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Impact of hypoxia on the survival, egg production and population dynamics of *Acartia tonsa* Dana

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Abstract

Concern for the increasing occurrence of coastal zone hypoxia has generally focused on the direct, short-term impact of reduced dissolved oxygen (DO) concentrations on the survival of commercially important species such as fish and crabs. Copepods, especially the naupliar stages, are important pelagic food web components, yet only a few studies have considered the effect of reduced DO concentrations on their survival and population dynamics. This study considers the impact of both lethal and sublethal DO concentrations on copepods. *Acartia tonsa* were reared at 25 °C at saturating DO (normoxic control) and reduced (hypoxic) DO concentrations of 1.5 or 0.7 ml l^{-1}. Oxygen concentrations were maintained in replicate flasks, by bubbling seawater with air (control), or mixtures of nitrogen and oxygen. Egg production, but not survival, was significantly higher in the controls compared to the 1.5 ml l^{-1} DO treatment. Survival and egg production were significantly lower at 0.7 ml l^{-1} DO compared to the control. The results suggest that the sublethal as well as the lethal effects of hypoxia may have important repercussions on population and community dynamics in coastal systems.
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Keywords: *Acartia tonsa*; Copepod; Hypoxia; Population dynamics; Sublethal; Zooplankton

1. Introduction

The existence of hypoxic/anoxic bottom waters in portions of the coastal zone is well documented (Diaz and Rosenberg, 1995). From a biological perspective, reduced dissolved

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oxygen (DO < 2.0 ml l^{-1}) is a major problem for the constituent biota because few metazoans can withstand long periods with little or no oxygen. Most studies of the impact of hypoxia/anoxia in the coastal zone have focused on the direct, short-term impact of reduced DO concentrations on the survival of commercially important invertebrate and fish species, e.g., blue crabs, shrimp, oysters and flounder (see Pihl et al., 1991; Diaz and Rosenberg, 1995; Miller et al., 2002). The results of these studies have shown that severe mortality of most species usually occurs when DO concentrations become hypoxic falling below 2.0 ml l^{-1}.

Only a few studies of the coastal zone have considered the impact of reduced DO concentrations on zooplankton mortality (e.g., Vargo and Sastry, 1977; Roman et al., 1993), yet copepods, especially the naupliar stages, are important prey items in the diets of many larval marine fish. No study has examined the sublethal effects of hypoxia on life history parameters (e.g., development and growth rates, egg production) of marine zooplankton or considered the potential impact of such responses on ecosystem community structure and processes. However, sublethal effects (e.g., reduced egg production) of hypoxia may be as important as survival rates, in terms of impact on zooplankton population dynamics. In the case of freshwater cladocerans, exposure to low DO concentrations can result in reduced growth rates and smaller individuals that produce smaller clutch sizes (Hanazato and Dodson, 1995; Hanazato, 1996). These reductions in egg production could lead to smaller population sizes in future generations. Shifts in zooplankton community structure due to hypoxia (whether from differential survival or chronic sublethal effects) could alter predator–prey interactions and thus affect the population dynamics of fish through not only reductions in prey density, but also through reductions in prey size, since many juvenile and adult fish are size-selective predators (Pepin and Penney, 1997). In addition, changes in grazing pressure on the phytoplankton community could alter the flux of organic matter to the seabed and thus biogeochemical cycling.

The sublethal effects of hypoxia on copepods may arise from many mechanisms. As an example, the physiological stress caused by exposure to hypoxic conditions may force organisms to expend energetic reserves on coping mechanisms (such as the production of hemoglobin), thereby diverting energy from expenditures on growth, development, and reproduction (Roff, 1992). This study examined the response of the cosmopolitan coastal copepod, *Acartia tonsa* to low DO concentrations. The concentrations of DO that were tested in this study are representative of the hypoxic conditions existing in systems inhabited by *A. tonsa*. For example, areas of Chesapeake Bay undergo seasonal bouts of hypoxia and anoxia (Officer et al., 1984), especially during the summer months, and this is a time of the year when *A. tonsa* is normally present (Roman et al., 1993). We show that reduced DO concentrations can lead to significant reductions in egg production in the absence of significant reductions in survival of the planktonic stages. Our empirical results were incorporated into a Leslie matrix model to predict long-term population growth; model results suggest that the combination of lethal and sublethal effects of hypoxia translate into reductions in population growth rate. The implications of these sublethal effects on copepod population dynamics are discussed in terms of possible impacts on coastal communities.

2. Materials and methods

2.1. Source of animals

Two experiments were initiated. For the first experiment, zooplankton were collected on August 8, 2001 by towing a 153-μm mesh, 0.5-m diameter net for approximately 2 min at two locations in the shallow coastal waters adjacent to the FSU Marine Laboratory at Turkey Point, FL (29°54′ N, 84°31′ W). The same protocol was followed on July 8, 2002 to collect samples for the second experiment. Surface seawater temperatures were recorded with a hand-held thermometer, surface salinity with a Reichert-Jung refractometer, and DO at 1.0 m depth with a YSI model-57 oxygen meter with a field probe. DO was not determined when samples were collected for the second experiment.

For both experiments, the contents of the cod end were placed in insulated coolers, diluted with ambient seawater, and transported to our main campus laboratory in Tallahassee, FL within 2 h. The 50–100-ml subsamples of the zooplankton in the cooler were anaesthetized with approximately 10.0–15.0 mg of 3-aminobenzoic acid ethyl ester, MS-222 (Sigma-Aldrich) to reduce swimming. Several hundred adult females and some males of *A. tonsa* were transferred to two 1-l beakers (~ 400 animals/beaker) containing 1 l of seawater filtered through glass fiber filters (GFF; Gelman A/E or Whatman GF/C) adjusted to a salinity of 30 ppt and 400 cells ml^{-1} of three dinoflagellate species (*Prorocentrum micans* (Proro), *Akashiwo sanguinea* (formerly *Gymnodinium sanguineum* (GSBL)) and *Scrippsiella trochoidea* (Peri)) for a final concentration of 1200 cells ml^{-1}. The dinoflagellates served as food for the copepods. The beakers were placed in an incubator set at 25 °C, 12 L–12 D (light-dark cycle). That evening at 19:00 h for the first experiment and 17:30 h for the second experiment, the contents of the beakers were filtered through nested sieves (153 and 48 μm) to separate the adults from the eggs. The fraction with the eggs was discarded; the medium and adults were rinsed back into the beakers, which were returned to the incubator.

The next morning at approximately 09:00 to 10:00 h, the contents of the beakers were filtered through a 153-μm mesh sieve to retain the adults and a 48-μm mesh sieve to retain the eggs, all of which were spawned within the 14–16-h period. The adults were discarded; the eggs were rinsed into 100 ml glass dishes and then distributed by micropipette into 90 petri dishes (50 eggs $dish^{-1}$). The contents of each petri dish were rinsed into individual 500-ml Erlenmeyer flasks containing 300 ml of GFF seawater adjusted to a salinity of 30 ppt. A food mixture of 200 cells ml^{-1} each of the dinoflagellates *P. micans*, *A. sanguinea* and *S. trochoidea* was added to the flasks, and the volume was brought up to 350 ml with GFF seawater. Each flask was capped with a rubber stopper with inlet and outlet ports, and attached to a gas manifold (see description below and Fig. 1). The manifolds and flasks were housed in a walk-in environmental chamber that was set at 25 °C and a 12-L–12-D. Every 2 days after the start of the experiment, the contents of each flask remaining on the manifolds were sieved through a 48-μm mesh sieve and the material retained on the sieve was rinsed into a flask containing clean GFF seawater adjusted to a salinity of 30 ppt (pre-bubbled for at least 1 h with the appropriate gas mixture) and food.

The temperature and salinity combination that we chose for the experiments was not selected to simulate the exact field conditions at the time animals were collected (see

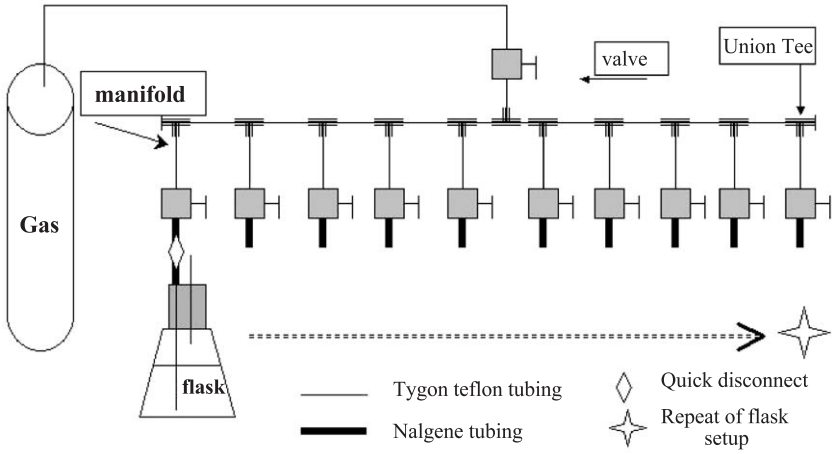

Fig. 1. Schematic of manifold system.

Results), but rather a common set of conditions that *A. tonsa* might experience during the summer period in the northern Gulf of Mexico. To determine the appropriate set of conditions, we examined the temperature and salinity records for the waters neighboring the FSU Marine Laboratory. Between July and September, temperatures generally range between 22 and 32 °C and salinity varies from 25 to 35 ppt. Higher salinity values occur during dry periods, whereas lower values are more common when rainfall, and therefore run-off, is heavy. Temperatures at the lower end of the range tend to coincide with lower salinity values.

2.2. Experimental equipment and design

The experiments were conducted with nine separate gas manifolds, each designed to deliver a desired gas mixture from a compressed gas cylinder to ten 500-ml Erlenmeyer flasks (Fig. 1). The gas cylinders were connected to the center point of manifold via needle valves to allow for the regulation of gas pressure from the cylinder. This central valve was connected to a series of 10 additional needle valves. The other end of each of these additional valves was connected to a Pasteur pipette using a piece of Nalgene tubing. The pipette was inserted through a stopper in the mouth of each flask. The pipette extended to within 13 mm of the flask bottom. In the second experiment, 2-ml polystyrene serological pipettes with a 100-μl tip were used instead of the Pasteur pipettes. Each piece of Nalgene tubing included a quick disconnect (Fisher cat #15-315-27C) to allow for easy removal of flasks. Each stopper was also fitted with a 10-cm long, 5-mm diameter glass tube as an exhaust port.

2.3. Gases and determination of DO concentrations

To achieve the desired level of DO in the flasks the contents were bubbled with compressed gas from the cylinders attached to each manifold. We used gas mixtures of

oxygen and nitrogen (Holox) for the hypoxic oxygen treatments and compressed air (Air Products) for the controls. The necessary gas mixtures of oxygen and nitrogen needed to achieve the target DO concentrations in the flasks were calculated based on Colt (1984).

The DO concentration of water in the flasks was periodically checked with a Clark mini-electrode that was connected to a Diamond General chemical micro-sensor (prod. #1231). The relationship between the electrical potential and the oxygen partial pressure is linear and allows for calibration by a two-point determination of DO. For the two-point method we used water that was bubbled with nitrogen to remove the DO (DO = 0%), and water that was saturated by bubbling with air (DO concentration of 21%, Colt, 1984).

There were three DO treatments: a control (saturation) and two with target hypoxic DO concentrations of 1.5 and 0.7 ml l^{-1}. According to Diaz and Rosenberg (1995), 1.0 ml l^{-1} DO is approximately equivalent to 1.4 mg l^{-1} DO. Each treatment was run in triplicate (3 manifolds $treatment^{-1}$).

2.4. Monitoring of survival and egg production

The first set of flasks from each treatment (one flask from each manifold) was removed from the manifolds after 24 h and the contents were washed through a 48-μm mesh sieve, preserved and stained in Lugol's iodine. The numbers of nauplii and unhatched eggs in these samples were determined by examination with a microscope and used to determine the initial hatching percentage. Thereafter a set of flasks was removed from the manifolds every 2–4 days, the contents were poured through a 48-μm mesh sieve, and the material remaining on the sieve was preserved in 10% formalin for subsequent examination with a microscope to determine survival. When the animals reached maturity as evidenced by the appearance of eggs, flasks were sampled as follows to determine egg production $female^{-1}$ day^{-1}. A set of flasks was removed from the manifolds, the contents were poured through a 153-μm mesh sieve and the animals remaining in the sieve were rinsed into a corresponding set of flasks, which was re-attached to the manifolds as described above. After 24 h, this set of flasks was removed and each flask was poured through 153- and 48-μm mesh sieves to retain and separate adults, and the eggs and nauplii produced in this 24-h period, respectively. The adults were rinsed into a dish with approximately 75.0 ml of seawater to which 2.5 ml of a solution of Neutral Red was added (stock solution, 0.1 g Neutral Red in 1-l distilled water). After 1 h, the adults were preserved in 10% formalin. Neutral Red is only absorbed by living organisms and was used therefore to distinguish live and dead adults. The eggs and nauplii were stained with Lugol's iodine solution and preserved. In Experiment 1, this egg production determination sequence was followed on days 8, 12, 16, 20, 24 and 28 for the control, days 10, 14, 18, 22 and 26 for the 1.5 ml l^{-1} treatment, and days 12, 14, 16, 18 and 20 for the 0.7 ml l^{-1} treatment. Egg collection did not commence in the 1.5 and 0.7 ml l^{-1} treatments until days 10 and 12, respectively, because development was delayed compared to the controls. In addition, the 0.7 ml l^{-1} treatment was sampled every 2 days because adult survival was much less than in the other treatments. We were concerned that, if we had longer sampling intervals, we would ultimately have fewer samples because the animals would have died. In Experiment 2, this egg production determination sequence was followed on days 13, 17, 21, 25 and 28 for the control and 1.5 ml l^{-1} treatments, and days 13, 15, 17 and 19 for the 0.7 ml l^{-1} treatment.

The preserved samples were analyzed as follows. The numbers of nauplii, copepodites and adults in each flask were counted. These values were used to calculate the mean number of individuals surviving in a set of flasks (three flasks per set) for each sampling date and treatment. The values were also converted to probits (assuming that 50 individuals were originally placed into each flask) and a regression analysis was performed with these values vs. the number of days (using a logarithmic transformation) since the start of the experiment. The regression equation was used to calculate the number of days required for survival to decrease to 50% (probit = 0) for each DO concentration. The numbers of live and dead adults (see above) were also determined at the time of an egg production assessment so that egg production per live female could be calculated. The number of eggs produced over each 24-h period in each flask was determined and expressed as eggs female^{-1} day^{-1}. A mean value was determined for the three flasks constituting a set for each day that egg production was determined. A grand mean of all egg production values for each treatment was also calculated, across days. The data for egg production were tested statistically using Levene's test for homogeneity of variances. Further analysis involved one-way analysis of variance (ANOVA), followed by Bonferroni *a posteriori* multiple comparison tests to determine if differences in the treatment mean values were significant at the $\alpha = 0.05$ level.

2.5. Model construction

We used the age-specific survival and egg production data from these two experiments to make long-term predictions of population dynamics, using a Leslie matrix modeling approach. These data were used to calculate the age-specific birth-flow survival probabilities (P_i) and fertilities (F_i) using Caswell's (2001, Eqs. (2.24) and (2.34)) birth-flow equations, where i is the age of individuals, at 2-day intervals. From this point onward, we distinguish between our empirically derived reproduction values (egg production) and these calculated values (fertilities). Fertility therefore refers to the mathematically calculated value of births (eggs hatched) at the midpoint of an age class, taking into account those eggs produced that do not successfully hatch and survive within the age class.

We constructed a separate Leslie matrix for each treatment (0.7 and 1.5 ml l^{-1}, control) and replicate, for a total of nine matrices for each experiment. Population growth rate was calculated as the dominant eigenvalue (λ) of each Leslie matrix; we present the means and standard deviations for each treatment.

We also conducted a decomposition analysis, which allows one to tease apart the relative contributions of survival and egg production to differences in population growth rates among treatments. Hypoxia could drive life history changes in either or both of these variables (survival and egg production) to varying degrees; the decomposition analysis uses the Leslie matrices constructed from our empirical data to determine which variable hypoxia altered more in terms of the resulting impact on population growth rate in the model. The time component is explicitly incorporated into this analysis, such that one can also pinpoint the organisms' ages at which hypoxia had its greatest effect on population growth rate. Greater impacts on population growth rate (λ) are calculated as greater contributions to changes in λ between the control and an experimental treatment. Because this analysis is

performed on the empirical data collected in our experiments, it provides us with very different information than the more theoretical elasticity analysis described below.

Our final step was to conduct elasticity analyses on our Leslie matrices. These procedures allow one to determine how population growth rate would be affected if the organisms in each treatment were further impacted by changes in their age-specific survival or egg production. The calculation is normalized such that direct comparisons can be made between how changes in survival and egg production at each age class will each impact the resulting population growth rate. This analysis is, therefore, a theoretical examination of which life history traits, if altered, would have the greatest impact on population dynamics. Greater elasticity values imply a larger proportional impact on population growth rate.

Both the decomposition and elasticity analyses were performed on the mean Leslie matrix for each treatment. All modeling was performed using the Maple mathematical software (version 8, Maplesoft, Waterloo, Canada).

2.6. Statistical analyses

Most results were analyzed by one-way analysis of variance (ANOVA) tests, at $\alpha = 0.05$. We also determined significance using the Bonferroni *a posteriori* multiple comparisons test procedure. The data analyzed by this method included the mean initial hatching percentage, the mean number of eggs produced female^{-1} day^{-1} and population growth rates. We also used probit analysis to estimate the number of days required for survival to decline to 50% for each of the DO treatments. The relative impact of the treatments was determined by calculating the relative median number of days for survival to decline to 50% and the associated 95% confidence intervals. The relative median number of days is the ratio of the number of days at each DO concentration that results in 50% mortality. If the associated confidence interval of a pair-wise comparison includes 1.00, then it is likely that the paired treatments have the same effect on mortality (SPSS, 1993). The data were analyzed using the SAS statistical software (version 8.0, SAS Institute, Cary, NC) and SPSS statistical software (version 11.5, SPSS, Chicago, IL).

3. Results

The temperature, salinity and DO concentration at the time the copepods were collected from the two sites in the field during August 2001 were 29 and 29 °C, 25 and 29 ppt, and 5.2 and 5.0 ml l^{-1} DO, respectively. The values for the second experiment were 29 and 30 °C and 32 and 32 ppt.

3.1. Experiment 1

The mean ($\pm$ S.D.) DO concentration achieved in the flasks was 6.2 (0.9), 1.8 (0.3) and 0.75 (0.2) ml l^{-1} for the control, 1.5 and 0.7 ml l^{-1} treatments, respectively.

Initial hatching success was evidenced by the number of nauplii and unhatched eggs recovered from the first set of flasks after 1 day. There were no significant differences in

Table 1
(A) Mean (± S.E.) percentage of eggs that had hatched by day one in each experiment and (B) mean (± S.E.) number of eggs female^{-1} day^{-1} in experiments with *A. tonsa* exposed to reduced (0.7 and 1.5 ml l^{-1}) and control (saturated) DO concentrations (one-way ANOVA, $\alpha = 0.05$, *a posteriori* Bonferroni multiple comparisons test)

Treatment	Experiment 1	Experiment 2
(A)		
Control	89 (3)[a]	56 (20)[a]
1.5 ml l^{-1}	93 (2)[a]	63 (21)[a]
0.7 ml l^{-1}	87 (9)[a]	53 (3)[a]
(B)		
Control	70.8 (7.1)[a]	93.7 (4.9)[a]
1.5 ml l^{-1}	28.8 (2.2)[b]	24.7 (2.1)[b]
0.7 ml l^{-1}	16.4 (5.1)[b]	2.0 (0.6)[c]

Treatments that share a letter superscript, within an experiment, are not significantly different.

hatching success among the treatments (Table 1A, one-way ANOVA, $\alpha = 0.05$; $p = 0.451$, $F_{2,\ 6} = 0.911$, MSE = 30.111). Survival of the nauplii, copepodites and adults in the flasks did not appear to differ between the 1.5 ml l^{-1} and control DO treatments (Fig. 2A). Since

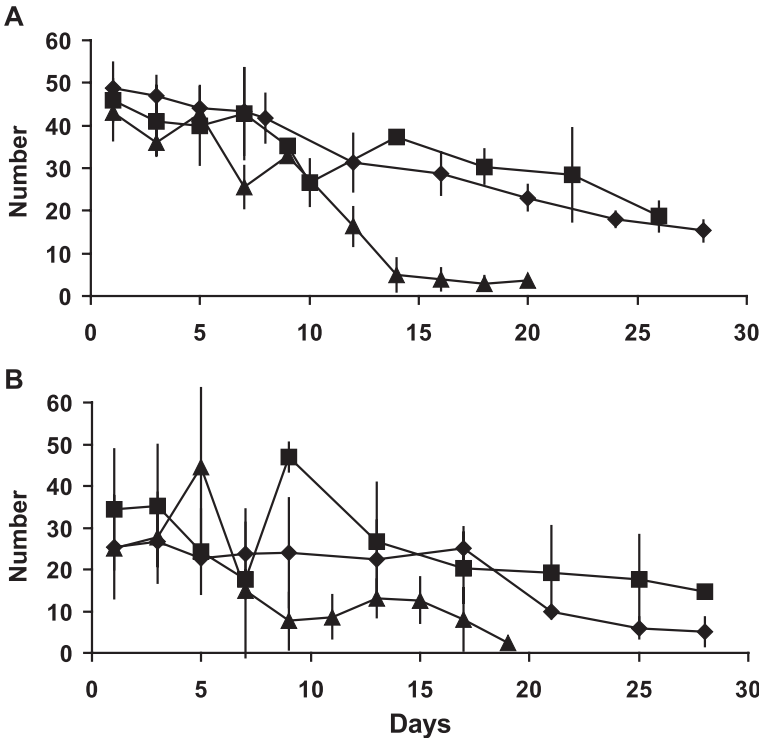

Fig. 2. *A. tonsa* survival in (A) Experiment 1 and (B) Experiment 2. Control treatment was saturating normoxic oxygen conditions (diamonds); reduced oxygen treatments included 1.5 (squares) and 0.7 ml l^{-1} (triangles).

Table 2
Number of days required for mortality to reach 50% in the control, 1.5 and 0.7 ml l^{-1} DO treatments in (A) Experiment 1 and (B) Experiment 2

Treatment	Days	95% Confidence interval	
		Low	High
(A)			
Control	18.53	13.99	25.48
1.5 ml l^{-1}	18.37	13.93	25.41
0.7 ml l^{-1}	5.88	4.35	7.70
(B)			
Control	3.13	1.16	5.93
1.5 ml l^{-1}	6.99	3.19	13.49
0.7 ml l^{-1}	1.20	0.29	2.59

the number of individuals surviving was not always determined on the same day for the three treatments, we used probit analysis to compare the effect of the treatments on survival (Table 2A). The results of this analysis indicated there was little difference in the time for mortality to reach 50% in the control and 1.5 ml l^{-1} treatments: 18.53 and 18.37 days, respectively. Survival in the 0.7 ml l^{-1} DO treatment appeared comparable to the other treatments through day 5, but thereafter appeared markedly less than in the 1.5 ml l^{-1} and control treatments (Fig. 2A). The results of the probit analysis indicated that unlike the control and 1.5 ml l^{-1} treatments, 50% of the animals exposed to 0.7 ml l^{-1} had died after only 5.88 days (Table 2A). The 95% confidence intervals for the number of days at which mortality reached 50% in the control and 1.5 ml l^{-1} treatments were almost identical, but were completely distinct from the 0.7 ml l^{-1} treatment (Table 2A). Based on the values of the relative median effective number of days for mortality to reach 50% (Table 3A), there is no reason to suspect a difference in the effect of the control and 1.5 ml l^{-1} treatments on survival (the associated confidence interval 0.68–1.51 contained 1.00),

Table 3
Relative median effective number of days for mortality to reach 50% in the control, 1.5 and 0.7 ml l^{-1} treatments in (A) Experiment 1 and (B) Experiment 2

Treatment	Estimate	95% Confidence interval	
		Low	High
(A)			
Control vs. 1.5 ml l^{-1}	1.01	0.68	1.51
Control vs. 0.7 ml l^{-1}	3.15	2.01	5.68
1.5 vs. 0.7 ml l^{-1}	3.13	2.03	5.47
(B)			
Control vs. 1.5 ml l^{-1}	0.45	0.12	1.10
Control vs. 0.7 ml l^{-1}	2.61	1.04	9.84
1.5 vs. 0.7 ml l^{-1}	5.84	2.11	34.82

If the confidence interval includes 1.00, then it is likely that the compared treatments have similar effects on survival (SPSS, 1993).

but there is reason to suspect that mortality is greater when exposed to 0.7 ml l^{-1} (the associated confidence intervals 2.01–5.68 and 2.03–5.47 did not include 1.00).

Estimates of the mean number of eggs produced female^{-1} day^{-1} were determined at periodic intervals after eggs were first observed in the flasks. In the control and 1.5 ml l^{-1} treatments, eggs were first observed on day 7. In the 0.7 ml l^{-1} treatment, eggs were first observed on day 9. Given the intervals of observation, this means that eggs were first produced sometime between days 5 and 7 for the control and 1.5 ml l^{-1} treatments, and between days 7 and 9 for the 0.7 ml l^{-1} treatment.

The numbers of eggs produced female^{-1} day^{-1} for the different treatments showed little variation among the collection days with the exception of day 8 for the control treatment (Fig. 3A). On this day, egg production female^{-1} day^{-1} was less than half that observed for the other days. Nevertheless, the overall mean number of eggs produced female^{-1} day^{-1} (70.8 ± 7.1) was significantly greater in the control treatment compared to the 1.5 and 0.7 ml l^{-1} treatments (28.8 ± 2.2 and 16.4 ± 5.1, respectively), while the mean number of eggs produced female^{-1} day^{-1} was not significantly different between the 0.7 and 1.5 ml l^{-1} treatments (Table 1B, one-way ANOVA, $\alpha = 0.05$; $p < 0.0001$, $F_{2,\ 42} = 26.97$, MSE = 472.12).

3.2. Experiment 2

The mean ($\pm$ S.D.) DO concentration achieved in the flasks was 4.9 (0.3), 1.5 (0.3) and 0.5 (0.2) ml l^{-1} for the control, 1.5 and 0.7 ml l^{-1} treatments, respectively.

There were no significant differences in the mean initial hatching success among the treatments (Table 1A, one-way ANOVA, $\alpha = 0.05$; $p = 0.775$, $F_{2,\ 6} = 0.266$, MSE = 276.33).

The number of animals surviving at each sampling varied considerably among replicates within treatments as evidenced by the large error bars (Fig. 2B). The results of the probit analysis indicated that mortality reached 50% after 3.13, 6.99 and 1.20 days in the control, 1.5 and 0.7 ml l^{-1} treatments, respectively (Table 2B). The 95% confidence intervals for the number of days at which survival had declined to 50% showed the greatest overlap between the control and 1.5 ml l^{-1} treatments. The 1.5 and 0.7 ml l^{-1} treatments did not overlap. Based on the values of the relative median effective number of days and the associated confidence intervals (Table 3B), there is confusing evidence for a difference in the effect of the treatments on survival. When the control was compared to the 1.5 and 0.7 ml l^{-1} treatments, the associated confidence intervals (0.12–1.10 and 1.04–9.84) included 1.00, suggesting no difference in the effects of these two low DO treatments on mortality compared to the controls. However, when the 1.5 and 0.7 ml l^{-1} treatments were compared to each other, the associated confidence interval (2.11–34.82) did not include 1.00, suggesting that mortality was greater when the animals were exposed to 0.7 ml l^{-1}.

Estimates of the mean number of eggs produced female^{-1} day^{-1} were determined at periodic intervals after eggs were first observed in the flasks. In the control and 1.5 ml l^{-1} treatments, eggs were first observed on day 9. In the 0.7 ml l^{-1} treatment, eggs were first observed on day 12. Given the intervals of observation, this means that eggs were first produced sometime between days 7 and 9 for the control and 1.5 ml l^{-1} treatments, and between days 11 and 12 for the 0.7 ml l^{-1} treatment.

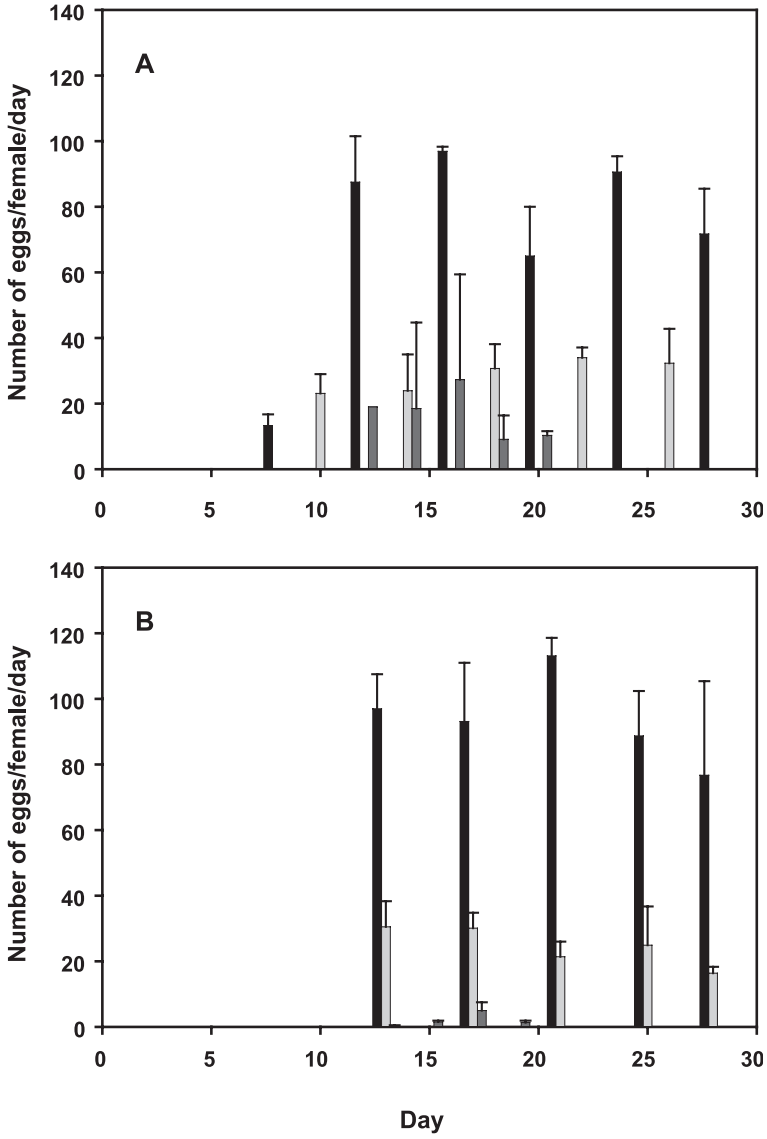

Fig. 3. *A. tonsa* mean (±S.D.) egg production female^{-1} day^{-1} for each day that eggs were collected for a determination in (A) Experiment 1 and (B) Experiment 2. Control treatment was saturating normoxic oxygen conditions (black); reduced oxygen treatments included 1.5 (light gray) and 0.7 ml l^{-1} (dark gray).

Since the egg production results of Experiment 1 suggested that only a few females had matured by day 8 in the control treatment, we waited to do the first determination of egg production female^{-1} day^{-1} in Experiment 2 until day 13. Indeed, the numbers of eggs produced female^{-1} day^{-1} for each of the treatments showed little variation among the collection days (Fig. 3B). Overall, the mean number of eggs produced female^{-1} day^{-1}

was significantly less in the 1.5 ml l^{-1} treatment compared to the control treatment, while the mean number of eggs produced female^{-1} day^{-1} was significantly less in the 0.7 ml l^{-1} treatment, compared to the control and 1.5 ml l^{-1} treatments (Table 1B, one-way ANOVA, $\alpha = 0.05$; $p < 0.0001$, $F_{2, 39} = 206.92$, MSE = 154.05).

During the second experiment, diatom contaminants were observed in some of the flasks. The number of diatoms was reduced each time the contents of the flasks were transferred to clean water, but they were never completely eliminated over the course of the experiment. We did not identify or quantify the contaminant.

3.3. Comparison of the experiments

Our experimental design enabled us to expose copepods to consistent concentrations of DO. The concentrations of reduced DO that were achieved in our experimental system differed by less than 30% from the initial target values of 1.5 and 0.7 ml l^{-1} (1.8 ± 0.3 and 0.75 ± 0.2 ml l^{-1}, and 1.5 ± 0.3 and 0.5 ± 0.2 ml l^{-1} for the first and second experiments, respectively). Hatching success as evidenced by copepod survival at the time of the first sampling interval was substantially lower in the second experiment compared to the first experiment (Table 1A). As time went on survival also was more variable within treatments in the second experiment compared to the first experiment (Fig. 2). However, in both experiments, the results of the probit analysis (Tables 2 and 3) indicated no difference in the impact of the control and 1.5 ml l^{-1} treatments on survival of the planktonic stages, whereas it is likely that the 0.7 ml l^{-1} had a significantly different and more negative effect on survival particularly in the first experiment. Despite the somewhat different results in survival of the planktonic stages, the trend of significantly greater egg production by animals in the control treatment and reduced egg production by animals in the 1.5 and 0.7 ml l^{-1} treatments was clearly evident in both experiments (Table 1B).

3.4. Model predictions

Population growth rate (λ) increased significantly with DO concentration in both experiments (one-way ANOVA, $\alpha = 0.05$; Experiment 1: $p < 0.0001$, $F_{2, 6} = 97.32$, MSE = 0.0110; Experiment 2: $p < 0.0001$, $F_{2, 6} = 167.41$, MSE = 0.0038). The pattern was remarkably consistent between the two experiments; λ in the low DO treatment (0.7 ml l^{-1}) was 51 % and 52 % of λ in the control treatment in Experiments 1 and 2, respectively, while λ in the 1.5 ml l^{-1} treatment was 80 % and 85 % of λ in the control treatment in Experiments 1 and 2, respectively (Fig. 4).

The decomposition analysis results are interpreted as follows: if there are no differences between the experimental treatment and the control treatment at a given age, the graph will show a contribution to changes in λ of 0.0. Negative contributions to a difference in λ occur when the variable (survival, egg production) is lower in the experimental treatment than in the control treatment; positive contributions imply greater values of the variable in the experimental treatment as compared to the controls. The results include the time component, such that one can pinpoint the age class at which the differences between a treatment and the controls were greatest (as seen by greater deviations from 0.0).

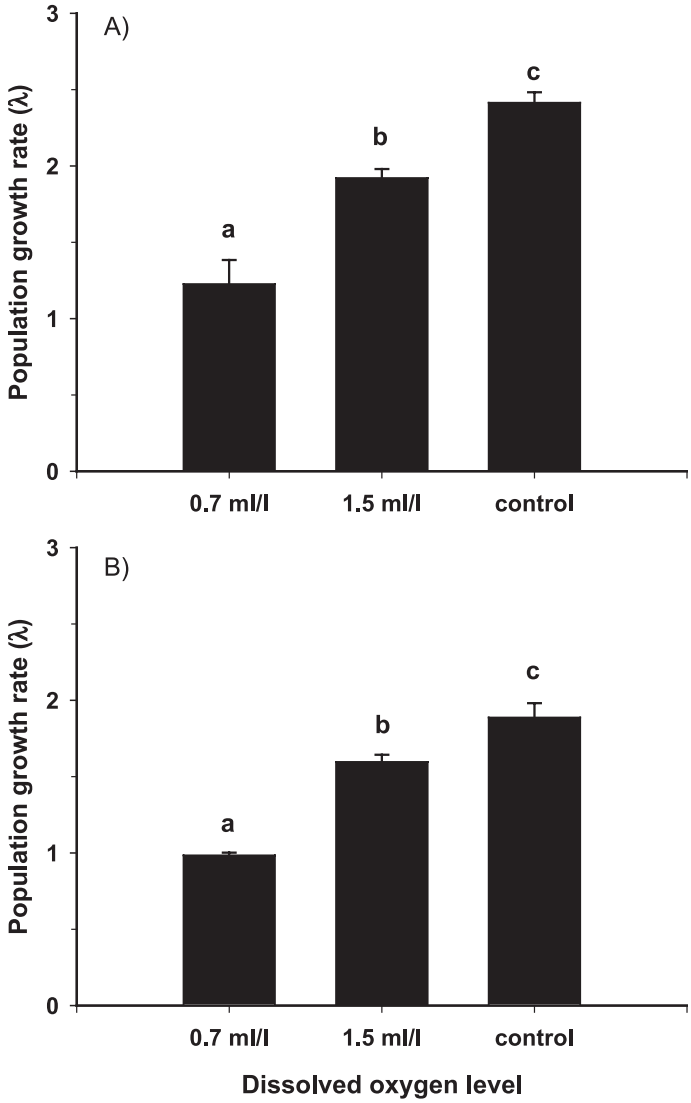

Fig. 4. Population growth rate (λ) (mean ± S.D.) of *A. tonsa* at reduced DO (0.7 and 1.5 ml l^{-1}) and saturated dissolved oxygen (control) in (A) Experiment 1 and (B) Experiment 2. Treatments that share a letter are not significantly different (one way ANOVA, $\alpha = 0.05$, *a posteriori* Bonferroni multiple comparisons test).

Looking at our results in Fig. 5, the greatest contributor to differences in λ between a treatment and the control is fertility, particularly between days 5 and 15. This negative spike in the decomposition analysis in both experiments, at both levels of hypoxia, implies that the reason we see significant reductions in population growth rates with reduced DO (both 0.7 and 1.5 ml l^{-1}) is due to lower egg production, particularly during the first 10 days after reaching reproductive maturity. We do, however, see earlier positive and

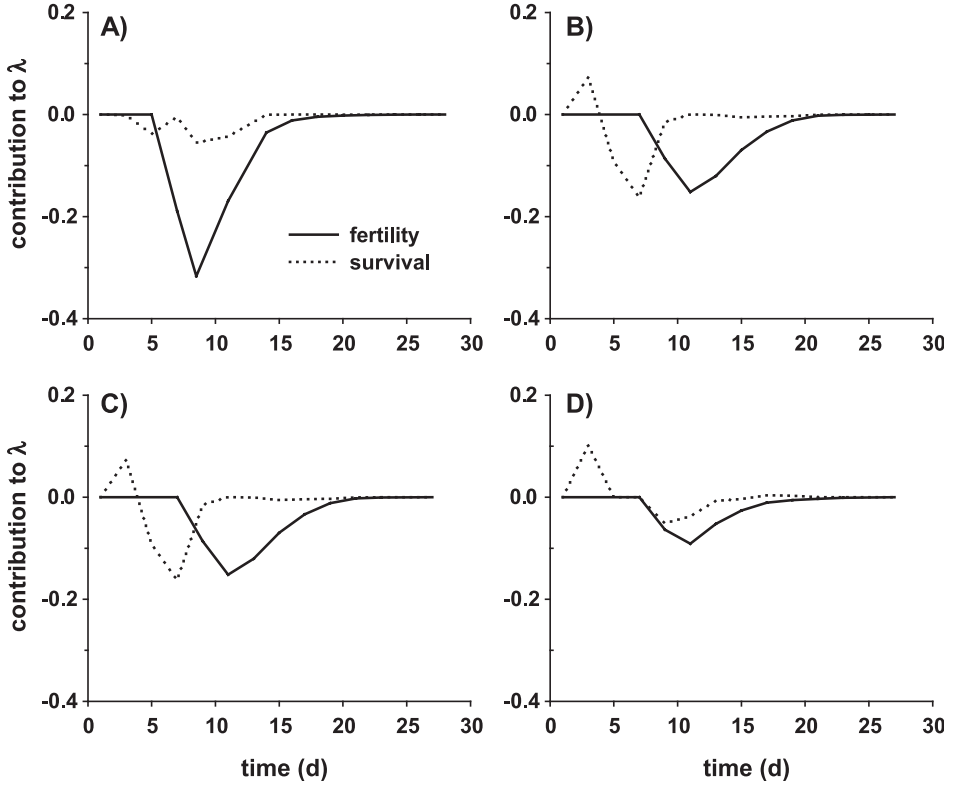

Fig. 5. Decomposition analyses based upon results from Experiment 1 (A, B) and Experiment 2 (C, D). Contributions of age-specific fertility (solid lines) and survival (dashed lines) to differences in population growth rate between control and 0.7 ml l^{-1} DO treatments (A, C) and between control and 1.5 ml l^{-1} DO treatments (B, D).

negative spikes due to a contribution of survival to the difference in population growth rate between the treatment and the control (Fig. 5b, c). These results correspond to pre-maturity survival, which is an important contributor to population growth rate early in life, but this is exchanged for the often greater and longer-lasting effect of hypoxia-reduced egg production on population growth rate. The magnitude of the contribution to changes in λ is diminished at the intermediate DO level (1.5 ml l^{-1}) relative to the magnitude at the lowest DO level (0.7 ml l^{-1}), which is consistent with our survival and egg production data: lower DO levels have a greater impact on these life history variables, which translates into lower population growth rates.

The elasticity analyses shows which vital rates (survival, egg production), if changed, would have the greatest impact on population growth rate. These calculations incorporate the time component, such that we are able to determine at what age class a vital rate would have its greatest impact on λ. Greater elasticities suggest a greater impact on population growth rate, if that vital rate were to be altered. Because elasticities are normalized, we can directly compare survival and fertility elasticities.

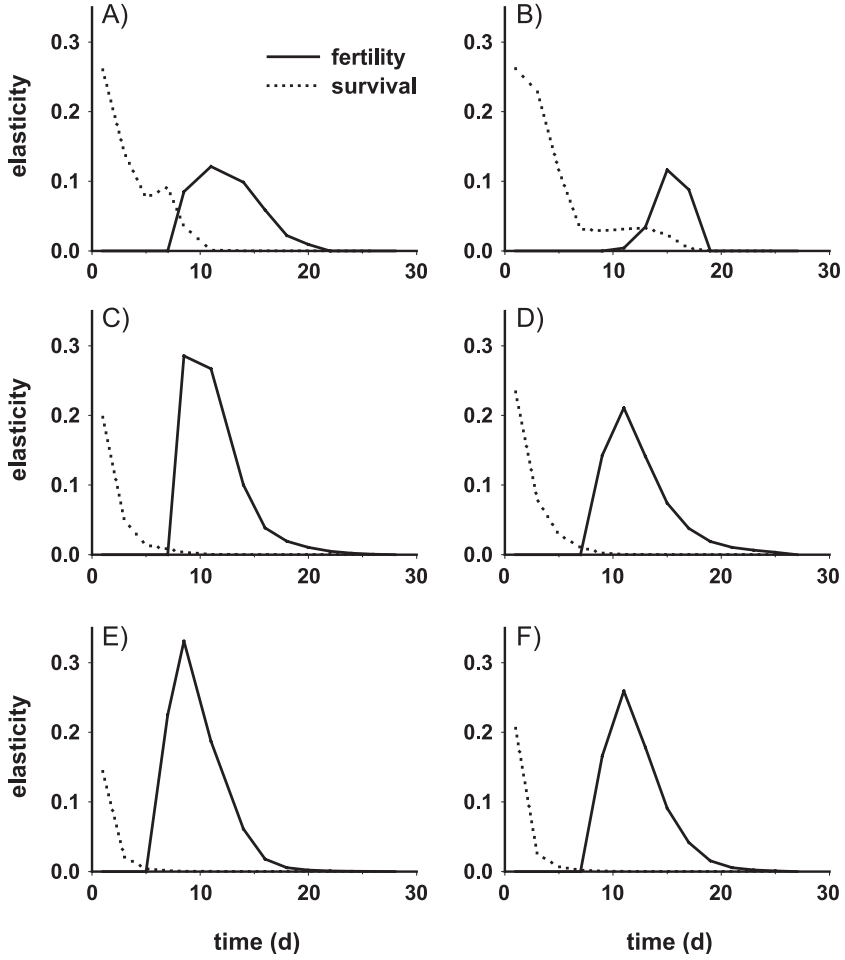

Fig. 6. Elasticity analyses based upon results from Experiment 1 (A, C, E) and Experiment 2 (B, D, F). Elasticities of fertility (solid lines) and survival (dashed lines) in the 0.7 ml l^{-1} DO treatment (A, B), the 1.5 ml l^{-1} DO treatment (C, D) and controls (E, F).

Elasticities calculated from data collected in both experiments suggest that the life history parameters having the greatest effects on population growth rate (λ) shift from pre-maturity changes in survival to post-maturity changes in fertility (Fig. 6). This pattern is consistent across experiments and treatments; however, the relative impacts of changes in survival and fertility are different among treatments. At the lowest DO level (0.7 ml l^{-1}), changes in survival early in life would have a far greater effect on λ than changes in fertility later in life (Fig. 6A–B). In the intermediate DO treatment (1.5 ml l^{-1}), changes in survival early in life and changes in fertility later in life would have relatively equivalent impacts on λ (Fig. 6C–D). Finally, in the control treatment, changes in survival early in life would have a relatively smaller impact on λ than changes in fertility later in life (Fig. 6E–F).

4. Discussion

Our findings clearly demonstrate that concentrations of DO that do not lead to mortality of the planktonic stages of the cosmopolitan marine copepod *A. tonsa* nevertheless have a substantial impact on egg production, an important life history parameter that relates to fitness. Similar results have been reported for the freshwater cladoceran, *Daphnia.* For example, Homer and Waller (1983) showed that the survival of *Daphnia* sp. was >85% at DO values >1.3 ml l^{-1}; however, reproduction was impaired below 1.9 ml l^{-1} and growth below 2.6 ml l^{-1}. Nebeker et al. (1992) also reported reduced reproductive effort in *D. pulex* at DO concentrations < 1.1 ml l^{-1}. Hanazato (1996) found that the growth rate of *D. pulex* that were exposed to reduced DO concentrations declined and maturation time was delayed. Hanazato and Dodson (1995) suggested that these changes could have been due to a reduction in the metabolic rate and/or a shift in energy allocation due to the cost of producing hemoglobin to facilitate oxygen uptake. In another study, Weider and Lampert (1985) found that clones of *Daphnia* varied in their response to low DO concentrations. While this variation was related to differences in hemoglobin concentration, the results suggested that organisms adjusted to reductions in DO concentrations through acclimation and adaptation. Recent studies have detected the production of hemoglobin by some deep-sea copepods, which are associated with hydrothermal vents (Hourdez et al., 2000; Sell, 2000), but there is no evidence for such oxygen-binding substances in coastal copepods such as *Acartia*.

While the results are clear in terms of an effect of hypoxia on egg production, the mechanism is not revealed by our studies. It may be that reduced DO concentrations cause a reduction in metabolism as observed for a wide variety of organisms. Further reductions in growth (somatic and reproductive) could arise if the reduction in metabolic rate is accompanied by reduced feeding, as observed for fish larvae exposed to low DO concentrations (Chabot and Dutil, 1999) and also for the common carp (Zhou et al., 2001). Since metabolic and grazing rates of copepods are also affected by temperature and food concentration, studies that take into account the interactive effects of these parameters are needed to accurately predict the impact of reduced DO in the field.

While survival may not be obviously affected by moderate hypoxia (e.g., 1.5 ml l^{-1} DO), longer-term chronic effects may impact the population dynamics of copepods, and this in turn may impact other elements of the food web through top-down and bottom-up effects. Our model results suggest that hypoxia can significantly reduce long-term population growth rate as a result of predominately sublethal impacts on *A. tonsa* egg production. The timing of hypoxic events relative to the life history of *A. tonsa* may play a role in determining the overall effect on copepod populations. Reductions in DO will have the greatest impacts on copepod population dynamics through lethal effects early in life, and through sublethal effects on egg production after maturity is reached. While the lethal effects of low DO can be substantial, it is important to reiterate that sublethal DO concentrations are enough to influence significant reductions in population growth rate.

Roman et al. (1993) reported that copepodite stages and nauplii of *A. tonsa* were not abundant in bottom waters of Chesapeake Bay during the summer when DO concentrations were < 1.0 mg l^{-1} (0.7 ml l^{-1}); however, the species did occur in the bottom waters when water column mixing led to higher DO concentrations in these layers. The

authors suggested that the decrease in copepod abundance resulted from reduced recruitment as a consequence of reduced egg hatching at such low DO concentrations. Keister et al. (2000) reported similar distributions for zooplankton in the Patuxent River (Chesapeake Bay). Copepod abundance, especially the naupliar stages, was reduced at DO concentrations <2.0 mg l^{-1} (1.4 ml l^{-1}), but copepods occurred in waters that were >2.0 mg l^{-1}. Though not identified in the paper to species, the copepods were most likely *A. tonsa* (D. Breitburg, personal communication). Although egg hatching may indeed be reduced at the lower of these concentrations, the results of our study suggest that reduced egg production by adult females is likely to be the most important factor contributing to reduced population growth under moderately hypoxic conditions. Because *A. tonsa* is ubiquitous, abundant and dominant in the plankton worldwide, the effects of hypoxia on these copepods is likely to have repercussions for many species in marine and estuarine food webs. More research that distinguishes the sublethal impacts of hypoxia on coastal organisms is needed.

Finally, a coincidental occurrence in our experiments has some relevance to the reported inhibitory effect of diatoms on the hatching success of copepod eggs (e.g., Ianora et al., 1995). In the second experiment, we noted the presence of diatom contaminants in some of the experimental flasks. It seems likely that these contaminants were introduced when the copepods used to initiate the experiment were collected in the field and subsequently transferred to beakers to obtain their eggs. This means that the copepods most likely experienced these diatoms in the wild. It may be that the initial hatching success of the eggs used to start the second experiment (53–63%) was substantially lower compared to the first experiment (87–93%) because the parents were exposed to these diatoms in the field. However, the presence of the diatoms during the second experiment did not alter the pattern of decreased egg production by copepods in response to hypoxic DO concentrations.

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