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# Developmental plasticity of shell morphology of quagga mussels from shallow and deep-water habitats of the Great Lakes

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## SUMMARY

**The invasive zebra mussel (*Dreissena polymorpha*) has quickly colonized shallow-water habitats in the North American Great Lakes since the 1980s but the quagga mussel (*Dreissena bugensis*) is becoming dominant in both shallow and deep-water habitats. While quagga mussel shell morphology differs between shallow and deep habitats, functional causes and consequences of such difference are unknown. We examined whether quagga mussel shell morphology could be induced by three environmental variables through developmental plasticity. We predicted that shallow-water conditions (high temperature, food quantity, water motion) would yield a morphotype typical of wild quagga mussels from shallow habitats, while deep-water conditions (low temperature, food quantity, water motion) would yield a morphotype present in deep habitats. We tested this prediction by examining shell morphology and growth rate of quagga mussels collected from shallow and deep habitats and reared under common-garden treatments that manipulated the three variables. Shell morphology was quantified using the polar moment of inertia. Of the variables tested, temperature had the greatest effect on shell morphology. Higher temperature (~18–20°C) yielded a morphotype typical of wild shallow mussels regardless of the levels of food quantity or water motion. In contrast, lower temperature (~6–8°C) yielded a morphotype approaching that of wild deep mussels. If shell morphology has functional consequences in particular habitats, a plastic response might confer quagga mussels with a greater ability than zebra mussels to colonize a wider range of habitats within the Great Lakes.**

Key words: biological invasions, bivalve, calcification, common-garden experiment, functional morphology, Great Lakes, moment of inertia, phenotypic plasticity, temperature.

## INTRODUCTION

Following their introduction into North America, zebra (*Dreissena polymorpha* Pallas) and quagga mussels (*Dreissena bugensis* Andrusov) have caused massive declines in native species and have cost an estimated $1 billion annually in removal and control (Pimentel et al., 2005). Of the two species, the zebra mussel has spread more quickly throughout the Great Lakes and inland waterways but the quagga mussel might eventually achieve a more extensive habitat range. Quagga mussels are displacing zebra mussels in shallow-water habitats (Jarvis et al., 2000; Mills et al., 1996; Mills et al., 1999; Stoeckmann, 2003) and have also colonized deep-water habitats (>50 m) in very high numbers. In contrast, zebra mussels have remained more restricted to shallow-water habitats (see Claxton et al., 1998; Mills et al., 1993). While differences in functional morphology between the two species have been hypothesized to affect their distribution (Claxton et al., 1998; Peyer et al., 2009), few comparative studies have examined this potential factor.

Shell morphology is likely to have functional and fitness consequences that limit the distribution of some molluscs (e.g. Bell and Gosline, 1997; Thayer, 1975; Trussell, 1997). For example, shell morphology that minimizes dislodgment from hard substrate was predicted or found to be favored in shallow wave-exposed habitats for the marine mussel *Mytilus californianus* (Bell and Gosline, 1997) and the marine snail *Littorina obtusata* (Trussell, 1997). In contrast, shell morphology of various species of bivalves and brachiopods might be adapted to soft sedimentary substrates in deep-water habitats in order to prevent sinking or to facilitate burrowing (Thayer, 1975).

Striking differences in shell morphology exist between shallow and deep-water populations of quagga mussels (Fig. 1) (Claxton et al., 1998; Dermott and Munawar, 1993; Spidle et al., 1994). Deep quagga mussels (Fig. 1A) have a morphotype in which the shells are more laterally flattened and ovular in shape than those from shallow-water habitats (Fig. 1B) (Dermott and Munawar, 1993; Claxton et al., 1998). In contrast, shallow quagga mussels (Fig. 1B) have a morphotype that largely resembles that of zebra mussels (Fig. 1C) [e.g. ratio of shell height to shell width (Claxton et al., 1998)]. Such difference in quagga mussel shell morphology between the two habitats might be induced during development in response to environmental variables (i.e. phenotypic plasticity) (see Claxton et al., 1998) or might result from genetic differentiation between the populations. Lack of genetic differentiation between shallow and deep populations of quagga mussels, based on mitochondrial COI sequences (Claxton et al., 1998) and microsatellite allele frequencies (C.E.L. and G. W. Gelembiuk, unpublished data), suggest that phenotypic plasticity might be responsible for the morphological difference. However, this hypothesis has never been tested.

Shallow and deep-water habitats of the Great Lakes differ in a number of environmental variables, such as temperature, food quantity, water motion, substrate, light and pressure. Of these variables, temperature (Aguirre et al., 2006; Trussell and Etter, 2001), food quantity (e.g. Seed, 1968) and water motion (i.e. velocity, wave impact, turbulence) (Aguirre et al., 2006; Fox and

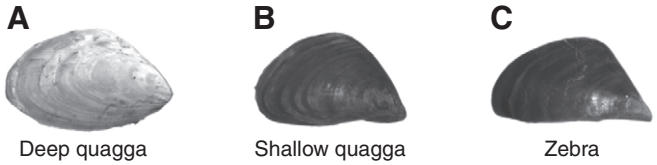


Fig. 1. Lateral shell views of (A) deep quagga, (B) shallow quagga and (C) zebra mussels.

Coe, 1943; Selin and Vekhova, 2003; Steffani and Branch, 2003) have been thought to affect shell morphology and growth of molluscs (e.g. *Brachidontes* sp., *L. obtusata*, *Mytilus edulis*, *M. californianus*, *Crenomitilus grayanus*, *Mytilus galloprovincialis*).

For example, temperature in shallow habitats varies seasonally but typically ranges between 8°C (Mills et al., 1993) and 25°C (Csanady, 1974) where both zebra and quagga mussels are found (e.g. Oswego Harbor, Lake Ontario, NY, USA). Deeper habitats colonized by quagga mussels (e.g. Olcott, Lake Ontario, NY, USA) are colder, typically in the range of 4–8°C (Mills et al., 1993). In addition, throughout the year, food concentration tends to be ~2 times higher in shallow (e.g. chlorophyll *a*: ~1–2.5 $\mu g\,l^{-1}$; water protein: 20–160 $\mu g\,l^{-1}$; 1.5 m depth in Lake Erie, North America) than in deep habitats (e.g. chlorophyll *a*: ~0.2–1.5 $\mu g\,l^{-1}$; water protein: 10–70 $\mu g\,l^{-1}$; 12–23 m depth in Lake Erie, North America) (Claxton and Mackie, 1998). Finally, shallow habitats tend to experience greater and more variable water velocities, with mean flow speeds of 8–20 $cm\,s^{-1}$ (Oswego Harbor, Lake Ontario, NY, USA), while deeper habitats are calmer and more constant, typically having flow speeds of 4 $cm\,s^{-1}$ (Olcott, Lake Ontario, NY, USA) (Csanady, 1974).

The goal of this study was to examine whether temperature, food quantity and water motion could induce plastic responses in shell morphology during development of quagga mussels collected from shallow and deep-water habitats of the Great Lakes. We reared juvenile mussels collected from both shallow and deep-water habitats in a laboratory common-garden experiment in which we manipulated the three environmental variables in a partial factorial design. Throughout the experiment, we quantified shell morphology and growth rate of the developing mussels reared under the different treatment conditions. We quantified shell morphology with the polar moment of inertia, which is a measurement that characterizes the distribution of the area of an object. We used the polar moment of inertia rather than other morphometric methods (e.g. landmark, Fourier), because it can be used as a surrogate for the mass moment of inertia. The mass moment of inertia is used extensively in physical models (see Hibbeler, 1989); therefore, we can directly examine the functional consequences of shell morphology that might affect a mussel's interaction with different substrates in shallow *versus* deep-water habitats (e.g. see Discussion).

If phenotypic plasticity contributes to the difference in quagga mussel shell morphology, we would expect juvenile quagga mussels collected from both shallow and deep-water habitats to develop a morphotype approaching that of (1) wild shallow quagga mussels (from <2 m depth), when reared under shallow-water conditions (high temperature, food quantity and water motion) and (2) wild deep quagga mussels (from >50m depth), when reared under deep-water conditions (low temperature, food quantity and water motion). Alternatively, the difference in shell morphology between shallow and deep quagga mussel populations might arise from genetic differences between populations at loci that affect morphology. In such a case, we would expect quagga mussels from shallow and deep-water habitats to resemble the morphotypes that are characteristic of their original habitats, regardless of the environmental conditions under which they were reared.

## MATERIALS AND METHODS

### Population sampling

We collected quagga mussels from two locations in Lake Ontario, North America. Shallow quagga mussels were collected in July 2006 from rocky substrate (1–2 m depth) in Oswego Harbor, NY, USA (~43°47′N, 76°49′W). We randomly sampled and removed mussels from rocks by cutting their byssal threads with a sharp knife. Deep quagga mussels were collected in April 2005 from Thirty Mile Point, NY, USA (43°24′N, 78°33′W) with a motor-driven ponar grab (65 m depth). After collection, mussels were wrapped in damp paper towels, sealed in plastic bags and placed on ice during transport. In the laboratory, mussels were temporarily housed in aquaria at ~6–8°C. For this experiment we used water collected from Racine Harbor, WI, USA, in Lake Michigan where both zebra and quagga mussels occur.

### Effects of temperature, food quantity and water motion on shell morphology

We used a laboratory common-garden experiment to test whether variation in shell morphology of quagga mussels could be induced during development in response to environmental variables of temperature, food quantity and water motion. We reared juvenile mussels (≤5 mm length; <1 year in age) from both shallow and deep-water habitats under identical treatment conditions. The treatments approximated field conditions where shallow-water habitats tend to have higher mean temperature, food quantity and water motion than deep-water habitats (>50 m). We reared mussels for two to three years under the treatment conditions. During this time, we fed mussels a commercial shellfish diet (*Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weissflogii*) from Reeds Mariculture, Inc. (Campbell, CA, USA). This diet was used previously to maintain zebra (Vanderploeg et al., 1996; Peyer et al., 2009) and quagga mussels (Peyer et al., 2009).

Our common-garden experiment consisted of rearing mussels collected from both shallow and deep-water habitats under four treatments that formed a partial factorial design (Table 1). For each treatment we reared 100 mussels each from shallow and deep-water habitats, equally distributed among ten 2-l beakers (i.e. 10 mussels per beaker) with rocky substrate typical of shallow-water habitats. The experimental variables were as follows: (1) temperature – temperature range was ~18–20°C for the high treatment and ~6–8°C for the low treatment. (2) Food quantity – we fed mussels in the high food treatment to saturation 3–4 days per week. During the first 6 months of growth, we fed mussels in each 2-l beaker 1–2 drops of food using a standard pipette. Each drop had a concentration of ~2 billion cells $ml^{-1}$. As the mussels grew, we increased this amount by 1 drop of food for every 6-month period of growth. We

Table 1. Experimental treatments of the laboratory common-garden experiment

| Treatment | Experimental conditions | Manipulated variable (relative to Treatment A) |
|---|---|---|
| A | 18–20°C$^S$, high food$^S$, 1 kPa$^D$ | Control |
| B | 6–8°C$^D$, high food$^S$, 1 kPa$^D$ | Temperature |
| C | 18–20°C$^S$, low food$^D$, 1 kPa$^D$ | Food quantity |
| D | 18–20°C$^S$, high food$^S$, 10 kPa$^S$ | Water motion |

Temperature, food quantity and water motion typical of shallow (S) and deep (D) water habitats are denoted by superscripts *S* and *D*, respectively.

fed mussels in the low food treatment 1/3 of the amount given to mussels in the high food treatment. (3) Water motion – to simulate turbulence, we used vigorous aeration (~10 kPa) for the high water motion treatment and calm aeration (~1 kPa) for the low water motion treatment.

We designated Treatment A (high temperature, high food quantity, low water motion) as an arbitrary control, to which we compared each of the other treatments. Each of the other Treatments B–D differed from Treatment A in only one variable (Table 1). We expected mussels reared at either Treatment A or D (high temperature, high food quantity, high water motion) to most resemble shallow quagga mussels. We did not include treatment conditions that deep quagga mussels would tend to experience in the wild (i.e. low temperature, low food quantity, low water motion), because growth rate would have been exceedingly slow under such conditions. Of the four treatments, we expected mussels reared at either Treatment B (low temperature, high food quantity, low water motion) or Treatment C (high temperature, low food quantity, low water motion) to most resemble deep quagga mussels. Photoperiod was constant across treatments with 15 h fluorescent light and 9 h dark exposure, resembling lighting conditions in shallow-water habitats.

### Quantifying shell morphology and growth rate

We quantified shell morphology and calculated growth rate of laboratory-reared mussels to determine their response to treatment conditions (Table 1). We used a Dragonfly IEEE-1394 digital camera from Point Grey Research (Vancouver, BC, Canada) to capture images of the lateral shell view (Fig. 1) of each mussel. To quantify shell morphology, we used IMAQ programming software for LabVIEW (National Instruments, 2003; Austin, TX, USA) and obtained the polar moment of inertia. The polar moment of inertia is a geometrical measurement that describes the distribution of the area of an object about a particular axis. The polar moment of inertia, $J_{zz}$ about the $z$-axis at the shell centroid is defined by:

$$J_{zz} = \int_A r^2\, dA\ , \qquad (1)$$

where $r$ is the distance from the centroid to a differential element of shell area, $dA$ (see Beer and Johnston, 1981). $J_{zz}$ describes the distribution of the shell area about the $z$-axis (Fig. 2). Thus, shell morphology affects $J_{zz}$ of mussels of a given shell area. In our LabVIEW program, we used the perpendicular axis theorem and calculated $J_{zz}$ as the sum of the moments of inertia, $I_{xx}$ and $I_{yy}$ (Fig. 2). For our two-dimensional image of a mussel, $I_{xx}$ and $I_{yy}$ are the distribution of the shell area about the principal $x$- and $y$-axes, respectively. The origin of the $x$- and $y$-axes is located at the centroid of the shell area. $I_{xx}$ and $I_{yy}$ are defined by the equations:

$$I_{xx} = \int_A y^2\, dA \qquad (2)$$

and

$$I_{yy} = \int_A x^2\, dA\ , \qquad (3)$$

respectively, where $y$ and $x$ are the distances from the $x$- and $y$-axes to $dA$.

Images of lateral shell views from the four laboratory treatments were captured at the beginning and end of the experiment to determine initial and final shell morphology. We calculated growth rate of each individual developing mussel as the difference between the final and initial shell areas divided by the total experimental time period. Unique patterns of stripes or coloration on the shells of each developing mussel allowed us to track individuals over time. To compare shell morphology between wild and laboratory-reared mussels, we also captured images of the lateral shell view of wild quagga mussels of various size (5–30 mm length), for 60 individuals collected each from shallow and deep-water habitats. We weighed wild mussels after collection and laboratory-reared mussels after two to three years of growth during the common-garden experiment to determine whether morphology changed as a function of mass.

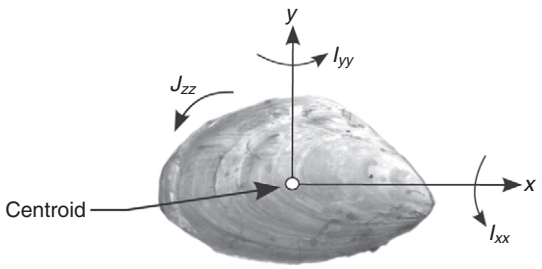


Fig. 2. Moments of inertia ($I_{xx}$ and $I_{yy}$) about the $x$- and $y$-axes, and polar moment of inertia ($J_{zz}$) about the $z$-axis at the shell centroid. Moments of inertia are the distributions of shell areas about their respective $x$-, $y$- or $z$-axes. $J_{zz}$ is the sum of the moments of inertia, $I_{xx}$ and $I_{yy}$.

### Data analysis

We used an analysis of covariance (ANCOVA), using the statistical package R (R Development Core Team, 2008), to test whether the polar moment of inertia depended on (1) the habitat from which the mussels were collected (i.e. shallow, deep), (2) mussel mass and (3) the environmental condition to which the mussels were exposed during development (i.e. wild, laboratory Treatments A–D). We were interested in whether the polar moment of inertia differed between wild and laboratory-reared mussels. We also were interested in whether the polar moment of inertia of laboratory-reared mussels differed between the control Treatment A and each of the other three Treatments B–D (Table 1). Our model for the polar moment of inertia, $J_{zz}$ as the dependent variable was:

$$J_{zz} = \beta_0 + \beta_1 x_h + \beta_2 x_m + \beta_3 x_{w/t}\,, \qquad (4)$$

where independent variables were quagga mussel habitat in the wild ($x_h$), mussel mass ($x_m$) and environmental condition (i.e. wild, laboratory treatment) under which the mussels developed ($x_{w/t}$). Maximum likelihood parameter estimates were represented by $\beta_0$–$\beta_3$. We also used an ANCOVA to test whether the polar moment of inertia as a function of mussel mass differed among replicates within each experimental treatment. We used the model:

$$J_{zz} = \beta_0 + \beta_1 x_r + \beta_2 x_m\,, \qquad (5)$$

where independent variables were treatment replicate ($x_r$) and $x_m$. Maximum likelihood parameter estimates were represented by $\beta_0$–$\beta_2$. All variables were treated as fixed effects. Mussel mass was raised to the 4/3 power in order to generate linear relationships with the polar moment of inertia.

We used a generalized linear model in R to test for differences in growth rate of shallow and deep quagga mussels reared under the control Treatment A *versus* each of the other three Treatments B–D. The maximum likelihood model for growth rate, $G_r$ as the dependent variable was:

$$G_r = \beta_0 + \beta_1 x_h + \beta_2 x_t\,, \qquad (6)$$

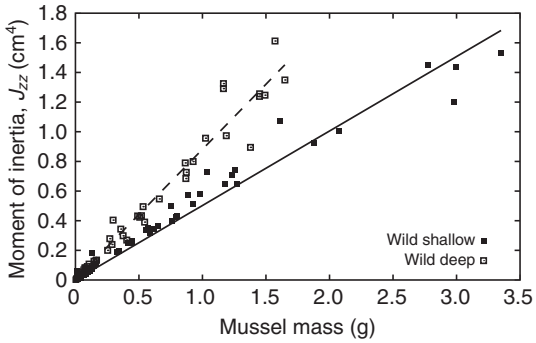


Fig. 3. Polar moment of inertia, $J_{zz}$, in response to mass ($x_m$) of wild quagga mussels collected from shallow ($J_{zz}$=0.48·$x_m$) and deep-water habitats ($J_{zz}$=0.88·$x_m$) ($N$=60 per morphotype). Mussel mass was raised to the 4/3 power.

where independent variables were $x_h$ and treatment condition from our laboratory common-garden experiment ($x_t$). Maximum likelihood parameter estimates were represented by $\beta_0$–$\beta_2$. We used an analysis of variance to test whether growth rate differed among replicates within each experimental treatment. All variables were treated as fixed effects.

## RESULTS

### Shell morphology of shallow *versus* deep quagga mussels

We found significant differences between shell morphology of wild populations of quagga mussels from shallow and deep-water habitats (Fig. 3; Table 2). The polar moment of inertia, our descriptor of shell morphology, increased significantly with increasing mussel mass (relative to zero slope) and was lower for wild shallow than for wild deep mussels (Fig. 3; Table 2 Comparison 1; Eqn 4). Thus, wild deep mussels, with higher polar moment of inertia, had higher distribution of shell area than wild shallow mussels of a given mass.

Of the variables tested in our common-garden experiment, temperature was the most important determinant of morphology, as mussels collected from both shallow and deep-water habitats developed a morphotype typical of wild shallow mussels when reared at high temperature (Table 1 Treatments A, C, D) but not at low temperature (Table 1 Treatment B). Values for polar moment of inertia for mussels reared at the four Treatments A–D typically fell between those of wild shallow and wild deep mussels (Figs 4, 5).

Quagga mussels reared at the control (Treatment A: high temperature, high food quantity, low water motion) developed a morphotype with lower polar moment of inertia, resembling wild shallow mussels (Fig. 4A, Fig. 5A). Mussels collected from both shallow and deep habitats and reared at Treatment A did not differ significantly in polar moment of inertia from that of wild shallow mussels of similar mass (Table 2 Comparisons 2, 3; Eqn 4).

### Temperature (Treatments B *versus* A)

Temperature level significantly affected shell morphology of developing quagga mussels. Mussels reared at low temperature (Treatment B) approached the higher polar moment of inertia of wild deep mussels (Fig. 4B, Fig. 5B). Mussels collected from both shallow and deep habitats and reared at low temperature (Treatment B; ~6–8°C), characteristic of deep water, developed significantly higher polar moment of inertia than those of similar mass reared at high temperature (Treatment A; ~18–20°C) (Table 2 Comparisons 4, 5A; Eqn 4). These mussels reared at low temperature also developed significantly higher polar moment of inertia than that of wild shallow mussels of similar mass (Table 2 Comparisons 6, 7; Eqn 4; Fig. 4A *versus* Fig. 4B, Fig. 5A *versus* Fig. 5B). One outlier mussel collected from the deep habitat and reared at low temperature was from the only replicate in our common-garden experiment that differed significantly from other replicates within a given treatment (with outlier: Student's $t$=2.30, $P$=0.025; without outlier: Student's $t$=0.79, $P$=0.43; Eqn 5). This mussel grew to a greater extent than other mussels (Fig. 5B at $x$=0.52, $y$=0.36). When we removed this outlier, mussels collected from the deep habitat and reared at low temperature (Treatment B) were still significantly higher in polar moment of inertia than mussels reared at high temperature (Treatment A) (Table 2 Comparison 5B; Eqn 4).

Table 2. Statistical comparisons of polar moment of inertia of wild quagga mussels and of quagga mussels collected as juveniles and reared at Treatments A–D in the common-garden experiment (see Table 1; Eqn 4; d.f.=728)

| Comparison | Student's $t$ | $P$-value |
|---|---|---|
| (1) Wild shallow *versus* wild deep | 39.4 | <0.00001 |
| (2) Treatment A shallow *versus* wild shallow | 1.14 | 0.26 |
| (3) Treatment A deep *versus* wild shallow | –0.68 | 0.49 |
| (4) Treatment B shallow *versus* Treatment A shallow | 2.14 | 0.032 |
| (5A) Treatment B deep *versus* Treatment A deep | 2.88 | 0.0041 |
| (5B) Outlier removed in 5A | 2.11 | 0.035 |
| (6) Treatment B shallow *versus* wild shallow | 4.12 | <0.00001 |
| (7) Treatment B deep *versus* wild shallow | 3.13 | 0.0018 |
| (8) Treatment B shallow *versus* wild deep | –6.56 | <0.00001 |
| (9) Treatment B deep *versus* wild deep | –6.57 | <0.00001 |
| (10) Treatment C shallow *versus* Treatment A shallow | –0.22 | 0.83 |
| (11) Treatment C deep *versus* Treatment A deep | 0.53 | 0.59 |
| (12) Treatment C shallow *versus* wild shallow | 0.009 | 0.99 |
| (13) Treatment C deep *versus* wild shallow | 0.39 | 0.70 |
| (14) Treatment D shallow *versus* A shallow | 0.37 | 0.71 |
| (15) Treatment D deep *versus* A deep | 1.84 | 0.066 |
| (16) Treatment D shallow *versus* wild shallow | 2.32 | 0.021 |
| (17) Treatment D deep *versus* wild shallow | 2.14 | 0.033 |
| (18) Treatment D shallow *versus* wild deep | –13.1 | <0.00001 |
| (19) Treatment D deep *versus* wild deep | –13.5 | <0.00001 |

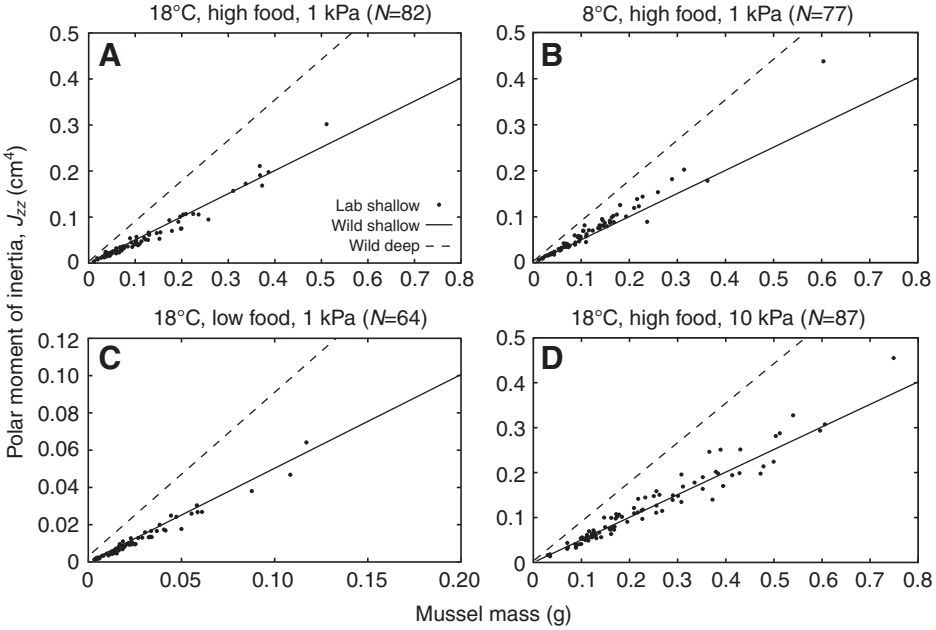


Fig. 4. Polar moment of inertia ($J_{zz}$) in response to mass ($x_m$) of quagga mussels collected from shallow-water habitats and reared under treatment conditions (see Table 1). Regressions of mussels reared under each treatment were (A) $J_{zz}=0.52\cdot x_m$, (B) $J_{zz}=0.63\cdot x_m$, (C) $J_{zz}=0.48\cdot x_m$ and (D) $J_{zz}=0.54\cdot x_m$. Regressions of wild shallow ($J_{zz}=0.48\cdot x_m$) and wild deep quagga mussels ($J_{zz}=0.88\cdot x_m$) are shown for comparison (see Fig. 3). Mussel mass was raised to the 4/3 power. Note that scales for *x*- and *y*-axes of Treatment C differ from other treatments.

### Food quantity (Treatments C *versus* A)

Level of food quantity did not have a significant effect on development of shell morphology of quagga mussels. Mussels collected from both shallow and deep habitats and reared at low food quantity (Treatment C), characteristic of deep-water habitats, did not differ significantly in polar moment of inertia from that of mussels of similar mass reared at high food quantity (Treatment A) (Table 2 Comparisons 10, 11; Eqn 4). Thus, low food conditions characteristic of deep habitats failed to induce a deep-water morphotype (Fig. 4A *versus* Fig. 4C, Fig. 5A *versus* Fig. 5C).

### Water motion (Treatments D *versus* A)

Level of water motion did not significantly affect development of shell morphology of mussels collected from shallow or deep habitats. Mussels collected from shallow and deep habitats and reared at high water motion (Treatment D), characteristic of turbulent shallow habitats, did not differ significantly in polar moment of inertia from that of mussels of similar mass reared at low water motion (Treatment A) (Table 2 Comparisons 14, 15; Eqn 4). While mussels collected from shallow and deep habitats and reared at high water motion (Treatment D) were significantly higher in polar moment of inertia than that of wild shallow mussels of similar mass (Table 2 Comparisons 16, 17; Eqn 4), they still approached the lower polar moment of inertia of wild shallow mussels more than that of wild deep mussels (Table 2 Comparisons 18, 19; Eqn 4; Fig. 4A *versus* Fig. 4D, Fig. 5A *versus* Fig. 5D).

### Growth rate of laboratory-reared mussels

Growth rate of mussels collected from shallow and deep habitats differed significantly between the control (Treatment A) and each of the other Treatments B–D in our common-garden experiment (Tables 3, 4; Eqn 6). Relative to the control with high temperature (Treatment A), growth rate at low temperature (Treatment B) was significantly lower for mussels collected from deep habitats (Table 4 Comparison 2) but did not differ significantly for mussels collected from shallow habitats (Table 4 Comparison 1). At low food quantity (Treatment C), growth rate was significantly lower than that of the control with high food quantity (Treatment A) for mussels collected from both shallow and deep habitats (Table 4 Comparisons 3, 4). At high water motion (Treatment D), growth rate was significantly higher than that of the control with low water motion (Treatment A) for mussels collected from both shallow and deep habitats (Table 4 Comparisons 5, 6).

## DISCUSSION

We found that quagga mussel shell morphology exhibited a significant plastic response to temperature, regardless of food quantity or level of water motion. Variation in shell morphology might have functional consequences for quagga mussels across diverse habitats. Thus, developmental plasticity of quagga mussel shell morphology in response to different environmental conditions might be an important trait that facilitates their colonization of shallow and deep-water habitats within the Great Lakes, influencing their competition with zebra mussels.

Table 3. Growth rate with standard error of the mean (s.e.m.) of quagga mussels reared in four common-garden treatments

| Treatment | Shallow quagga growth rate ($mm^2\,month^{-1}$) | Deep quagga growth rate ($mm^2\,month^{-1}$) |
|---|---|---|
| A (18°C, high food, 1 kPa) | 1.1 (s.e.m.=0.087; *N*=77) | 1.8 (s.e.m.=0.074; *N*=72) |
| B (**8°C**, high food, 1 kPa) | 1.3 (s.e.m.=0.0068; *N*=96) | 1.5 (s.e.m.=0.0067; *N*=87) |
| C (18°C, **low food**, 1 kPa) | 0.46 (s.e.m.=0.042; *N*=64) | 0.48 (s.e.m.=0.045; *N*=63) |
| D (18°C, high food, **10 kPa**) | 2.3 (s.e.m.=0.10; *N*=82) | 2.1 (s.e.m.=0.089; *N*=67) |

The bold font of Treatments B–D indicates the variable that differs from Treatment A.

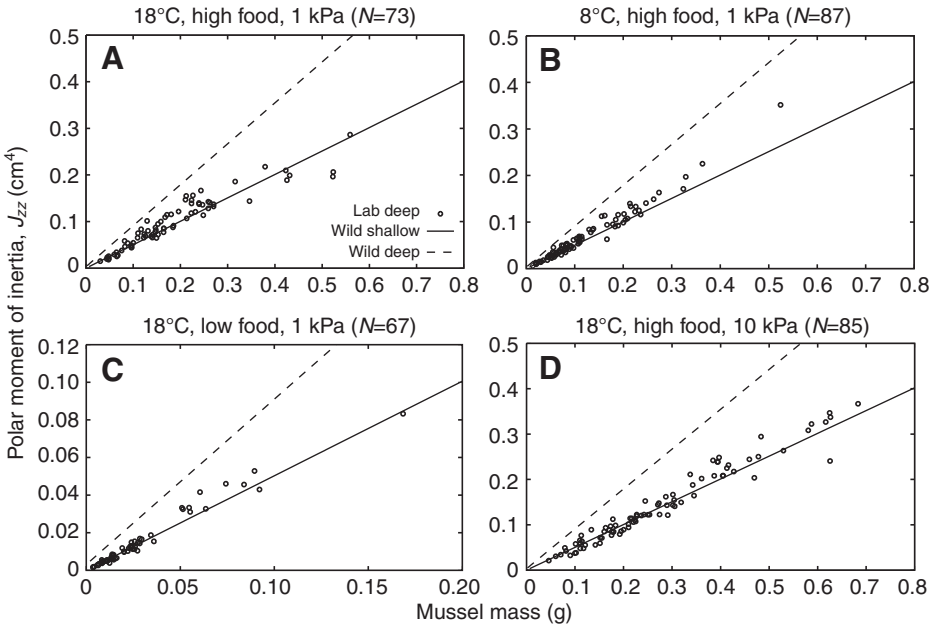


Fig. 5. Polar moment of inertia ($J_{zz}$) in response to mass ($x_m$) of quagga mussels collected from deep-water habitats and reared under treatment conditions (see Table 1). Regressions of mussels reared under each treatment were (A) $J_{zz}=0.46\cdot x_m$, (B) $J_{zz}=0.61\cdot x_m$, (C) $J_{zz}=0.54\cdot x_m$ and (D) $J_{zz}=0.54\cdot x_m$. Regressions of wild shallow ($J_{zz}=0.48\cdot x_m$) and wild deep quagga mussels ($J_{zz}=0.88\cdot x_m$) are shown for comparison (see Fig. 3). Mussel mass was raised to the 4/3 power. Note that scales for *x*- and *y*-axes of Treatment C differ from other treatments.

### Morphological plasticity in response to temperature, food quantity and water motion

We tested whether a difference in shell morphology between shallow and deep quagga mussel populations could be induced by developmental plasticity in response to three environmental variables. Our results showed support for developmental plasticity as a mechanism of morphological divergence (Figs 4 and 5). In addition, quagga mussels from shallow and deep-water habitats were not genetically differentiated based on mitochondrial COI sequences (Claxton et al., 1998) or allele frequencies at five microsatellite loci (C.E.L. and G. W. Gelembiuk, unpublished data), suggesting that the populations are not genetically subdivided. However, there still could be genetically based morphological (quantitative genetic) variation that contributes to the morphological divergence between shallow and deep populations.

Of the three environmental variables examined in our study, temperature was a key factor that induced plasticity of quagga mussel shell morphology. Most strikingly, juvenile mussels collected from both shallow and deep-water habitats developed lower polar moment of inertia, resulting in a morphotype typical of wild shallow quagga mussels, when reared at a high temperature, typical of the shallow-water habitats (Fig. 4A,C,D and Fig. 5A,C,D). It was only when reared at low temperature, typical of deep-water habitats, that quagga mussels collected from both shallow and deep-water habitats developed a higher polar moment of inertia that approached a morphotype typical of wild deep quagga mussels (Fig. 4B and Fig. 5B). The significant effect of temperature on shell morphology was apparent even after removal of an outlier in the low temperature treatment for quagga mussels collected from the deep habitat. This outlier mussel grew to a greater extent than the others and was most similar in polar moment of inertia to wild deep quagga mussels (Fig. 5B at $x$=0.52, $y$=0.36). After removal of this outlier, quagga mussels reared at low temperature (Treatment B; ~6–8°C) still showed a significant plastic response to temperature, developing significantly higher polar moment of inertia than mussels reared at high temperature (Treatment A; ~18–20°C).

Although quagga mussels reared at low temperature showed a significant plastic response, they did not generate as high a polar moment of inertia as that of wild deep quagga mussels (Fig. 4B, Fig. 5B; Table 2 Comparisons 8, 9; Eqn 4). Several confounding factors might have affected the development of shell morphology in the laboratory. For example, field temperatures of deep-water habitats are slightly colder (~4–8°C) (Mills et al., 1993) than the lowest temperatures that we used in our common-garden experiment (~6–8°C). Quagga mussels might be able to survive at even lower temperatures of 0.5–3°C, although with very limited growth and development (see Karatayev et al., 1998). We also eliminated the treatment that was most likely to characterize deep-water habitats, of low levels of temperature, food quantity and water motion, under which growth of mussels would have been excessively slow. However, shell morphology under these rearing conditions might have more definitively resembled that of wild deep quagga mussels, especially if there were any additive effects of low temperature and low food quantity. Because our experiment used juvenile mussels (≤5 mm in length) that were collected from the field, rather than larvae hatched in the laboratory, we cannot be certain that other

Table 4. Statistical comparisons of growth rate of quagga mussels collected as juveniles and reared at Treatment A *versus* Treatments B–D in the common-garden experiment (see Table 1; Eqn 6; d.f.=600)

| Comparison | Student's *t* | *P*-value |
|---|---|---|
| (1) Treatment B shallow *versus* Treatment A shallow | 1.74 | 0.082 |
| (2) Treatment B deep *versus* Treatment A deep | –3.68 | 0.00026 |
| (3) Treatment C shallow *versus* Treatment A shallow | –5.82 | <0.00001 |
| (4) Treatment C deep *versus* Treatment A deep | –11.83 | <0.00001 |
| (5) Treatment D shallow *versus* Treatment A shallow | 11.11 | <0.00001 |
| (6) Treatment D deep *versus* Treatment A deep | 2.27 | 0.024 |

variables, including food quantity and water motion, would not have served as cues affecting shell morphology during early development.

Growth rate has been thought to affect shell morphology of mussels [e.g. *M. edulis* (Seed, 1968)] but we did not find this to be the case with quagga mussels. In our laboratory common-garden experiment, mussels reared at the different treatments grew at significantly different rates, which were lower at low food quantity (Treatment C) than at high food quantity (Treatments A, B, D). Among the high food treatments, growth rate was highest when mussels were reared at high water motion (Treatment D), possibly because of more efficient food delivery with increased turbulence. Such differences in growth rate of mussels among treatments did not affect shell morphology of quagga mussels, at least for the duration of our study of two to three years. For example, quagga mussels reared at high (Fig. 4A, Fig. 5A) and low food quantity (Fig. 4C, Fig. 5C), with constant temperature and water motion, tended to follow the same developmental trajectory of shell morphology in spite of significant differences in growth rate.

### How temperature might induce morphological plasticity

Although we found temperature to be a likely environmental variable that affects shell morphology of quagga mussels, the mechanisms by which temperature might affect shell morphology are not entirely known. Temperature affects a number of biological processes that might induce morphological change. However, the effects of these processes on shell morphology would need to be specifically examined.

Variation in shell morphology might arise from diverse crystal morphologies, which are thought to depend, in part, on the rate of temperature-induced crystal growth (Howe and Marshall, 2002; Wada, 1961; Watabe and Wilbur, 1966; Wilbur and Saleuddin, 1983). In addition, physiological processes involved in mineralization of mussel shells, including respiration (Barbariol and Razouls, 2000; Stoeckmann, 2003), filtration (Karatayev et al., 1998) and growth rates (Fox and Coe, 1943; Karatayev et al., 1998; Mestre et al., 2009), have generally been found to decline with decreasing temperature. Temperature has also served as a proximate cue for morphological change in other species (e.g. Appleby and Credland, 2007) and might serve as a reliable cue for inducing an adaptive morphotype in quagga mussels if it is correlated with the critical variable that differs between shallow and deep-water habitats. Finally, shell morphology of quagga mussels might be affected by byssal thread production, as a poorly developed muscle associated with byssal thread attachment might affect mussel shell morphology (Selin and Vekhova, 2003). Temperature could have an indirect effect on shell morphology given that byssal thread production decreases with temperature in zebra mussels (Clarke and McMahon, 1996).

### Functional consequences of shell morphology

Different quagga mussel morphotypes in the wild might have functional consequences in shallow *versus* deep-water habitats; however, such studies have yet to be performed. Quagga mussels in deep-water habitats have been hypothesized to be adapted to living on soft sedimentary substrates, because their low density and elongated shells might prevent them from sinking into such substrates (Claxton et al., 1998). A morphotype that facilitates locomotion might also be beneficial for quagga mussels in deep-water habitats (e.g. see Thayer, 1975) where they have adopted an infaunal lifestyle (Dermott and Munawar, 1993). Zebra mussels are known to move in response to environmental conditions (Kobak, 2001; Toomey et al., 2002; Burks et al., 2002). Shell morphology might affect such movement and have important consequences for habitat selection and survival of both zebra and quagga mussels. The polar moment of inertia as a descriptor of shell morphology can serve as a surrogate for the mass moment of inertia (assuming uniform mussel density). The mass moment of inertia is a parameter in equations of motion (Hibbeler, 1989) and can be used to describe a mussel's resistance to rotational acceleration, providing a direct way to determine the effect of shell morphology on locomotion.

### Future studies involving morphological plasticity and quagga mussel range expansion

Aside from the variables we examined in this study, shallow and deep-water habitats differ in other environmental variables that might contribute to the difference in shell morphology of quagga mussels. Substrate type differs between shallow and deep-water habitats and might be a cue for morphological change. Differences in shell morphology have been described in molluscs (e.g. *Brachidontes* sp., *Mya arenaria*, *Crenomitilus grayanus*, *Mytilids*) (see Aguirre et al., 2006; Newell and Hidu, 1982; Selin and Vekhova, 2003; Stanley, 1972). Deep-water habitats also have low light level and high pressure relative to shallow-water habitats. Little is known about the effect of sunlight on calcium carbonate deposition in mollusc shells. However, research has revealed the importance of vitamin D, which can be synthesized by exposure to sunlight, on calcium absorption for bone health in humans and other vertebrates (Blunt and Cowan, 1930; Mark et al., 2008; Webb and Holick, 1988; Wolff et al., 2008), and on spicule formation in the gorgonian coral, *Leptogorgia virgulata* (Kingsley et al., 2001). The effect of pressure on shell morphology of quagga mussels also has not been examined but has been shown to affect growth and normal development of the blue mussel, *M. edulis*, except at a low temperature of 5°C (Mestre et al., 2009).

While a number of environmental variables are likely to affect shell morphology of quagga mussels, our results indicated that quagga mussels could achieve significantly different morphotypes through developmental plasticity by manipulating temperature alone. If greater variation in shell morphology has functional benefits, implications for quagga mussel habitat expansion might be serious. Context-dependent and environmentally induced changes in shell morphology might facilitate their colonization of shallow and deep-water habitats. Although developmental plasticity of zebra mussel shell morphology has not been tested, thus far, only a shallow-water morphotype has been described. Whether morphological differences have fitness consequences in the wild has yet to be determined. Subsequent studies that focus on functional morphology might reveal whether morphological variation between shallow and deep-water quagga mussels (e.g. through developmental plasticity) could enable them to colonize a broader range of habitats and impact their competition with zebra mussels.

### LIST OF ABBREVIATIONS

| | |
|---|---|
| $A$ | shell area |
| $dA$ | differential element of shell area |
| $G_r$ | growth rate of mussels |
| $I_{xx}$ | moment of inertia about $x$-axis |
| $I_{yy}$ | moment of inertia about $y$-axis |
| $J_{zz}$ | polar moment of inertia about $z$-axis |
| $r$ | distance from centroid of shell area to $dA$ |
| $x$ | distance from $y$-axis to $dA$ |
| $x_h$ | quagga mussel habitat in wild |
| $x_m$ | mussel mass |
| $x_r$ | treatment replicate |

| | |
|---|---|
| $x_t$ | treatment condition from laboratory common-garden experiment |
| $x_{w/t}$ | condition under which the mussels developed (i.e. wild, laboratory treatment) |
| $y$ | distance from $x$-axis to $dA$ |
| $\beta_0$–$\beta_3$ | maximum likelihood parameter estimates |

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