

The effects of a harmful alga on bivalve larval lipid stores

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ABSTRACT

Marine invertebrates often have complex life histories that include a swimming planktivorous larval stage, at which time they are vulnerable to a variety of stressors, including those associated with nutritional stress and harmful algal blooms. Lipid stores have been shown to be especially important for post-metamorphic survivorship and growth in a variety of marine invertebrates. We investigated the effects of the harmful brown tide alga *Aureococcus anophagefferens* on the lipid stores and growth of larvae of the hard clam (northern quahog, *Mercenaria mercenaria*), a dominant bivalve in many western Atlantic bays and estuaries. *M. mercenaria* was the dominant bivalve in Great South Bay, Long Island, until the mid-1970s, but very few larvae are presently found in these waters. Recent brown tide blooms have been hypothesized to pose a barrier to recovery of *M. mercenaria* populations and hinder recent restoration efforts by negatively affecting clam larvae. To test whether a diet of the brown tide alga affects the accumulation of beneficial lipid stores, we fed larvae one of three diets representing equal biovolumes of *Isochrysis galbana*, a nutritious control alga; *A. anophagefferens*, the brown tide alga for which nutritional quality is not presently known; or a mixture of the two. Larvae fed only brown tide had significantly less lipid stores than those in the other dietary treatments. In addition, brown tide negatively affected larval size. We also tested for evidence of tradeoffs between larval growth and lipid stores, predicting that when the diet was less nutritious as in the brown tide treatments, larval size and lipids would be negatively correlated. In contrast, we found that larvae fed a mixed algal diet or only *A. anophagefferens* showed a significant positive correlation between lipid stores and size, suggesting that some larvae were simply better at obtaining food and associated nutrients. Larval success likely depends on a complex interplay between genetic and environmental factors. Our study suggests that poor nutrition associated with a harmful alga can have negative effects on larval size and lipids stores, which in turn are mediated by the inter-individual variability in the ability to grow and accumulate necessary lipid stores. Phytoplankton quality is likely to be important for the sustainability of bivalve populations even when it primarily impacts the larval phase; and a diet of brown tide algae may have lasting legacies for juveniles and adults.

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1. Introduction

Planktotrophic larvae of marine invertebrates must acquire adequate nutrition and energy storage for survival, growth, metamorphosis, and initiation of juvenile growth. At the same time, they are exposed to multiple and often interacting biotic and abiotic stressors, which can have dramatic effects on larval growth and survival (Przeslawski, 2004; Padilla and Miner, 2006; Pechenik, 2006), particularly through their effects on larval nutrition. Lipids and fatty acids play an important role in the development of marine invertebrate larvae and are linked to food

quantity (Gallager and Mann, 1986) and quality (Vanderploeg et al., 1996). Neutral lipids are particularly important during larval development, especially embryogenesis and metamorphosis (Sewell, 2005 and references therein) (e.g., triglycerides in oysters (Gallager et al., 1986) and scallops (Pernet et al., 2003)). Long-chain polyunsaturated fatty acids (PUFAs) are also important for the survival and development of aquatic larvae of many species, including freshwater (Vanderploeg et al., 1996) and marine bivalves (Delaunay et al., 1993; Soudant et al., 1996; Milke et al., 2004).

In addition to immediate nutritional needs, the storage of lipids has also been shown to be important for post-metamorphic survival and growth in a variety of invertebrates, especially bivalves (Wikfors et al., 1992; Phillips, 2002, 2006; Wacker and von Elert, 2002; Emlet and Sadro, 2006). For the northern quahog, or hard clam, *Mercenaria mercenaria*, the accumulation of lipids and

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proteins during the larval stage is strongly correlated with juvenile growth rates (Wikfors et al., 1992). Thus, nutritional experiences during the larval stage can provide longer term legacies, impacting later life stages (Padilla and Miner, 2006; Pechenik, 2006).

Larval nutrition may be negatively affected by harmful algal blooms, including brown tide (Ward et al., 2000; Gobler et al., 2005). The picoplanktonic brown tide alga, *Aureococcus anophagefferens*, first appeared in bloom concentrations in mid-Atlantic U.S. waters in 1985, and blooms have since periodically occurred in several bays in this region (reviewed in Bricelj and Lonsdale, 1997; Nuzzi and Waters, 2004; Gobler et al., 2005), including a recent bloom in Great South Bay in May 2008, the furthest west yet detected (R. Waters, Suffolk County Department of Health Services, unpublished data). *A. anophagefferens* has negative impacts on a variety of bivalves and has been linked to the collapse of the bay scallop industry and loss of important eelgrass habitat (Bricelj and Lonsdale, 1997). This alga inhibits movement of gill cilia of some juvenile and adult bivalves (Gainey and Shumway, 1991), resulting in species-specific feeding suppression that is often more pronounced in juveniles (Bricelj et al., 2001). At present we do not know the nutritional characteristics of this species.

In the mid-Atlantic, natural populations of several ecologically and commercially important species of bivalves have recently collapsed, including *M. mercenaria*. Once among the most abundant bivalves in the eastern U.S., supporting an extensive fishery, populations of hard clams in Great South Bay, Long Island, NY, collapsed during the 1970s, likely due to overfishing (Kraeuter et al., 2005), and other stressors including pollution, habitat loss, and changes in phytoplankton communities, including harmful algal blooms (Weiss et al., 2007 and references therein). Suspension-feeders such as *M. mercenaria* play key ecological roles by regulating water quality and phytoplankton communities, and they also play an integral role in nutrient cycling through benthic–pelagic coupling (reviewed in Newell, 2004; Dame and Olenin, 2005).

Despite recent restoration efforts and harvesting limits, larval abundance (Perino, 2006) and recruitment (Kraeuter et al., 2005) of *M. mercenaria* in the field remain low. Brown tide blooms may coincide with hard clam spawning (Kassner and Malouf, 1982; M. Doall, unpublished data) and could pose a barrier to recovery of *M. mercenaria* populations by negatively impacting the larval stage. Laboratory studies have shown that larval growth is consistently retarded by *A. anophagefferens* (Padilla et al., 2006; Bricelj and MacQuarrie, 2007). Recent observations also suggest that *A. anophagefferens* may negatively affect lipid stores of hard clam larvae, although this hypothesis was not directly tested (Padilla et al., 2006). We conducted an experiment to directly test the hypothesis that *A. anophagefferens* negatively impacts lipid stores and growth of *M. mercenaria* larvae prior to metamorphosis. We also tested whether there were immediate tradeoffs between larval growth and lipid storage. If there were such a tradeoff, we would expect lipid stores to be negatively correlated with larval size during periods of limited resources. If, however, nutritional resources are not limiting, then such tradeoffs may not be seen, especially if there are intrinsic differences among larvae such that those with fast growth rates are also the best at accumulating lipid stores. This study is the first to investigate nutritional effects of a harmful alga on lipid stores of marine invertebrate larvae.

2. Methods

2.1. Algal cultures

A. anophagefferens (strain CCMP1708, maintained by D. Lonsdale, Stony Brook University) was used, as previous research

indicates that this strain is highly toxic to juvenile bivalves (Bricelj et al., 2001) and affects larval growth rates (Padilla et al., 2006; Bricelj and MacQuarrie, 2007). *Isochrysis galbana* (strain CCMP1323) was used as a control diet because it is rich in long-chain PUFAs and triglycerols (Volkman et al., 1989; Brown et al., 1997), hard clam larvae have been shown to thrive on this diet (Kraeuter and Castagna, 2001), and it has been used as a control diet in related studies (Padilla et al., 2006; Bricelj and MacQuarrie, 2007).

Both algal species were cultured in sterile 0.45- μm filtered seawater. *I. galbana* was cultured in standard f/2 medium at 24 °C at 14 h light:10 h dark cycles. *A. anophagefferens* was grown in a modified-batch culture (Hoff and Snell, 2001) at 20 °C at 16 h light:8 h dark cycles with constant air-bubbling using a modified f/2 medium with citric acid, iron (FeCl_3), selenium, and β -glycerophosphate (Bricelj et al., 2001). Previous research has shown that hard clam larvae do not grow well when fed slow or stationary phase *A. anophagefferens* cultures (Padilla et al., 2006). To ensure cultures were in exponential phase, *A. anophagefferens* cell density was monitored with a fluorometer and by direct counts with a hemacytometer. *I. galbana* cell densities were counted daily with a hemacytometer.

2.2. Clam spawning

Adult clams were collected from Great South Bay (40.8N, 72.5W) and Shinnecock Bay (40.7N, 73.1W) Long Island, NY, in December 2006–January 2007 and held at 16 °C for at least 3 weeks to condition them for spawning. During this time they were fed a marine microalgal concentrate (Shellfish Diet 18,100 by Reed Mariculture Inc.) administered via a drip bag at a rate of 1 l d⁻¹ at 6×10^6 cells ml⁻¹ for the first 3 weeks and then 1 l d⁻¹ at 10×10^7 cells ml⁻¹ until spawning.

To induce spawning, a small hole was drilled in the shell of each clam, 0.4 ml of 0.2 mM serotonin was injected into the anterior adductor muscle (Gibbons and Castagna, 1985), and clams were placed in individual 1 l beakers with filtered seawater. Clams that did not spawn within an hour of injection were subjected to a thermal shock cycle of 30-min periods of 10 and 30 °C (Padilla et al., 2006), and sperm stripped from a dissected male clam were added to the water to help induce spawning. When individuals spawned, the seawater containing sperm or eggs was filtered through a 150- μm mesh to remove mucus and feces. Water containing sperm was diluted 1:1 with 0.45 μm filtered seawater, and 10 ml of this dilution was added to 1 l beakers with eggs resuspended in filtered seawater. Containers were stirred for 5 min, and fertilized eggs were sieved at 50 μm to remove excess sperm and rinsed into 4-l glass jars with filtered seawater. A subsample of fertilized eggs was stained for lipid analysis.

2.3. Larval culture

Embryos and larvae were held in loosely covered 4-l glass jars for the first 24 h at 22 °C. The next day, viable larvae were easily detected as swimming trochophores, and these were randomly allocated to 1-l containers at 120 larvae/l. In addition, a sample of trochophores was stained for lipid analysis to quantify maternal investment. Larval culture procedures followed Padilla et al. (2006), modified from Strathmann (1987), including use of embryologically clean materials and antibiotics.

Larvae were fed for the first time 24 h after fertilization according to their specified dietary treatment. Larvae were then fed daily and transferred into clean beakers with freshly filtered water twice a week. The experiment ended when pediveligers were first detected in the control *Isochrysis*-only treatment (Day

14). At the end of the experiment, all larvae were immediately stained for lipid analysis and photographed.

2.4. Dietary treatments

Larvae were fed one of three diets daily (four replicate beakers per treatment), such that there were a total of 12 beakers, each with approximately 120 larvae. Dietary treatments were equal biovolumes of algal food, based on Strathmann (1967) for *I. galbana* and Bricelj et al. (2001) for *A. anophagefferens*: (1) 100% *I. galbana* (2×10^4 cells/ml), (2) 50% *I. galbana* (1×10^4 cells/ml) and 50% *A. anophagefferens* (8×10^4 cells/ml), and (3) 100% *A. anophagefferens* (1.6×10^5 cells/ml).

Cell densities of live algae used to feed larvae were counted daily with a hemacytometer, and the volume of culture needed to obtain the required cell number in each treatment was calculated. Because brown tide algal medium has a significant negative impact on hard clam growth (Padilla et al., 2006), calculated volumes of cultures were centrifuged ($G = 1855$ for 25 min) and the pellet resuspended in filtered seawater prior to feeding larvae.

2.5. Lipid analysis and image analysis

Nile Red was used to stain for lipids. This stain binds to neutral lipids and fluoresces under an FITC filter on a fluorescent microscope (Castell and Mann, 1994; Phillips, 2002). Preliminary experiments revealed that larvae should be immediately analyzed for lipid content: we detected significantly less lipids in 24-h-old larvae that had been stored for a week at -80°C (0.6000 ± 0.0408 , mean lipid index \pm S.E.M.) than larvae were stained and analyzed immediately for lipids (0.7653 ± 0.0130) (ANOVA, $F = 14.9268$, $p = 0.0003$, $n = 30$).

For lipid analyses, 1.25 mg of Nile Red crystals were dissolved in 100-ml acetone to make a stock solution, which can be kept refrigerated in the dark for up to 28 days (Tankersley, 2000). Immediately prior to use, a 1:9 dilution was made of the stock solution and filtered seawater. Larvae were exposed to this dilution ($1.25 \mu\text{g}$ Nile Red/ml) for 1.5 h (Castell and Mann, 1994; Phillips, 2002). Larvae were then rinsed with filtered seawater and digitally photographed under a fluorescent microscope to observe lipids. Twenty randomly selected veligers from each replicate container (four replicates per treatment) were photographed. From the photographs, larval shell length, total projected shell area, and the fluorescent area were quantified with image analysis software (Image J, National Institute of Health). Images were converted to black and white, and standardized thresholds were determined based on visual estimation of optimal detection of fluorescing lipids (larval area: threshold = 20, lipid area: threshold = 110). Pilot data comparing other close thresholds revealed no difference in the overall analysis. Lipid index was defined as the fluorescent area of the larval shell divided by the projected area (representing neutral lipids), thereby allowing direct comparisons among treatments independent of larval growth (Phillips, 2006).

2.6. Statistical analyses

We used nested ANOVAs to test for the effects of diet on growth (larval shell length) and lipid accumulation (size-independent lipid index), as individual larvae grown within a single replicate container may not be independent. Therefore, the effect of diet was tested using the among-containers variance in each treatment from the ANOVA, rather than the error variance. For all analyses with significant differences among treatments, Tukey's HSD multiple comparison tests were used to test for specific differences among treatments. An arcsin transformation was applied to lipid

indices to correct for non-normal distribution and heterogeneous variances of the data (Sokal and Rohlf, 1995).

To determine if there was evidence of a tradeoff between larval growth and storage of lipids, we used correlation analyses to test for the relationship between lipid stores and larval size for each treatment. A sequential Bonferroni (Sokal and Rohlf, 1995) was used to correct the critical alpha level for multiple testing. ANOVAs were performed using Statistica 6.1 with $\alpha = 0.05$, and correlations were performed with JMP 4.1.

3. Results

3.1. Lipid index

Dietary treatment significantly affected lipid index (d.f. = 2, 9; $F = 4.7829$; $p = 0.0384$). Larvae fed only *A. anophagefferens* had significantly lower average lipid indices (0.1262 ± 0.0137 , mean lipid index \pm S.E.M.) than larvae fed mixed algae (0.1751 ± 0.0148) or only *I. galbana* (0.1970 ± 0.0210) (Fig. 1). Although we could not incorporate eggs and trochophores into formal statistical analyses due to lack of replicate containers for these stages, larvae in all dietary treatments had far lower lipid indices than eggs (0.9110 ± 0.2037) and trochophores (0.7644 ± 0.0142) (Fig. 1).

3.2. Size

Diet significantly affected larval size (d.f. = 2, 9; $F = 25.125$; $p < 0.0001$). Larval size after 14 days was significantly different among all dietary treatments (Tukey's HSD test, $\alpha < 0.05$), with larvae fed only *A. anophagefferens* the smallest ($134.4 \pm 21.4 \mu\text{m}$, mean larval size \pm S.E.M.), larvae fed mixed algae intermediate size ($157.2 \pm 26.0 \mu\text{m}$) and larvae fed only *I. galbana* the largest ($199.8 \pm 26.9 \mu\text{m}$) (Fig. 2). Larvae in all dietary treatments were much larger than eggs and trochophores (Fig. 2).

3.3. Size and lipid stores

Lipid stores and size were significantly positively correlated for larvae fed mixed algae ($r = 0.3697$, $p = 0.0007$) and *A. anophagefferens* ($r = 0.4270$, $p < 0.0001$) (Table 1, Fig. 3). Thus, for these two treatments larger larvae had more lipid stores than smaller larvae. In contrast, larvae fed only *I. galbana* showed no significant

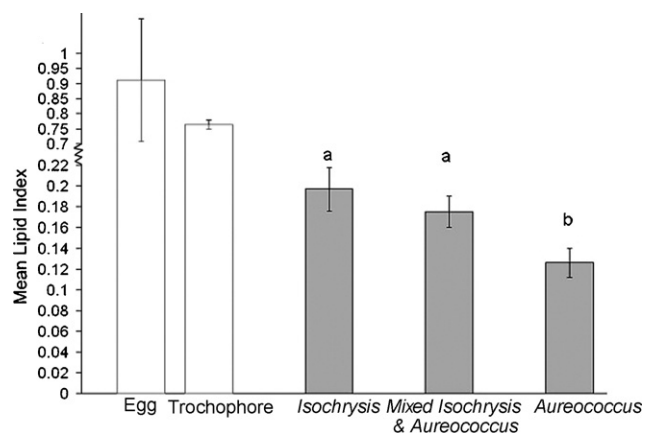


Fig. 1. Lipid stores of 14-day-old larvae fed different diets. Each shaded bar represents the mean lipid index of 80 larvae from four containers. Lipid indices of eggs and trochophores are provided for comparison and provide a measure of initial inter-individual variation in resource allocation. Eggs ($n = 20$) were stained immediately after fertilization; and trochophores ($n = 20$) at 24 h post-fertilization. Letters indicate relationships that are not significantly different (Tukey's HSD test, $\alpha < 0.05$). Error bars are S.E.M.

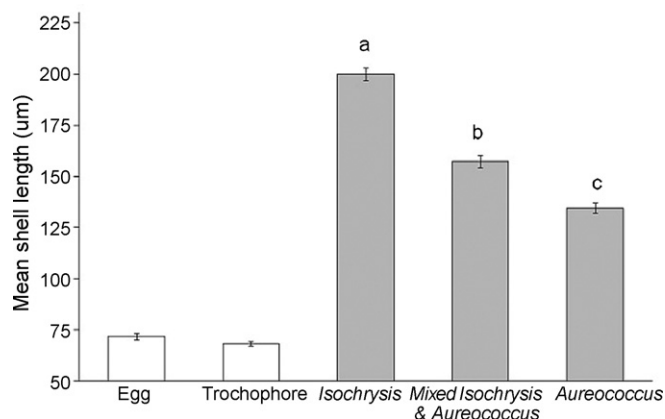


Fig. 2. Shell lengths of 14-day-old larvae fed different diets. Each shaded bar represents the mean shell length of 80 larvae from four containers. Sizes of eggs and trochophores are provided for comparison and provide a measure of inter-individual variation. Diameter of eggs ($n = 20$) was measured immediately after fertilization; and maximum length of trochophores ($n = 20$) at 24 h post-fertilization. Letters indicate relationships that are not significantly different (Tukey's HSD tests, $\alpha < 0.05$). Error bars are S.E.M.

Table 1

Correlation analyses between lipid stores and larval size among dietary treatments, with sequential Bonferroni corrections

	<i>R</i>	<i>p</i>	Adjusted critical alpha
<i>Isochrysis</i>	0.023087	0.8389	0.05
Mixed <i>Isochrysis</i> and <i>Aureococcus</i>	0.369745	0.0007	0.025321
<i>Aureococcus</i> only	0.426988	<0.0001	0.016952

Bold text indicates significant relationships.

relationship between lipid stores and larval size ($r = 0.0231$, $p = 0.8389$) (Fig. 3).

4. Discussion

This study shows that *M. mercenaria* larvae fed a diet of the brown tide alga *A. anophagefferens* are not only smaller, but also have lower lipid stores, which are known to be important for post-

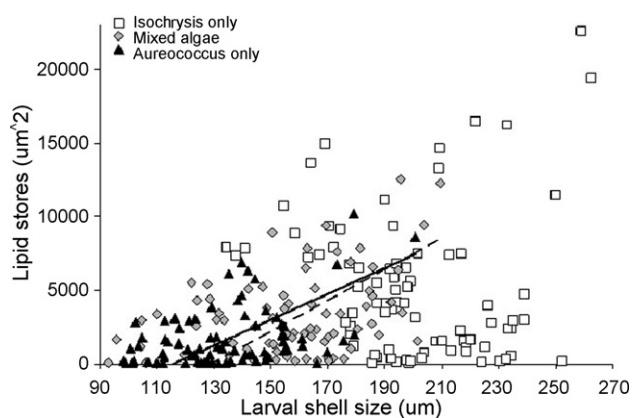


Fig. 3. Relationship between lipid stores and larval shell length for each dietary treatment, the control alga *Isochrysis*, the brown tide alga *Aureococcus*, and a mixed diet of each in equal amounts ($n = 20$ larvae per each of the three replicate containers for each treatment). Lines are significant linear relationships (Reduced Major Axis regression, Sokal and Rohlf, 1995), with the solid black line for the *Aureococcus*-only treatment and the dashed line for the mixed algal treatment. For larvae fed *Isochrysis*, there was no significant association between lipid content and larval size.

metamorphic survival and juvenile growth. Accordingly, brown tide may pose a barrier to the recovery of natural populations of hard clams due to its effect on larval growth and nutrition as well as legacy effects on post-larval stages (Padilla and Miner, 2006; Pecehnik, 2006). These results are consistent with studies that found that *A. anophagefferens* negatively impacts hard clam larval growth (Padilla et al., 2006; Bricelj and MacQuarrie, 2007), and they represent the first study to identify negative effects of brown tide on larval lipid stores.

Lipid stores are crucial for embryogenesis and morphogenesis, and lipids accumulated during the larval stage have also been shown to be critical for post-metamorphic survival and early juvenile performance for a range of marine invertebrates, particularly bivalves (Bochenek et al., 2001; Phillips, 2002; Wacker and von Elert, 2002). Lingering nutritional effects may persist in juveniles for 2–3 times longer than the duration of nutritional deficit actually experienced by larvae (Emler and Sadro, 2006), and this period may actually be far longer, particularly for less obvious effects on reproductive traits and longevity (Hadfield and Strathmann, 1996). Unfortunately, the lipid staining method used in the current study precluded larval rearing and associated quantification of potential legacy effects such as decreased juvenile growth rate and survivorship. Once exposed to the staining solution, all D-stage and umbo larvae died within 2 days, even with shorter exposure times to the staining solution; and 90% of pediveligers died within a week (Przeslawski, unpublished data).

We found no evidence of a tradeoff between the accumulation of lipid stores and larval growth; larval size and lipid stores were not negatively correlated when nutrients were limited. Indeed, the relationship between size and lipid stores in larvae fed brown tide or mixed algae was positive. This may be due to genetic differences or initial maternal allocation differences (Marshall and Keough, 2007), which meant that some larvae may simply have been better than others at feeding, growing, or storing lipids (Pernet et al., 2006). When resources were limited (i.e., brown tide diet treatments), the effects due to these differences were pronounced, with stronger larvae storing more lipids and growing faster than their weaker siblings. When resources were not limited (e.g., control *Isochrysis* treatment), these effects were less obvious (Fig. 3). Although variation in 24-h trochophores was relatively low, the variation in lipid stores among eggs was high, ranging between 0.82 and 1.00, indicating high variance in initial maternal investment (Fig. 1). Future research investigating the relationships between initial maternal investment, larval lipid stores, and larval growth rates under various food rations across several larval cohorts would help further reveal how genetic and maternal investment differences may affect larval fitness.

Although the differences in lipid index were relatively small among treatments, the results from Phillips (2002), in which she used the same method of lipid quantification as we did (lipid index), suggest that the differences we detected are biologically significant. She found that a difference in mean lipid index between 0.11 and 0.25 during pre-pediveliger stages of a mussel resulted in a significant and large difference in juvenile growth rates (approximately threefold). In our study, lipid indices ranged between 0.13 and 0.20. It therefore seems likely that this difference would result in differences in juvenile growth, based on similar results to that observed in Phillips (2002), including the step-wise decrease in lipids across nutrition-mediated treatments (Fig. 1) and similar observed effects on larval size (Fig. 2).

Differences between experiments conducted on *M. mercenaria* larvae are intriguing. Previous studies found that brown tide affects hard clam larval growth at 26 °C in bloom concentrations but not in more moderate concentrations (Padilla et al., 2006). In contrast, we found these moderate concentrations affect larval size

when larvae are reared at 22 °C (Fig. 2). Other studies found either no effect or a negative effect of the brown tide alga on survivorship (Padilla et al., 2006; Bricelj and MacQuarrie, 2007). These differences suggest that the effects of brown tide on *M. mercenaria* larvae may be dependent on a variety of parameters including: algal quality, rearing temperature, and differences in parental stock used to generate gametes. Biochemical composition of microalgae can vary across strains as well as under different culture conditions, according to growth phase, light regime, temperature and media (Brown et al., 1997 and references therein). Rearing temperature may also affect sensitivity of larvae to brown tide, as temperature may exacerbate stress associated with low-quality food and other environmental stressors (Folt et al., 1999). Variability in the quality and performance of larvae could also come from environmental conditioning or genetic differences among adults used for egg and sperm sources for experiments. Adult diet or environmental conditions can affect gamete quality, particularly of eggs (Gimenez, 2006), and smaller eggs have been shown to develop into larvae or juveniles with lower fitness than their counterparts from larger eggs (Allen et al., 2006). Genetic differences may also contribute to differential response to brown tide observed among this study and similar experiments (Padilla et al., 2006; Bricelj and MacQuarrie, 2007). Variation between larval cohorts of bivalves can be extreme (Gimenez, 2006; Pernet et al., 2006; Phillips, 2007; Marshall and Keough, 2007) and is likely due to an interplay among genetic and environmental factors. More experiments controlling for parental environmental history, maternal provisioning, and genetic quality of larvae need to be conducted to test the relative importance of these factors.

The challenge for all laboratory studies is to translate results into likely effects and mechanisms in field populations. This is essential not only for understanding population dynamics, but also for management and restoration of native species. Our study clearly shows that the brown tide alga affects lipid stores and growth of hard clam larvae when reared in a controlled laboratory environment. However, larvae in the field are exposed to variable environments and multiple, often simultaneous stressors (Przeslawski, 2004). Larval survival and development may be affected by interactions between diet and salinity (Gimenez, 2006), temperature (Vagner et al., 2007), light intensity (Puvanendran and Brown, 2002), and bacteria (Plante et al., 2007). Interactions between these stressors may exacerbate the negative effects of brown tide on larvae and require further exploration to estimate their relative importance. In addition, it is unlikely that most larvae will be exposed only to a diet of the brown tide alga for the duration of their planktonic stage, except during the most extreme bloom events. The duration of brown tide blooms varies in Great South Bay, lasting anywhere from a week to over a month and occurring at concentrations over 10^6 cells ml⁻¹ between 17.5 and 28.6 °C (Bricelj and Lonsdale, 1997; data from Suffolk County Department of Health Services). Comparatively, larval duration of *M. mercenaria* lasts 10–23 days at typical summer water temperatures of 22–28 °C in Great South Bay (M. Doall, unpublished data; Loosanoff et al., 1951; Padilla et al., 2006), with developmental rate varying according to food availability and other environmental conditions (Davis and Calabrese, 1964; Przeslawski, 2004). However, even if larvae are not exposed to the brown tide alga for the duration of their development, the alga may negatively affect their lipid stores and growth as the primary food source for even part of the larval stage. Phillips (2004) found that juvenile mussels exposed to low food concentrations only during early larval stages had lower survivorship and growth rates than juveniles exposed to low food only during later larval

stages, despite no differences in larval size or lipid stores. Other studies have similarly found that episodes of starvation or low-quality food differentially affect larvae, based on the timing and duration of exposure within the larvae stage (Gallager and Mann, 1986; Bos et al., 2007). Thus, legacies in juveniles from episodic larval exposure to brown tide may play an important role in population dynamics.

The quality of planktonic food is clearly important and likely to impact the restoration potential for hard clams, as well as the continued maintenance of sustainable populations of other bivalves in these bay systems. During a harmful algal bloom, only one species of phytoplankton will form a large portion of the larval diet. Even disregarding its potential toxicity, brown tide may negatively impact population recovery of hard clams and possibly other invertebrates due to its low nutritional quality for developing larvae. Although legacies of larval exposure to brown tide remain unknown, lower lipid stores and smaller size at metamorphosis may prove a formidable barrier to success or recovery of vulnerable populations.

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References

- Allen, J.D., Zakas, C., Podolsky, R.D., 2006. Effects of egg size reduction and larval feeding on juvenile quality for a species with facultative-feeding development. *J. Exp. Mar. Biol. Ecol.* 331, 186–197.
- Bochenek, E.A., Klinck, J.M., Powell, E.N., Hofmann, E.E., 2001. A biochemically based model of the growth and development of *Crassostrea gigas* larvae. *J. Shellfish Res.* 20, 243–265.
- Bos, O.G., Philippart, C.J.M., van der Meer, J., 2007. Effects of temporary food limitation on development and mortality of *Macoma balthica* larvae. *Mar. Ecol. Prog. Ser.* 330, 155–162.
- Bricelj, V.M., Lonsdale, D.J., 1997. *Aureococcus anophagefferens*: causes and ecological consequences of brown tides in U.S. mid-Atlantic coastal waters. *Limnol. Oceanogr.* 42, 1023–1038.
- Bricelj, V.M., MacQuarrie, S.P., 2007. Effects of brown tide (*Aureococcus anophagefferens*) on hard clam *Mercenaria mercenaria* larvae and implications for benthic recruitment. *Mar. Ecol. Prog. Ser.* 331, 147–159.
- Bricelj, V.M., MacQuarrie, S.P., Schaffner, R.A., 2001. Differential effects of *Aureococcus anophagefferens* isolates ("brown tide") in unialgal and mixed suspensions on bivalve feeding. *Mar. Biol.* 139, 605–615.
- Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. *Aquaculture* 151, 315–331.
- Castell, L.L., Mann, R., 1994. Optimal staining of lipids in bivalve larvae with Nile Red. *Aquaculture* 119, 89–100.
- Dame, R., Olenin, S., 2005. The comparative roles of suspension-feeders in ecosystems. NATO Science Series IV: Earth and Environmental Sciences, vol. 47. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Davis, H.C., Calabrese, A., 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. *Fish. Bull. Fish Wildlife Serv.* 63, 643–655.
- Delaunay, F., Marty, Y., Moal, J., Samain, J.-F., 1993. The effect of monospecific algal diets on growth and fatty acid composition of *Pecten maximus* (L.) larvae. *J. Exp. Mar. Biol. Ecol.* 173, 163–179.
- Emlet, R.B., Sadro, S.S., 2006. Linking stages of life history: how larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*). *Integr. Comp. Biol.* 46, 334–346.
- Folt, C.L., Chen, C.Y., Moore, M.V., Burnaford, J., 1999. Synergism and antagonism among multiple stressors. *Limnol. Oceanogr.* 44, 864–877.
- Gainey, L.F., Shumway, S.E., 1991. The physiological effects of *Aureococcus anophagefferens* ("brown tide") on the lateral cilia of bivalve mollusks. *Biol. Bull.* 181, 298–306.

- Gallager, S.M., Mann, R., 1986. Individual variability in lipid content of bivalve larvae quantified histochemically by absorption photometry. *J. Plankton Res.* 8, 927–937.
- Gallager, S.M., Mann, R., Sasaki, G.C., 1986. Lipid as an index of growth and viability in three species of bivalve larvae. *Aquaculture* 56, 81–103.
- Gibbons, M.C., Castagna, M., 1985. Responses of the hard clam *Mercenaria mercenaria* (Linne) to induction of spawning by serotonin. *J. Shellfish Res.* 5, 65–67.
- Gimenez, L., 2006. Phenotypic links in complex life cycles: conclusions from studies with decapod crustaceans. *Integr. Comp. Biol.* 46, 615–622.
- Gobler, C.J., Lonsdale, D.J., Boyer, G.L., 2005. A review of the causes, effects, and potential management of harmful brown tide blooms caused by *Aureococcus anophagefferens* (Hargaves et Sieburth). *Estuaries* 28, 726–749.
- Hadfield, M.G., Strathmann, M.F., 1996. Variability, flexibility and plasticity in life histories of marine invertebrates. *Oceanol. Acta* 19, 323–324.
- Hoff, F.H., Snell, T.W., 2001. Microalgae culture. In: Neslen, J. (Ed.), *Plankton Culture Manual*. 5th ed. Florida Aqua Farms Inc., Dade City, pp. 27–59.
- Kassner, J., Malouf, R.E., 1982. An evaluation of 'spawner transplants' as a management tool in Long Island's hard clam fishery. *J. Shellfish Res.* 2, 165–172.
- Kraeuter, J.N., Buckner, S., Powell, D.N., 2005. Notes on a spawner-recruit relationship for a heavily exploited bivalve: the case of northern quahogs (hard clams), *Mercenaria mercenaria*, in Great South Bay New York. *J. Shellfish Res.* 24, 1043–1052.
- Kraeuter, J.N., Castagna, M., 2001. *Biology of the Hard Clam*. Elsevier, Amsterdam.
- Loosanoff, V.L., Miller, W.S., Simth, P.B., 1951. Growth and settling of larvae of *Venus mercenaria* in relation to temperature. *J. Mar. Res.* 10, 59–81.
- Marshall, D.J., Keough, M.J., 2007. The evolutionary ecology of offspring size in marine invertebrates. *Adv. Mar. Biol.* 53, 1–60.
- Milke, L.M., Bricelj, V.M., Parrish, C.C., 2004. Growth of postlarval sea scallops, *Placopecten magellanicus*, on microalgal diets, with emphasis on the nutritional role of lipids and fatty acids. *Aquaculture* 234, 293–317.
- Newell, R.I.E., 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *J. Shellfish Res.* 23, 51–61.
- Nuzzi, R., Waters, R.M., 2004. Long-term perspective on the dynamics of brown tide blooms in Long Island coastal bays. *Harmful Algae* 3, 279–293.
- Padilla, D.K., Doall, M.H., Gobler, C.J., Hartson, A., O'Boyle, K., 2006. Brown tide alga, *Aureococcus anophagefferens*, can affect growth but not survivorship of *Mercenaria mercenaria* larvae. *Harmful Algae* 5, 736–748.
- Padilla, D.K., Miner, B.G., 2006. Legacies in life histories. *Integr. Comp. Biol.* 46, 217–223.
- Pechenik, J., 2006. Larval experience and latent effects-metamorphosis is not a new beginning. *Integr. Comp. Biol.* 46, 323–333.
- Perino, L., 2006. Using PCR to determine the accuracy of morphological identification of *Mercenaria mercenaria* (L.) larvae. MS Thesis, Stony Brook University, Stony Brook, NY.
- Pernet, F., Bricelj, V.M., Cartier, S., 2006. Lipid class dynamics during larval ontogeny of sea scallops, *Placopecten magellanicus*, in relation to metamorphic success and response to antibiotics. *J. Exp. Mar. Biol. Ecol.* 329, 265–280.
- Pernet, F., Tremblay, R., Bourget, E., 2003. Biochemical indicator of sea scallop (*Placopecten magellanicus*) quality based on lipid class composition. Part II. Larval growth, competency and settlement. *J. Shellfish Res.* 22, 377–388.
- Phillips, N.E., 2002. Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. *Ecology* 83, 2562–2574.
- Phillips, N.E., 2004. Variable timing of larval food has consequences for early juvenile performance in a marine mussel. *Ecology* 85, 2341–2346.
- Phillips, N.E., 2006. Natural variability in size and condition at settlement of 3 species of marine invertebrates. *Integr. Comp. Biol.* 46, 598–604.
- Phillips, N.E., 2007. High variability in egg size and energetic content among intertidal mussels. *Biol. Bull.* 212, 12–19.
- Plante, S., Pernet, F., Hache, R., Ritchie, R., Ji, B.J., McIntosh, D., 2007. Ontogenetic variations in lipid class and fatty acid composition of haddock larvae *Melanogrammus aeglefinus* in relation to changes in diet and microbial environment. *Aquaculture* 263, 107–121.
- Przeslawski, R., 2004. A review of the effects of environmental stress on embryonic development within intertidal gastropod egg masses. *Mol. Res.* 24, 43–63.
- Puvanendran, V., Brown, J.A., 2002. Foraging, growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. *Aquaculture* 214, 131–151.
- Sewell, M.A., 2005. Utilization of lipids during early development of the sea urchin *Evechinus chloroticus*. *Mar. Ecol. Prog. Ser.* 304, 133–142.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry*, 3rd ed. WH Freeman and Company, New York.
- Soudant, P., Marty, Y., Moal, J., Samain, J., 1996. Fatty acids and egg quality in great scallop. *Aquacult. Int.* 4, 191–200.
- Strathmann, M.F., 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press, Seattle.
- Strathmann, R.R., 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol. Oceanogr.* 12, 411–418.
- Tankersley, R.A., 2000. Fluorescence techniques for evaluating the lipid content of larval and juvenile freshwater mussels. In: *Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium*, 1998. pp. 115–125.
- Vagner, M., Robin, J.H., Infante, J.L.Z., Ruyet, J.P.L., 2007. Combined effects of dietary HUFA level and temperature on sea bass (*Dicentrarchus labrax*) larvae development. *Aquaculture* 266, 179–190.
- Vanderploeg, H.A., Liebig, J.R., Gluck, A.A., 1996. Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena polymorpha*): the importance of size and polyunsaturated fatty acid content. *J. Gt. Lakes Res.* 22, 36–45.
- Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* 128, 219–240.
- Wacker, A., von Elert, E., 2002. Strong influences of larval diet history on subsequent post-settlement growth in the freshwater mollusc *Dreissena polymorpha*. *Proc. R. Soc. Lond. B* 269, 2113–2119.
- Ward, L.A., Montagna, P.A., Kalke, R.D., Buskey, E.J., 2000. Sublethal effects of Texas brown tide on *Streblospio benedicti* (Polychaeta) larvae. *J. Exp. Mar. Biol. Ecol.* 248, 121–129.
- Weiss, M.B., Curran, P.B., Peterson, B.J., Gobler, C.J., 2007. The influence of plankton composition and water quality on hard clam (*Mercenaria mercenaria*) populations across Long Island's south shore lagoon estuaries (New York, USA). *J. Exp. Mar. Biol. Ecol.* 345, 12–25.
- Wikfors, G.H., Ferris, G.E., Smith, B.C., 1992. The relationship between gross biochemical composition of cultured algal foods and growth of the hard clam *Mercenaria mercenaria* (L.). *Aquaculture* 108, 135–154.