

## Research Article

## Variability in phenotypic tolerance to low oxygen in invasive populations of quagga and zebra mussels

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Received: 17 July 2015 / Accepted: 1 March 2016 / Published online: 7 April 2016

Handling editor: Vadim Panov

### Abstract

Novel biotic or abiotic conditions can cause invasive species to evolve rapidly in their newly invaded habitats and are important factors when predicting species invasions. Zebra mussels (*Dreissena polymorpha*) have a relatively long invasion history in Western Europe, whereas quagga mussels (*Dreissena rostriformis bugensis*) began spreading about a decade ago. In a previous invasion to North America, quagga mussels repeatedly colonized stratified lakes at greater depth than zebra mussels. It would be important to know if the same invasion pattern is expected to repeat in Western Europe, as the quagga are expected to reach deep stratified lakes in the near future. This might require quagga mussels to be more tolerant of the low oxygen conditions at depth than zebra mussels. Therefore, using a fully factorial design, we tested survival of different zebra and quagga mussel populations from Western Europe at four oxygen levels (6%, 33%, 66% and 90%) and two temperature regimes (11°C and 18°C). Surprisingly, survival differences among oxygen and temperature treatments depended more on population origin than on species identity. This finding suggests that populations have undergone rapid and convergent adaptation to local conditions after invasion, in particular to low oxygen. We also found that population-by-environment interactions were more variable among quagga than zebra mussel populations. Our results suggest that rapid evolutionary adaptation to low oxygen conditions needs to be considered when predicting the further spread of zebra and quagga mussels.

**Key words:** *Dreissena polymorpha*, *Dreissena rostriformis bugensis*, niche shifts, temperature, population-by-environment interactions

### Introduction

Recognizing that eco-evolutionary dynamics may be important for natural adaptation has alerted invasive species ecologists to consider ecological and evolutionary processes such as phenotypic plasticity, developmental plasticity and local adaptation when predicting the future range and ecological impact of non-native species (Lambrinos 2004; Lee 2002). Consequently, newly established invasive populations might diverge rapidly in their tolerance to various environmental factors (Pearman et al. 2008; Prentis et al. 2008; Sexton et al. 2009). For example the copepod *Eurytemora affinis*, which is a native salt water species, evolved adaptations to interstitial ion

regulation within a few generations of invading freshwater lakes on the east coast of North America (Lee et al. 2012). In another example Huey et al. (2000) found that upon introduction to North America the native European fruit fly, *Drosophila subobscura*, evolved gradual phenotypic adaptation in wing size in response to temperature along the invasion route in less than two decades. These examples emphasize that non-native species might adapt their environmental niche along with the invasion process in response to environment. Such adaptations are known to require sufficient heritable genetic variation, which in non-native species depends on propagule number, source of the introduction and details of the invasion history (Brown and Stepien 2010; Ficetola et al. 2008; Roman and Darling 2007).

The zebra mussel, *Dreissena polymorpha* (Pallas, 1771), and the quagga mussel, *Dreissena rostriformis bugensis* (Andrusov, 1897) are two closely related species originating from the Ponto-Caspian region. Both are invasive in Europe and North America and exhibit extremely high population densities and strong ecological and economic impacts in their invasive range (Higgins and Vander Zanden 2010; Pimentel et al. 2005; Strayer 2009). The zebra mussel has spread widely across Western Europe since the early 19th century (Kinzelbach 1992) and is now present in most larger rivers and lakes. The quagga mussel has a much shorter invasion history in Western Europe, starting in 2004 with potentially multiple introductions from the Netherlands and the Main – Rhine – Danube canal (Heiler et al. 2013; Imo et al. 2010; Molloy et al. 2007). Presently, quagga mussels are expected to spread further to the deep stratified lakes in vicinity of the alps, which often show pronounced oxygen depletion at depth (Matthews et al. 2014).

Both quagga and zebra mussel populations have high gene flow and express high genetic diversity in their invasive range (Brown and Stepien 2010; Imo et al. 2010; Muller et al. 2002; Therriault et al. 2005; Wilson et al. 1999). Therefore, populations of either species supposedly have a high potential for genetic adaptation. European quagga mussels show limited genetic differentiation in neutral nuclear markers (Imo et al. 2010; Therriault et al. 2005), whereas zebra mussels express clear divergence among European populations (Muller et al. 2002; Pollux et al. 2003; Rajagopal et al. 2009). These findings are likely to reflect the longer invasion history of the zebra mussel in Western Europe, which has had more time to differentiate and adapt to local conditions. Although quagga mussels started to spread later than zebra mussels in Western Europe, the invasion front is proceeding rapidly (Matthews et al. 2014) and the quagga mussel seems the stronger competitor when both species are present (Karatayev et al. 2011b; Mills et al. 1996; Orlova et al. 2005). The invasion history and population ecology of these species predicts that sufficient genetic variation for evolutionary adaptation should be present, but evidence for evolutionary adaptation in invasive populations has been lacking so far.

In this study, we investigated among-population differences in survival of quagga and zebra mussels in low and high oxygen conditions under two temperature regimes, with the aim of detecting physiological adaptation to low-oxygen conditions. The deep lakes of the alpine region are still free of quagga mussels and often show prolonged phases of oxygen depletion (of variable severity depending on the lake) during

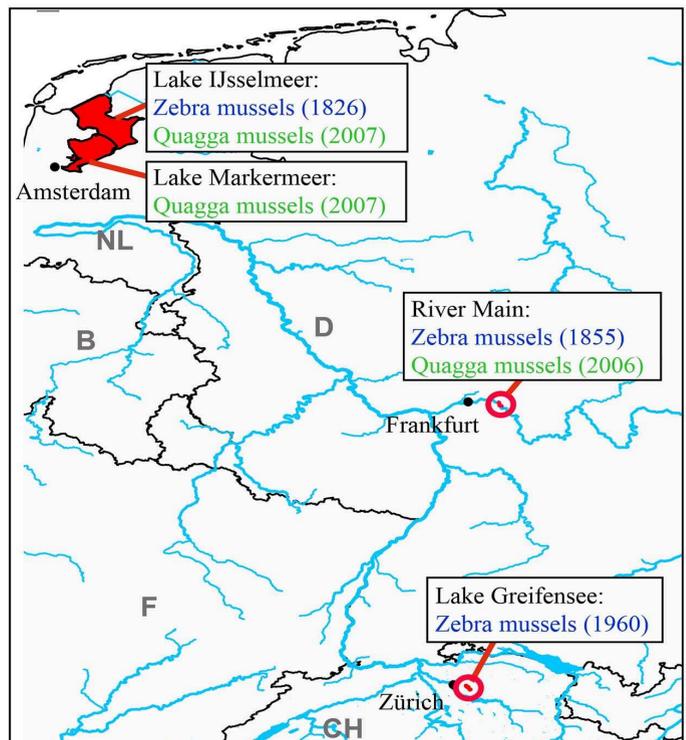
summer stratification. The recently invaded shallow lakes in the Netherlands on the other hand show only short phases (several days) of hypoxic conditions during extreme summer heat events. One study showed that zebra mussels are poor oxygen regulators but depending on the temperature are able to survive in low oxygen conditions for a number of days (Johnson and McMahon 1998). Moreover, Stoeckmann and colleagues (2003) showed that quagga mussels of Lake Erie had a lower respiration rate and consumed less oxygen than the sympatric zebra mussels over a range of temperatures. It is not clear if this means that quagga mussels have a higher phenotypic tolerance to low oxygen conditions compared to zebra mussels and whether this might facilitate colonization of the deep hypoxic zones of stratified lakes. As both species colonized water bodies of very different oxygen conditions, with quagga mussels often outcompeting zebra mussels when both species are present, we wanted to know whether differences in colonization patterns arise due to differences in phenotypic tolerance to low oxygen conditions or due to evolutionary adaptation.

We analyzed the population reaction norms for survival in response to oxygen and temperature among Western European populations of quagga and zebra mussels to evaluate the population-by-environment interactions, which can indicate if local adaptation has taken place post-invasion. Manipulating oxygen and temperature regimes, we simulated the conditions above and below the summer thermocline in a stratified lake and hypothesized that quagga mussels should show higher survival under these conditions than zebra mussels. In contrast to the very recent (only about one decade) introduction of quagga mussel populations, the time since the introduction of the tested zebra mussel populations was longer, but variable (150–40 years). Thus, we hypothesized further that due to their longer invasion history zebra mussel populations would show stronger population-specific adaptive environmental responses.

## Methods

### *Origin of mussels*

Samples from six populations of mussels were collected from four locations (Figure 1). Both species were collected from River Main (50.111140N, 8.916910E, Hanau, Germany), and Lake IJsselmeer (52.709983N, 5.493267E, Netherlands). Additionally, zebra mussels were collected from Lake Greifensee (47.349075N, 8.690081E, Switzerland) and quagga mussels from Lake Markermeer (52.531667N, 5.231083E, Netherlands). The study lakes have



**Figure 1.** Sampling locations (marked in red) with zebra mussel (*Dreissena polymorpha*) in blue and quagga mussel (*D. rostriformis bugensis*) in green. Lake Markermeer and Lake IJsselmeer are large shallow eutrophic lakes, while Lake Greifensee is a smaller stratified, eutrophic lake. The year of invasion for each population is given in brackets and was retrieved from Kinzelbach (1992) for zebra mussels, and from Heiler et al. (2013) for quagga mussels. Lake IJsselmeer and Lake Markermeer were only constructed in 1932 by cutting the former Zuiderzee (brackish water) from the sea with a dam but zebra mussels had already been present in the surrounding area (Zuiderzee) before that time.

a history of oxygen depletion in the deeper regions. Lake Markermeer and Lake IJsselmeer are large shallow (3.5 m depth and 5 to 6 m depth, respectively) eutrophic lakes in the Netherlands while Lake Greifensee is a smaller and deeper (30 m depth) stratified eutrophic lake in Switzerland. Lake Greifensee shows pronounced oxygen depletion in the hypolimnion during the summer months. For Lake Markermeer and Lake IJsselmeer, Noordhuis (2014) reported short periods of stratification and oxygen depletion (a few days) in 2011 and 2012 and mass mortalities of fish and dreissenid mussels were likely due to prolonged stratification and oxygen depletion in the deep zones of the lakes during the extreme summer heat-wave in 2006. Oxygen levels were also reduced in River Main during the summer months but the oxygen concentration never reached levels below 30% oxygenation in the non-stratified River Main (Supplementary material, Figure S1).

River Main and Lake Greifensee mussels were picked haphazardly from stones near the shore line at roughly 1 m water depth in May 2012 and transported in cooling boxes to the laboratory in Wageningen. In mid-June 2012, we collected mussels from Lake Markermeer (at ca. 3.5 m depth) and Lake IJsselmeer (at ca. 4 m depth) with a bottom dredge

(metal frame of 35 × 60 cm, mesh size 5 mm) and sorted them by species. For each population of mussels, we collected over 2000 individuals of shell length larger than 8 mm. All mussels were acclimated to lab conditions for at least one month before the experiment (started end of July 2012) in aerated ground water at 15°C and fed with 5 ml Shell Fish Diet (Reed Mariculture Inc.) per 1000 mussels per day.

### Experiment

We assessed the survival of mussels from the six study populations under different oxygen and temperature conditions in a mesocosm experiment. We conducted a fully factorial experiment including four replicates of two temperature treatments (11°C and 18°C) crossed with four oxygen levels (6%, 33%, 66% and 90% oxygen saturation) in a total of 32 experimental plastic aquaria (34 × 25 × 16 cm, 9 L, Figure S2). Aquaria were held in water baths (glass aquaria of 185 × 50 × 50 cm, equipped with a cooling and heating system, NIOO Institute, Wageningen) to adjust the temperature. Oxygen levels were controlled in a separate tank (20 L plastic bottle) for each oxygen level by bubbling either nitrogen (for the levels of 6%, 33% and 66% oxygen saturation) or

**Table 1.** Mean shell length (mm) and shell volume (mm<sup>3</sup>, estimated as  $4/3 \times \pi \times \text{length}/2 \times \text{height}/2 \times \text{width}/2$ ), the corresponding standard deviation and the total number of experimental mussels (N) for each population, listed as a combination of sampling site (origin) and species.

| Origin     | Species        | N   | Variable     | Unit            | Mean    | Standard Dev. |
|------------|----------------|-----|--------------|-----------------|---------|---------------|
| Greifensee | Zebra mussels  | 384 | Shell length | mm              | 13.40   | 3.69          |
| IJsselmeer | Quagga mussels | 384 | Shell length | mm              | 20.75   | 4.77          |
| IJsselmeer | Zebra mussels  | 384 | Shell length | mm              | 13.99   | 3.14          |
| Main       | Quagga mussels | 384 | Shell length | mm              | 19.74   | 5.52          |
| Main       | Zebra mussels  | 384 | Shell length | mm              | 16.06   | 3.96          |
| Markermeer | Quagga mussels | 384 | Shell length | mm              | 13.30   | 3.27          |
| Greifensee | Zebra mussels  | 384 | Shell volume | mm <sup>3</sup> | 421.47  | 422.52        |
| IJsselmeer | Quagga mussels | 384 | Shell volume | mm <sup>3</sup> | 1252.55 | 590.89        |
| IJsselmeer | Zebra mussels  | 384 | Shell volume | mm <sup>3</sup> | 383.18  | 239.81        |
| Main       | Quagga mussels | 384 | Shell volume | mm <sup>3</sup> | 1645.09 | 1309.74       |
| Main       | Zebra mussels  | 384 | Shell volume | mm <sup>3</sup> | 736.06  | 539.18        |
| Markermeer | Quagga mussels | 384 | Shell volume | mm <sup>3</sup> | 268.88  | 208.35        |

oxygen (for 90% oxygen saturation) into the tank. The outflow of each tank was directed to the corresponding aquaria with PVC tubes. Each experimental aquarium was completely filled with water and closed with a lid in order to minimize gas exchange with the surrounding air. The excess water discharged from the aquaria to the surrounding water bath, while the outflow from the water baths was collected in a common tank and from there pumped back to the four different tanks where the four oxygen levels were controlled. In this way all aquaria were connected to the same closed water circulation system, because we wanted to minimize the nesting effect of the separate oxygen regulating tanks and water baths in the experiment. The flow of nitrogen to each of the oxygen controlling tanks was controlled with a manometer and the resulting oxygen levels were measured with an optical oxygen sensor (LDO101, HACH Company, USA) in the experimental aquaria. The experimental setup is further described in Supplemental material Figures S2 and S3. The flow speed at the inflow to the experimental aquaria was set between 90 and 110 ml per minute with screw clamps. Flow, temperature and oxygen levels were checked regularly (daily during the first two weeks of the experiment and biweekly later on) in all aquaria and adjusted if deviation from the target value was larger than 1°C for temperature or 5% for oxygen. The experimental setup was filled and run without mussels for three weeks before the start of the experiment in order to adjust experimental conditions. The oxygen concentrations and temperatures monitored in the aquaria throughout the experiment are shown in the Supplementary material Figure S4 and Table S1 and Figure S5, respectively.

Each aquarium received 12 randomly selected mussels from each population (12 individuals  $\times$  6 populations  $\times$  32 aquaria = 2304 mussels). Experi-

mental mussels were selected such that they were representative for the size distribution of each mussel population with shell length > 8 mm (Table 1). The experimental size distributions of each population were not different between treatments (Supplementary material Figure S6). For the quagga mussel populations from River Main and Lake IJsselmeer a stratified random sampling was used in order to ensure that each aquarium contained both small and large mussels. All mussels were marked individually with a color and number using bee tags (Geller GbR) and measured for shell length, width and height with calipers. We tagged and measured one population per day and kept the tagged mussels under acclimatization conditions until the start of the experiment. An additional 60 “baseline” mussels per population were measured and tagged in the same way as the experimental mussels. On the starting day these “baseline” mussels were stored in -80°C for subsequent analysis of tissue dry weight and all experimental mussels were distributed to their corresponding aquaria of defined temperature and oxygen level. A food mixture consisting of 7.2 ml of Shell Fish Diet and 4 ml of Rotifer Diet (Reed Mariculture Inc.) suspended in aerated groundwater was equally distributed to the 32 aquaria daily. Every second day we checked all aquaria for dead mussels and removed them. We considered mussels to be dead when they stayed wide open and did not close their shells upon touching. After 65 days the experiment was stopped because almost all mussels had died in some of the tanks.

#### Statistical analysis

We analyzed the right censored survival of mussels throughout the experiment with a parametric accelerated failure time (AFT) model using Weibull error distribution as recommended in Kleinbaum and

Klein (2005). As fixed factors we included oxygen, temperature and either population or species and all two-way interactions. In both models, including either species or population as an explanatory variable, the three-way interactions were not significant and omitted ( $p = 0.38$  and  $p = 0.72$ , respectively). In order to avoid convergence problems resulting from high survival in high oxygen treatments we pooled the data for the three highest oxygen levels (33%, 66% and 90%) as opposed to the low oxygen treatment (6% oxygen). This simplification was justified, because the survival in the three higher oxygen levels did not differ significantly (Weibull model,  $p = 0.89$ ) and the model for the data set with only two oxygen treatment classes gave a slightly reduced AIC (Akaike's Information Criterion). Additionally, we included aquarium as the frailty term (random effect) to account for the variability between experimental units. As mussel volume (or shell length) did not significantly affect mussel survival, it was not included in the final models (Supplementary material Table S2). We confirmed the assumptions of the AFT Weibull model graphically by plotting the log of the negative log of the survival function (Kleinbaum and Klein 2005). The lines for the different populations were roughly straight, but not parallel, which is in accordance with assumptions of the Weibull model. We calculated the median predicted survival days for each population and all treatment combinations from each of the two survival models: one including populations and one including species. We then used these predictions to compare the reaction norms between the different populations and species.

In order to control for differences in survival due to condition differences of mussels after the acclimatization period, we calculated the condition index (CI, see also Lawrence and Scott 1982) for sixty "baseline" mussels per population. For all "baseline" mussels the tissue was retrieved from the shell, freeze dried for three days and the tissue dry mass (mg) was weighed on a high precision scale. The volume ( $\text{mm}^3$ ) was calculated as using the approximation of  $4/3 \times \Pi \times \text{length}/2 \times \text{height}/2 \times \text{width}/2$  and the condition index (CI) as tissue dry weight (mg) divided by volume ( $\text{mm}^3$ ). The CI was then compared between populations in a linear regression model. The influence of the factors species and population origin was additionally examined in a linear mixed effects model, where location was considered as a nested effect under species.

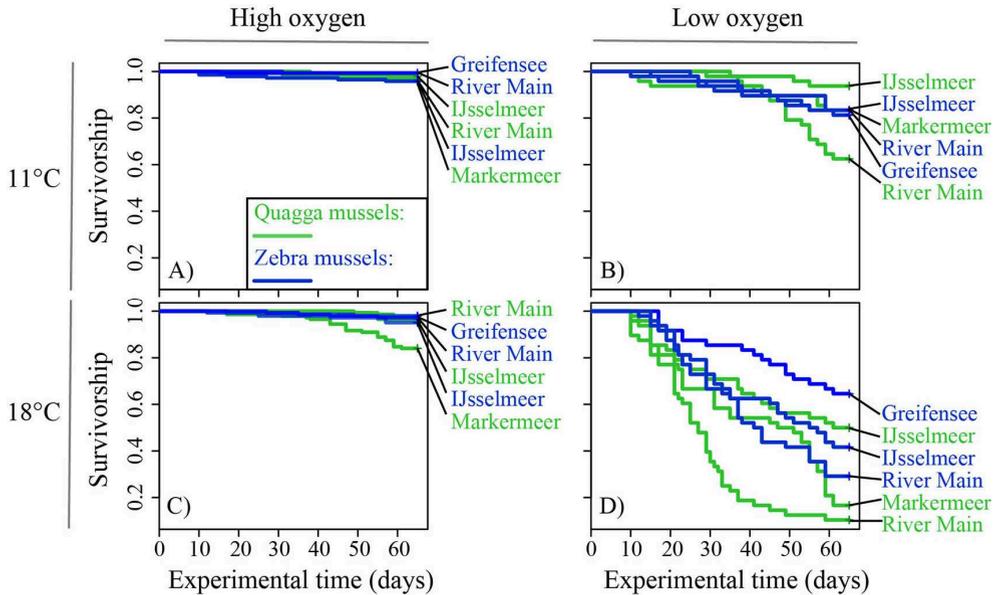
All analyses were performed in R (R-Core-Team 2014) and the "survival" - package (Therneau 2015) was used for the survival analysis and the "nlme" - package (Pinheiro et al. 2013) for mixed linear models.

## Results

Oxygen and temperature had strong and significant effects on the survival of mussels (Figure 2, Table 2A). The predicted median survival of mussels in the lowest oxygen treatment was 96 days (SE = 16 days) translating to four times lower survival rate than in the high oxygen treatment (389 days, SE = 103 days,  $p < 0.0001$ , Table 2A). Higher mortality in the low oxygen treatment was observed for all populations in both temperature regimes but there were no significant differences in survival by populations among the three high oxygen treatments 33%, 66% and 90% ( $p = 0.88$ , data not shown). The predicted mussel survival was 27% lower at 18°C when compared to 11°C (267 days, SE = 65.2 compared to 364.4 days, SE = 97.7,  $p < 0.0001$ , Table 2A) including all mussels from all populations and treatments. The reduction in survival due to low oxygen was also more pronounced at 18°C in all populations (Figure 2). Against our expectations the survival rate of quagga mussels was significantly lower compared to zebra mussels (272 days, SE = 62.5 days compared to 359 days, SE = 100.4 days,  $p < 0.001$ , Table 2B).

We also found strong and significant differences in phenotypic response to oxygen and temperature treatments among populations (Figure 3). Significant interaction between oxygen level and population in survival ( $p < 0.0001$ , Table 2A) implies origin-specific tolerance differences to oxygen depletion. The model with population as a factor fitted the data better (AIC = 3603) than the model including species (AIC = 3667), suggesting that the differences among species were less pronounced than the differences among study populations. Sensitivity to lowered oxygen varied strikingly between populations showing a strong population-by-environment interaction in both temperatures (Figure 3). The slopes of the reaction norms varied between sites of origin but less so between populations of species coexisting at the same site (Figure 3), showing that responses were more similar within locations than within species. Analyzing survival rates at low oxygen levels separately revealed that in the high temperature treatment both quagga and zebra mussels from River Main survived relatively poorly (Figure 2D) while both Lake IJsselmeer populations had higher tolerance of oxygen and temperature stress. In the low oxygen treatment, the survival profiles differed strongly among both zebra and quagga mussel populations at 18 °C, while differences in survival were less pronounced among zebra mussel populations than among quagga mussel populations at 11°C (Figure 2B).

The condition indices (CI) for 'baseline' samples were significantly different between populations ( $p < 0.0001$ ,



**Figure 2.** Experimental survivorship curves for the four treatment combinations, after the three highest oxygen levels were pooled: **A)** High oxygen and low temperature, **B)** low oxygen and low temperature, **C)** high oxygen and high temperature, **D)** low oxygen and high temperature. Curves for zebra mussels are depicted in blue and for quagga mussels in green and the populations are indicated on the right of each plot, with populations showing highest survival on top and populations showing lowest survival at the bottom of each list.

Table S3), but did not predict their ranking in survival at low oxygen in the experiment. Quagga mussels differed more from each other in CI than zebra mussels and location paired populations better than species (Figure 4, Figure S7). In the mixed effects model, population origin explained 70% of the variation in CI while species identity explained only a minor fraction of the variation ( $1 \times 10^{-5}\%$ , Table S4).

## Discussion

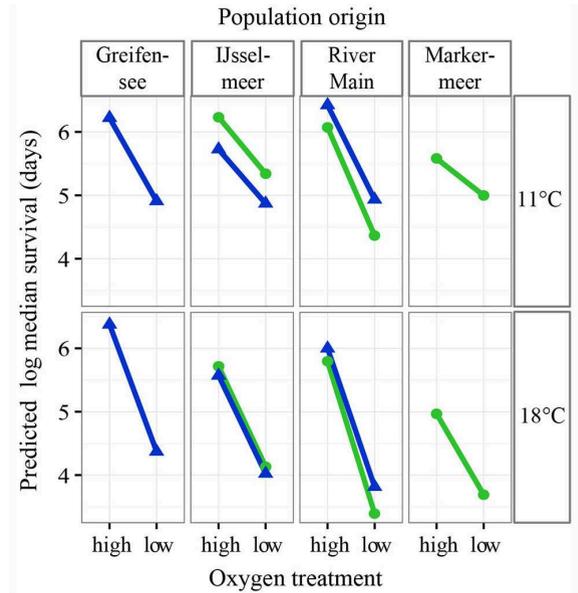
We found that the survival of both quagga and zebra mussels was strongly reduced at low oxygen with higher temperature (18°C). Comparable reductions of survivorship due to chronic hypoxia at different temperatures were found experimentally by Johnson and McMahon (1998) for zebra mussels from the Niagara River, Buffalo, New York. Unexpectedly, our results revealed that the survival reaction norms between oxygen treatments depended more on population origin than on species identity. In more detail, we found that the population-by-oxygen interaction differed more among quagga than among zebra mussel populations (Figure 3). Similarly, the origin of the populations explained the variation in recorded condition indices (CIs) better than species identity, but CI did not correlate with the survivorship of the populations in the experiment (Figure 2,

Figure 4). In fact, among-population differences in survival under low oxygen reflect the environmental oxygen levels recorded at each sampling location. Under low oxygen conditions both species from River Main showed the lowest survival rate, followed by mussels from the Dutch lakes, while zebra mussels from Lake Greifensee showed the highest survival (Figure 2D). This corresponds to environmental data where Lake Greifensee shows strongest oxygen depletion at greater depths during summer months, while Lake Markermeer and Lake IJsselmeer experienced more pronounced phases of oxygen depletion at depth than River Main (Figure S1 and Noordhuis et al. 2014). Hence, the observed pattern might be an indication of local adaptation in the selected populations as the locally experienced environmental conditions are reflected in the reaction norms. Therefore, population origin matters more than species identity in explaining the experimental survival data. As external fertilization in the water column mixes local genotypes in every reproductive event and free-floating larvae have only a very limited ability to select their settling place, it is unlikely that the different sampling depths of the populations influences their genetic background. Of course, we cannot exclude the possibility of developmental phenotypic differences as our experimental mussels were wild-caught.

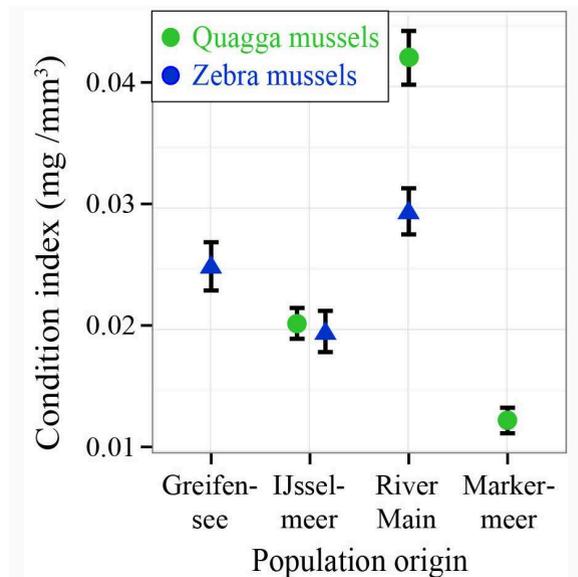
Zebra and quagga mussel populations from North America and Europe are reported to be genetically diverse outcrossing populations suggesting that genetic bottlenecks should not be limiting adaptation (Brown and Stepien 2010; Imo et al. 2010; Muller et al. 2002; Therriault et al. 2005; Wilson et al. 1999). Western European zebra mussel populations showed clear genetic differentiation (Muller et al. 2002; Pollux et al. 2003; Rajagopal et al. 2009), indicating that the local population genetic structure has emerged through restricted gene-flow, drift and possibly local evolutionary processes in these post-invasion populations. Local adaptation of zebra mussel populations to temperature regimes has already been suggested by Elderkin and Klerks (2001, 2005) who showed a gradient in allele frequency along the Mississippi River, which corresponded to latitudinal distance.

German quagga populations, that were established only one decade ago (Heiler et al. 2013; Molloy et al. 2007), do not show genetic differentiation (Imo et al. 2010). In the face of their recent invasion history it seems remarkable that quagga populations show more variance in response to oxygen levels than zebra mussels and seem to be better adapted to the variable oxygen conditions in the different invaded habitats. The examined populations represent the current invasion front of quagga mussels in Western Europe, which implies that potential adaptive processes can happen in only few generations. This is in accordance with the prediction that rapid local adaptation should be more frequent in populations at the species' range limits (Sexton et al. 2009). A complementary explanation for differences between the species might be that propagule exchange (gene flow and migration) is lower for quagga mussels than for zebra mussels (Karatayev et al. 2011a) leading to faster genetic divergence among populations at the invasion front. Therefore, we should not exclude the possibility of some degree of rapid local adaptation in quagga mussels, even though the populations are recent. Alternatively, the population differences may also have arisen through pre-invasion local adaptation of different source populations. For example, Heiler et al. (2013) suggested that repeated introductions of quagga mussels from potentially different source populations have occurred in the delta of the River Rhine in the Netherlands and the Rhein – Danube canal in Germany. Distinguishing the two alternatives may not be possible based on the results of this study, but certainly is an important point for future studies and predictions.

Quagga and zebra mussels showed similar sensitivities to low oxygen but on average quagga mussels had a somewhat higher mortality than zebra



**Figure 3.** Reaction norms for the natural logarithm of the predicted median survival against high and low oxygen treatment class. Reaction norms for zebra mussels (blue, triangles) and quagga mussels (green, circles) are shown separately for each of the populations and the two temperatures.



**Figure 4.** Mean condition index, calculated as soft tissue dry weight (mg) divided by shell volume (mm<sup>3</sup>), for zebra mussels (blue triangles) and quagga mussels (green circles) against population origins. Error bars represent standard errors.

**Table 2. A)** Deviance and likelihood statistics and statistical significance of the factors in the Weibull-survival models including oxygen, temperature and population as explanatory factors, **B)** Deviance and likelihood statistics and statistical significance of the factors in the Weibull-survival models including species instead of population as an explanatory variable. Tables show, the explanatory variables and the interactions included, degrees of freedom (D.f.), deviance, residual degrees of freedom,  $-2 \times \log$ -likelihood ( $-2 \times LL$ ) and corresponding p-values (Pr (>Chi)). All significant effects have p-values < 0.001 and are indicated with \*\*\*. The AIC gives the value of the Akaike information criterion as a measure of the relative model quality and goodness of fit.

| <b>A)</b>                         | D.f.  | Deviance | Resid. D.f. | ( $-2 \times LL$ ) | Pr (>Chi) | Sig. |
|-----------------------------------|-------|----------|-------------|--------------------|-----------|------|
| Intercept                         | NA    | NA       | 2302        | 4331               | NA        |      |
| Oxygen level                      | 1.00  | 471.4    | 2301        | 3859               | <0.00001  | ***  |
| Temperature                       | 1.00  | 146.3    | 2300        | 3713               | <0.00001  | ***  |
| Population                        | 5.00  | 68.4     | 2295        | 3645               | <0.00001  | ***  |
| Frailty term (aquarium)           | 22.49 | 83.0     | 2273        | 3562               | <0.00001  | ***  |
| Oxygen level $\times$ temperature | -0.75 | -0.1     | 2273        | 3562               | 0.59      |      |
| Oxygen level $\times$ population  | 5.44  | 30.1     | 2268        | 3532               | <0.00001  | ***  |
| Temperature $\times$ population   | 5.17  | 10.1     | 2263        | 3522               | 0.079     |      |
| AIC = 3603                        |       |          |             |                    |           |      |
| <b>B)</b>                         | D.f.  | Deviance | Resid. D.f. | ( $-2 \times LL$ ) | Pr (>Chi) | Sig. |
| Intercept                         | NA    | NA       | 2302        | 4331               | NA        |      |
| Oxygen level                      | 1.00  | 530.1    | 2299        | 3692               | <0.00001  | ***  |
| Temperature                       | 1.00  | 97.5     | 2300        | 4222               | <0.00001  | ***  |
| Species                           | 1.00  | 11.3     | 2301        | 4319               | 0.0010    | ***  |
| Frailty term (aquarium)           | 21.96 | 78.2     | 2277        | 3614               | <0.00001  | ***  |
| Oxygen level $\times$ temperature | -0.99 | -0.1     | 2276        | 3611               | 0.71      |      |
| Oxygen level $\times$ species     | 1.01  | 0.7      | 2275        | 3611               | 0.42      |      |
| Temperature $\times$ species      | 1.05  | 2.4      | 2276        | 3611               | 0.13      |      |
| AIC = 3667                        |       |          |             |                    |           |      |

mussels in the low oxygen treatment, independent of temperature. Zebra mussels were found to have similarly low survival and low oxygen regulatory capacities under hypoxic conditions (Johnson and McMahon 1998). Garton et al. (2013) suggested similar oxygen regulatory capacities for zebra and quagga mussels based on unpublished results by Johnson and McMahon, who found somewhat lower median lethal times ( $LT_{50}$ ) compared to our data, with slightly but consistently higher  $LT_{50}$  values for zebra mussels. Stoeckmann (2003) showed that quagga mussels of Lake Erie had a lower respiration rate and consumed less oxygen than the sympatric zebra mussels over a range of temperatures. They suggested that in Lake Erie, this difference in respiration rate translated into higher growth and reproductive rates and that this has been one factor promoting the competitive exclusion of zebra mussels by the recently invaded quagga mussel. Assuming that generally lower respiration rates could also be found in the quagga mussel popu-

lations tested in our experiment, our results suggest that such differences would not generally translate into better tolerance of low oxygen conditions.

European lakes colonized so far are rather shallow, often well mixed across the water column and experience only short periods of oxygen depletion in summer (Figure S1). In contrast, the deep lakes in the vicinity of the Alps, where quagga mussels are expected to invade in the near future, show variably strong stratification and oxygen depletion below the thermocline for several months during summer. These hypolimnetic zones are also characterized by lower temperatures and low nutrient conditions. Our results indicate that quagga mussels of Western Europe are more sensitive to lower oxygen levels than sympatric zebra mussels, suggesting that alternative factors should be considered when predicting species-specific depth distribution and the potential displacement of zebra mussels by quagga mussels, which has repeatedly been observed in North American and European lakes. Such alternatives

could be better survival and reproduction at lower temperatures (Roe and MacIsaac 1997) and better tolerance of low nutrient conditions. Quagga mussels were found to filter seston, including bacterioplankton, more efficiently than zebra mussels at low seston concentrations (Baldwin et al. 2002; Stoeckmann 2003). A generally higher somatic growth and survivorship in freshwater (Karatayev et al. 2011b) might also give quagga mussels a competitive advantage over zebra mussels. All these factors may allow quagga mussels to better colonize the deeper zones of lakes (as long as they show only moderate levels of hypoxia) and reach higher densities compared to zebra mussels. Yet, the above cited studies did not take the potentially different population background into account.

Assuming that source populations are of Western European origin and adapted to shallow lakes, we predict that invading quagga and zebra mussels should initially have a similar tolerance to low oxygen conditions. In a field experiment by Verhofstad et al. (2013) zebra mussels survived even better than quagga mussels in the deep zones of a Dutch Lake (Lake Cuijk), potentially due to their better tolerance of hypoxic conditions. Nevertheless, quagga mussels might gain an additional competitive advantage over zebra mussels for reaching the deeper zones of these lakes through better post-invasion adaptation to low oxygen conditions given sufficient time.

To conclude, we found more pronounced phenotypic variation among populations than among species which highlights the possibility for post-invasion evolutionary response. Alternatively, these patterns of phenotypic divergence might have arisen pre-invasion through local adaptation of different source populations followed by separate invasions to the investigated locations, or through canalized phenotypic plasticity to environmental conditions at the studied location. The population-by-environment interactions call for further studies where canalized developmental plasticity should be contrasted with adaptive divergence using F1 and F2 lab-reared offspring in a classic common garden design. Different life cycle stages of zebra and quagga mussel may have different ecological requirements and further studies are needed to examine the environmental niche of different life-cycle stages. Despite these constraints, local adaptation in quagga and zebra mussels might promote environmental niche shifts along their invasion fronts in Europe and elsewhere and thus seem to be important for predictive models of future quagga and zebra mussel distributions. Furthermore, our results stress the importance of using multiple populations of a species when the environmental niches of invasive species (or species in general) are investigated in experiments.

## Acknowledgements

We thank two anonymous reviewers for the constructive comments on our manuscript. This work was financed by Eawag discretionary fund (5221.00492.005.10\_Invasive Mussel) and hosted and supported by the Netherlands Institute of Ecology (NIOO). Environmental data was provided by the Canton of Zürich, Bayerisches Landesamt für Umwelt, Rijkswaterstaat and Ruurd Noordhuis (Deltares). Special thanks go to Bas Ibelings for advice and Nico Helmsing and Koos Swart for practical support.

## References

- Baldwin BS, Mayer MS, Dayton J, Pau N, Mendilla J, Sullivan M, Moore A, Ma A, Mills EL (2002) Comparative growth and feeding in zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*): implications for North American lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 680–694, <http://dx.doi.org/10.1139/f02-043>
- Brown J, Stepien C (2010) Population genetic history of the dreissenid mussel invasions: expansion patterns across North America. *Biological Invasions* 12: 3687–3710, <http://dx.doi.org/10.1007/s10530-010-9763-2>
- Elderkin CL, Klerks PL (2001) Shifts in allele and genotype frequencies in zebra mussels, *Dreissena polymorpha*, along the latitudinal gradient formed by the Mississippi River. *Journal of the North American Benthological Society* 20: 595–605, <http://dx.doi.org/10.2307/1468090>
- Elderkin CL, Klerks PL (2005) Variation in thermal tolerance among three Mississippi River populations of the zebra mussel, *Dreissena polymorpha*. *Journal of Shellfish Research* 24: 221–226, [http://dx.doi.org/10.2983/0730-8000\(2005\)24\[221:VITAT\]2.0.CO;2](http://dx.doi.org/10.2983/0730-8000(2005)24[221:VITAT]2.0.CO;2)
- Ficetola GF, Bonin A, Miaud C (2008) Population genetics reveals origin and number of founders in a biological invasion. *Molecular Ecology* 17: 773–782, <http://dx.doi.org/10.1111/j.1365-294X.2007.03622.x>
- Garton DW, McMahon R, Stoeckmann AM (2013) Limiting Environmental Factors and Competitive Interactions between Zebra and Quagga Mussels in North America. *Quagga and Zebra Mussels*. CRC Press, pp 383–402
- Heiler KCM, Bij de Vaate A, Ekschmitt K, von Oheimb PV, Albrecht C, Wilke T (2013) Reconstruction of the early invasion history of the quagga mussel (*Dreissena rostriformis bugensis*) in Western Europe. *Aquatic Invasions* 8: 53–57, <http://dx.doi.org/10.3391/ai.2013.8.1.06>
- Higgins SN, Vander Zanden MJ (2010) What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. *Ecological Monographs* 80: 179–196, <http://dx.doi.org/10.1890/09-1249.1>
- Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287: 308–309, <http://dx.doi.org/10.1126/science.287.5451.308>
- Imo M, Seitz A, Johannesen J (2010) Distribution and invasion genetics of the quagga mussel (*Dreissena rostriformis bugensis*) in German rivers. *Aquatic Ecology* 44: 731–740, <http://dx.doi.org/10.1007/s10452-009-9311-2>
- Johnson PD, McMahon RF (1998) Effects of temperature and chronic hypoxia on survivorship of the zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*). *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1564–1572, <http://dx.doi.org/10.1139/f98-030>
- Karatayev AY, Burlakova LE, Mastitsky SE, Padilla DK, Mills EL (2011a) Contrasting rates of spread of two congeners, *Dreissena polymorpha* and *Dreissena rostriformis bugensis*, at different spatial scales. *Journal of Shellfish Research* 30: 923–931, <http://dx.doi.org/10.2983/035.030.0334>
- Karatayev AY, Mastitsky SE, Padilla DK, Burlakova LE, Hajduk MM (2011b) Differences in growth and survivorship of zebra and quagga mussels: size matters. *Hydrobiologia* 668: 183–194, <http://dx.doi.org/10.1007/s10750-010-0533-z>
- Kinzelbach R (1992) The main features of the phylogeny and dispersal of the zebra mussel (*Dreissena polymorpha*). In: Neumann D, Jenner HA (eds), *Zebra Mussel Dreissena Polymorpha – Ecology, Biological Monitoring and First Applications in the Water Quality Management*. Gustav Fischer Verlag, Stuttgart, pp 5–17

- Kleinbaum DG, Klein M (2005) *Survival Analysis: A Self Learning Text*. Springer Science + Business Media, New York, NY
- Lambrinos JG (2004) How Interactions between Ecology and Evolution Influence Contemporary Invasion Dynamics. *Ecology* 85: 2061–2070, <http://dx.doi.org/10.1890/03-8013>
- Lawrence D, Scott G (1982) The determination and use of condition index of oysters. *Estuaries* 5: 23–27, <http://dx.doi.org/10.2307/1352213>
- Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution* 17: 386–391, [http://dx.doi.org/10.1016/S0169-5347\(02\)02554-5](http://dx.doi.org/10.1016/S0169-5347(02)02554-5)
- Lee CE, Posavi M, Charmantier G (2012) Rapid evolution of body fluid regulation following independent invasions into freshwater habitats. *Journal of Evolutionary Biology* 25: 625–633, <http://dx.doi.org/10.1111/j.1420-9101.2012.02459.x>
- Matthews J, Van der Velde G, Bij de Vaate A, Collas FPL, Koopman KR, Leuven RSEW (2014) Rapid range expansion of the invasive quagga mussel in relation to zebra mussel presence in The Netherlands and Western Europe. *Biological Invasions* 16: 23–42, <http://dx.doi.org/10.1007/s10530-013-0498-8>
- Mills EL, Rosenberg G, Spidle AP, Ludyanskiy M, Pligin Y, May B (1996) A review of the biology and ecology of the quagga mussel (*Dreissena bugensis*), a second species of freshwater dreissenid introduced to North America. *American Zoologist* 36: 271–286, <http://dx.doi.org/10.1093/ich/36.3.271>
- Molloy DP, Bij de Vaate A, Wilke T, Giamberini L (2007) Discovery of *Dreissena rostriformis bugensis* (Andrusov 1897) in Western Europe. *Biological Invasions* 9: 871–874, <http://dx.doi.org/10.1007/s10530-006-9078-5>
- Muller JC, Hidde D, Seitz A (2002) Canal construction destroys the barrier between major European invasion lineages of the zebra mussel. *Proceedings of the Royal Society B: Biological Sciences* 269: 1139–42, <http://dx.doi.org/10.1098/rspb.2002.1994>
- Noordhuis R, Simon G, Miguel DP, Maaike M (2014) Wetenschappelijk eindadvies ANT-IJsselmeergebied. [Scientific advice IJsselmeer-Markermeer-IJmeer. A five year research study on ecosystem opportunities to achieve Natura 2000 goals (in Dutch)]. Deltares report 1207767, Delft
- Orlova MI, Theriault TW, Antonov PI, Shcherbina GK (2005) Invasion ecology of quagga mussels (*Dreissena rostriformis bugensis*): a review of evolutionary and phylogenetic impacts. *Aquatic Ecology* 39: 401–418, <http://dx.doi.org/10.1007/s10452-005-9010-6>
- Pearman PB, Guisan A, Broennimann O, Randin CF (2008) Niche dynamics in space and time. *Trends in Ecology & Evolution* 23: 149–158, <http://dx.doi.org/10.1016/j.tree.2007.11.005>
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273–288, <http://dx.doi.org/10.1016/j.ecolecon.2004.10.002>
- Pinheiro J, Bates D, DebRoy S, Sarkar D (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-113 edn.
- Pollux B, Minchin D, Van Der Velde G, Van Alen T, Moon-Van der Staay SY, Hackstein J (2003) Zebra mussels (*Dreissena polymorpha*) in Ireland, AFLP-fingerprinting and boat traffic both indicate an origin from Britain. *Freshwater Biology* 48: 1127–1139, <http://dx.doi.org/10.1046/j.1365-2427.2003.01063.x>
- Prentis PJ, Wilson JR, Dormontt EE, Richardson DM, Lowe AJ (2008) Adaptive evolution in invasive species. *Trends in Plant Science* 13: 288–294, <http://dx.doi.org/10.1016/j.tplants.2008.03.004>
- R-Core-Team (2014) Development Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.
- Rajagopal S, Pollux BJA, Peters JL, Cremers G, Moon-van der Staay SY, van Alen T, Eygensteyn J, van Hoek A, Palau A, de Vaate AB, van der Velde G (2009) Origin of Spanish invasion by the zebra mussel, *Dreissena polymorpha* (Pallas, 1771) revealed by amplified fragment length polymorphism (AFLP) fingerprinting. *Biological Invasions* 11: 2147–2159, <http://dx.doi.org/10.1007/s10530-009-9495-3>
- Roe SL, MacIsaac HJ (1997) Deepwater population structure and reproductive state of quagga mussels (*Dreissena bugensis*) in Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 2428–2433, <http://dx.doi.org/10.1139/f97-151>
- Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution* 22: 454–464, <http://dx.doi.org/10.1016/j.tree.2007.07.002>
- Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009) Evolution and Ecology of Species Range Limits. *Annual Review of Ecology Evolution and Systematics* 40: 415–436, <http://dx.doi.org/10.1146/annurev.ecolsys.110308.120317>
- Stoekmann A (2003) Physiological energetics of Lake Erie dreissenid mussels: a basis for the displacement of *Dreissena polymorpha* by *Dreissena bugensis*. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 126–134, <http://dx.doi.org/10.1139/f03-005>
- Strayer DL (2009) Twenty years of zebra mussels: lessons from the mollusk that made headlines. *Frontiers in Ecology and the Environment* 7: 135–141, <http://dx.doi.org/10.1890/080020>
- Therneau TM (2015) A Package for Survival Analysis in S. Version 2.38 edn.
- Theriault TW, Orlova MI, Docker MF, Macisaac HJ, Heath DD (2005) Invasion genetics of a freshwater mussel (*Dreissena rostriformis bugensis*) in eastern Europe: high gene flow and multiple introductions. *Heredity* 95: 16–23, <http://dx.doi.org/10.1038/sj.hdy.6800691>
- Verhofstad MJM, Grutters BMC, van der Velde G, Leuven RSEW (2013) Effects of water depth on survival, condition and stable isotope values of three invasive dreissenid species in a deep freshwater lake. *Aquatic Invasions* 8: 157–169, <http://dx.doi.org/10.3391/ai.2013.8.2.04>
- Wilson AB, Naish KA, Boulding EG (1999) Multiple dispersal strategies of the invasive quagga mussel (*Dreissena bugensis*) as revealed by microsatellite analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 2248–2261, <http://dx.doi.org/10.1139/f99-162>

The following supplementary material is available for this article:

- Table S1.** The overall mean oxygen saturation for each combination of oxygen level and temperature in the experiment.
- Table S2.** Deviance and likelihood statistics and statistical significance of the factors in the Weibull-survival models.
- Table S3.** Linear regression model explaining the condition index (CI) of experimental mussel populations.
- Table S4.** The mixed effects model explaining the condition index (CI) by species and population origin as nested effects.
- Figure S1.** Environmental oxygen concentrations and water temperatures in studied water bodies.
- Figure S2.** Schematic view and pictures of the experimental setup.
- Figure S3.** Schematic top view of how the oxygen regulating tanks, the water baths and aquaria were arranged.
- Figure S4.** Experimental oxygen levels measured in the aquaria during the course of the experiment.
- Figure S5.** Experimental temperatures measured in the aquaria during the course of the experiment.
- Figure S6.** Mean shell length and estimated mean shell volume for each experimental population (origin, species) and all treatment combinations.
- Figure S7.** Condition index (CI) as linear regression of tissue dry weight by volume for each population of quagga mussels and zebra mussels.

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