axis represents PC1 values and the “y” axis PC4 values.

Figure 7. Differences in outline length of incisor ridge to the corrected value in different sites.

**Sexes**

There are clear differences in mandibles in relation to the sex based on principal components (Fig 8) and outline length results (Fig 9). Indeed MANOVA confirm the results showing significant differences between sexes ($p = 0.0001$). Males PC1 and PC4 results present a more saw-like shape in accordance to our assumptions whereas females present a more blade-like shape (Fig 8). Outline length method confirms these results (Fig 9). The previous results show that blade-like shape could be the most profitable to feed on zebra mussel. However males being more aggressive and having access to the most
preferred food present more saw-like shape. Zebra mussel presence favours the abundance in benthic invertebrates which could be more preferred food for crayfish (Ward and Ricciardi, 2007). Thereby males will feed more on other benthic invertebrates and females will acquire a blade-like shape to feed on zebra mussel. Crushing mussel shells could be more difficult than to feed on other organisms so males may feed on the less costly organisms.

Figure 8. Principal components (PC1 and PC4) results in relation to sex. The “x” axis represents PC1 values and the “y” axis PC4 values.

Figure 9. Differences in outline length of incisor ridge to the corrected value in different sexes.
Site & sex

MANOVA results shows that interaction of sex and site gives no significant differences (p = 0.544) meaning that sex results do not depend on site and vice versa. This fact verifies the assumption that zebra mussel density and sex of crayfish are two independent variables which affect mandible shape.

Crayfish size

PC1 results show no relation between crayfish length and mandible shape (Fig 10). The results are similar using PC4 and outline length as indicators of the shape. Conversely to our hypothesis length of crayfish do not influence mandible shape in this lake. Juvenile and adult crayfish have different feeding habits (Loya-Javellana, 1997). Big crayfish feed on bigger organisms requiring a more specific mandible whereas small crayfish feed on small food. However our results show no relation between crayfish size and mandible shape. Many individuals with the same carapace length present opposite mandible shapes associated to a different feeding. Thereby crayfish do not adapt its mandible when they grow in order to feed on more zebra mussel.

CONCLUSION

Our results indicate that the shape of crayfish mandibles is affected by the presence of zebra mussels. The different shapes are related with changes in feeding habits. Thereby we claim that the presence of an invasive species is likely to trigger evolutionary responses in feeding apparatus of another invasive species who feed on them following diet specialization (Mooney and Cleland, 2001). The crayfish had more blade-like shape in the mandibles in sites with high zebra mussel densities,
which was against our expectations. It could mean that blade-like shape is more advantageous than saw-like shape to overcome zebra mussel shells and feed on them. However it is unclear why the blade-like shape would be the best to feed on zebra mussel. More research is necessary to clarify that.

In accordance to our hypothesis males and females present different mandible shapes. The different shapes correspond to different feeding habits. The more aggressive males have a more saw-like shape in order to feed on the most preferred and softer body preys. Against our expectations they seem to prefer other preys than zebra mussels. Little is known about to which extent crayfish feed on zebra mussels (Schreiber, 1998) and more research should be done. Consequently, females that are subordinated to males feed more on zebra mussel acquiring a more blade-like shape. Mandible shape is independent of crayfish size. Opposite to our expectations individuals with similar size present different mandible shapes.

Elliptic Fourier analysis was the most reliable method with the more confident results. The Fourier analysis also enabled visualization of the shape changes. The outline length method confirmed the elliptic Fourier analysis results. Hence, this investigation found some new and unexpected patterns in crayfish mandible shape, clearly related to the presence of zebra mussels and the sex of crayfish. The reasons behind these results still remain unclear and need to be elucidated by further research on food preferences and feeding mechanisms of signal crayfish.

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