

# Growth and reproduction of gelatinous zooplankton exposed to low dissolved oxygen

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**ABSTRACT:** The lobate ctenophore *Mnemiopsis leidyi* and the scyphomedusan jellyfish *Chrysaora quinquecirrha* are seasonally important consumers in the food web of Western Atlantic and Gulf of Mexico estuaries, including Chesapeake Bay. The abundance and importance of these gelatinous species may be increasing as a result of anthropogenic alteration of these systems, particularly the increasing severity and extent of low dissolved oxygen. Ctenophores and jellyfish are more tolerant of hypoxia than co-occurring finfish, and can sustain high feeding rates in hypoxic waters. We examined the effects of hypoxia exposure on *M. leidyi* and *C. quinquecirrha* growth rates and *M. leidyi* reproduction over 4 d periods in 1 m<sup>3</sup> mesocosms at a range of natural prey densities. Both small (0.2 to 2.0 ml biovolume) and larger (8.0 to 17.6 ml biovolume) ctenophores had significantly reduced growth at oxygen levels of 1.5 and 2.5 mg l<sup>-1</sup> as compared to air-saturated water, especially at high prey densities. Egg production by large ctenophores was also significantly reduced by exposure to low dissolved oxygen concentrations. In contrast, *C. quinquecirrha* growth rates were unaffected by low dissolved oxygen concentrations tested. These results are counter-intuitive as *M. leidyi* preferentially utilizes moderately hypoxic bottom waters in the field, while *C. quinquecirrha* avoids such waters. Our findings suggest that hypoxia may differentially affect population growth of these dominant gelatinous species.

**KEY WORDS:** Hypoxia · Gelatinous zooplankton · Growth · Reproduction · Chesapeake Bay · *Chrysaora quinquecirrha* · *Mnemiopsis leidyi*

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## INTRODUCTION

Gelatinous zooplankton species, especially lobate ctenophores in the genus *Mnemiopsis* and the scyphomedusan jellyfish *Chrysaora quinquecirrha* (sea nettles), are important components of the pelagic food web of Chesapeake Bay and other estuaries along the Atlantic and Gulf of Mexico coasts of the United States (Baird & Ulanowicz 1989, Kremer 1994, Graham 2001, Sullivan et al. 2001). In Chesapeake Bay, *M. leidyi* abundances dramatically increase in spring and early summer, and these ctenophores can be significant predators on mesozooplankton, fish eggs and fish larvae (Cowan & Houde 1993, Purcell et al. 1994a,b, Purcell et al. 2001a). During the summer, *C. quinquecirrha* appears

to reduce ctenophore abundance through direct predation (Miller 1974, Purcell & Cowan 1995). Sea nettles also act as direct competitors of *M. leidyi* for the same zooplankton and ichthyoplankton food sources (Purcell et al. 1994a,b, Purcell et al. 2001b).

Anthropogenic influences, especially in the form of altered oxygen availability in Bay waters, may be changing the relative importance of gelatinous zooplankton in the Chesapeake Bay food web (Breitburg et al. 1997, 2003). Both the severity of bottom water oxygen depletion and the percent of the water column that is hypoxic vary considerably within the Chesapeake Bay system. Bottom layer oxygen concentrations in the most severely oxygen depleted regions of the mesohaline mainstem bay can remain near zero through much

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of the summer, with as much as 80% of the water column affected (Hagy et al. 2004). In contrast, in the upper and lower mainstem bay, the shallower regions of the mesohaline mainstem, and the tributaries, hypoxia tends to be less severe, more temporally variable and affect a smaller percent of the water column (Breitburg 1990, Breitburg et al. 2003). Hypoxia and anoxia are most severe during the summer months when density stratification is greatest (Taft et al. 1980, Sanford et al. 1990), and gelatinous zooplankton are most abundant. Regions of hypoxia (often defined as dissolved oxygen concentrations of  $<2 \text{ mg l}^{-1}$ ) and even anoxia (i.e.  $0 \text{ mg l}^{-1}$ ) probably occurred intermittently in the mainstem bay in pre-colonial times. However, the spatial and temporal extent, and the severity of low dissolved oxygen have greatly increased as a result of increased anthropogenic nutrient inputs to the bay and have worsened in both the mainstem Chesapeake bay and its tributaries during the 20th century (Cooper & Brush 1991, Adelson et al. 2001, Cronin & Vann 2003, D'Elia et al. 2003, Hagy et al. 2004).

Low dissolved oxygen (DO) levels can influence food webs in a number of ways because of variation among species in survival, feeding rates, growth, reproduction of organisms, and behavioral avoidance of hypoxic bottom waters. There is considerable variation in tolerance to low oxygen exposure among species in the Chesapeake bay ecosystem (Breitburg et al. 2003). Fish such as the bay anchovy *Anchoa mitchilli* and striped bass *Morone saxatilis* are among the most sensitive species and have 12 to 24 h  $\text{LC}_{50}$  values between 2 and 3  $\text{mg O}_2 \text{ l}^{-1}$  for various life stages (Chesney & Houde 1989, Miller et al. 2002). In contrast, *Chrysaora quinquecirrha* medusae and *Mnemiopsis leidyi* survive well when exposed to DO levels as low as  $0.5 \text{ mg l}^{-1}$  (Breitburg et al. 2003), although *C. quinquecirrha* polyp survival is reduced at higher oxygen concentrations (Condon et al. 2001). The sensitivity of non-gelatinous zooplankton, such as the copepod species *Acartia tonsa* common in the Chesapeake bay during summer months, tends to be intermediate to that of gelatinous zooplankton and fish species (Roman et al. 1993, Stalder & Marcus 1997). The predation rates of various Chesapeake bay species are also differentially affected by hypoxia. Predation by *C. quinquecirrha* and *M. leidyi* on ichthyoplankton and zooplankton and *C. quinquecirrha* predation on *M. leidyi* remain unchanged or increase at oxygen concentrations that reduce feeding rates of co-occurring finfish (Breitburg et al. 1997, 2003, Decker et al. 2004, S. Kolesar unpubl. data). The tolerance of *C. quinquecirrha* and *M. leidyi* to low oxygen exposure, and their high feeding rates under hypoxic conditions, may account, at least in part, for the high abundance of gelatinous zooplankton in systems such

as Chesapeake bay where hypoxia is common (Breitburg et al. 1997, Purcell et al. 2001a).

The effects of hypoxia on survival and feeding may contribute to the patterns of vertical distributions seen in pelagic Chesapeake bay species during summer months when hypoxia and anoxia are widespread. Fish larvae, (Breitburg 1994), copepods (Decker et al. 2003), and gelatinous zooplankton (D. Breitburg & R. Morris unpubl. data) are all behaviorally capable of regulating their depth in the water column and avoiding hypoxic or anoxic bottom waters, but do not always exhibit complete avoidance in the field. Fish larvae, copepods, and sea nettles all tend to show an increasing use of bottom waters as bottom DO increases and increasing avoidance of bottom waters with DO concentrations less than  $3 \text{ mg l}^{-1}$  (Keister et al. 2000, Breitburg et al. 2003). For fish larvae, this pattern may result from avoidance of areas where survival and feeding rates are low. In general, fish tend to avoid oxygen concentrations resulting in reduced growth rates (Breitburg 2002).

The extent to which hypoxia-induced feeding or growth impairment influences vertical distributions of gelatinous zooplankton in the field is not clear. Sea nettles require higher DO for survival during long-term exposures (Breitburg et al. 2003) and exhibit avoidance of bottom waters at higher DO levels than do *Mnemiopsis leidyi*. However, survival and predation by *Chrysaora quinquecirrha* is high under hypoxic conditions in which nettles are uncommon in the field; their preferential use of more oxic pycnocline and surface waters may result from following fish larvae and copepods into areas more preferred by these prey species. In contrast to sea nettles, *M. leidyi* preferentially use bottom waters when the DO concentration is between 1 and  $3 \text{ mg l}^{-1}$ , and only avoids areas with DO concentrations  $<1 \text{ mg l}^{-1}$  (Keister et al. 2000, Breitburg et al. 2003). In spite of their tolerance to low DO, this preferential use of hypoxic waters by *M. leidyi* is somewhat surprising since prey densities are substantially reduced as DO declines below  $2 \text{ mg l}^{-1}$ .

The effects of these habitat usage patterns on the population dynamics of gelatinous zooplankton species and the subsequent pattern of energy transfer in the bay food web remain uncertain. This is due largely to our current lack of understanding of how hypoxia affects the growth and reproduction of many species, including gelatinous zooplankters. Deep-sea medusae may maintain relatively high metabolic rates by switching between aerobic and anaerobic metabolism as they pass through oxygen minimum zones (Thuesen & Childress 1994), although there is little information about the effects of extended exposure to hypoxia on gelatinous zooplankton metabolism. The effects of hypoxia on ctenophore metabolism may be lessened by the fact that the primary energy demand for these

animals, i.e. the ctenes used in swimming, is located superficially, presenting a reduced oxygen diffusion distance between mitochondria and the outside environment (Childress & Seibel 1998).

This study was designed to investigate the effects of exposure to hypoxia on the growth and reproduction of *Mnemiopsis leidyi* and the growth of *Chrysaora quinquecirrha*. The response of these species was tested in mesocosm-sized enclosures over a range of food densities and DO concentrations, commonly experienced in the field. Given the very limited effect of hypoxia exposure on ctenophore feeding rates and the strong selective pressure that should be placed on these animals to be adapted to the conditions of a habitat they appear to be actively selecting, we expected minimal impact of hypoxia exposure on *M. leidyi* growth and reproduction. In contrast, we expected *C. quinquecirrha* to show greater reduction in growth than *M. leidyi* because the medusa is slightly more sensitive to low oxygen exposure and shows avoidance of bottom waters at higher oxygen concentrations. Our results differed considerably from these *a priori* predictions.

## MATERIALS AND METHODS

**General experimental design.** All experiments were performed in 12 outdoor 1 m<sup>3</sup> cylindrical fiberglass mesocosms (107 cm diameter × 122 cm high) submerged to a depth of 90 cm in raceways with flowing Patuxent River water to keep temperatures in the mesocosms similar to that of the river. Prior to the start of any trial, the mesocosm walls were washed with dilute hydrochloric acid and rinsed with freshwater to reduce algal growth that would have interfered with oxygen level regulation. The mesocosms were filled over the course of several days with water drawn from the Patuxent River that had been nominally filtered to 0.5 µm.

Water inside the mesocosms was exchanged at 10% per day with 0.5 µm filtered river water and continuously mixed using 4-blade PVC paddlewheels suspended horizontally over the tanks on fiberglass rods. Paddlewheels rotated at 4 rpm for a period of 6 h, after which they would stop for 5 min, and then reverse direction for the next 6 h. This paddlewheel system provided natural turbulence levels and effectively mixed the water to the bottoms of the mesocosms (M. Bundy & L. Sanford unpubl. data)

Fiberglass lids were placed over the mesocosms and taped down to minimize gas exchange between the enclosed headspace and the surrounding air. Lids extended several centimeters above the paddlewheels and enclosed a headspace of 0.3 m<sup>3</sup>. A hinged door provided access to the interior of each mesocosm for sampling. Black plastic sheeting covered the outside of

the mesocosms and lid tops to reduce light penetration and phytoplankton growth. White sheets were draped over the lids to reduced light absorbance and heating in the headspaces.

Target DO concentrations were achieved prior to the addition of organisms by vigorously bubbling mesocosms with N<sub>2</sub> and were maintained during trials by regulating DO levels in the headspace using a method modeled after Greccay & Stierhoff (2002). Microelectrode sensors (Microelectrodes Inc.) were suspended inside the headspace of the mesocosms assigned to reduce DO treatments. At least once per day, these sensors were calibrated to determine the relationship between the sensor voltage output and DO levels in the calibration vessels. The slope and intercept of these relationships were then entered into the computer control program (LabView: National Instruments) and used to convert the voltage output of the appropriate sensor into a DO reading for the mesocosm headspace. If the headspace DO level was above or below the target level by more than 0.2 mg l<sup>-1</sup>, the LabView control program opened a solenoid. This allowed either nitrogen gas or compressed air, as appropriate, to be added to the headspace via tubing. Gas addition was limited to no more than 90 s, which meant that the headspace of each reduced DO mesocosm was checked and adjusted at least once every 10 to 12 min over the course of the 4 d experiment. The rotation of the paddlewheels served to mix the gas added to the headspace into the water and to change the DO level in the water, albeit with some lag time. Dissolved oxygen levels and water temperatures were checked in all mesocosms approximately every 4 h from 08:00 to 24:00 h using either a YSI model 95 or 85 DO meter (Yellow Springs Instruments).

The hypoxia exposure period of 4 d used here should be realistic given the temporal duration of hypoxia events in areas such as the Patuxent River (Breitburg et al. 2003). This represents a significant duration given the ability of small ctenophores to more than double their body size per day under optimal conditions (Reeve et al. 1978) and the fact that egg production is sensitive to environmental changes (e.g. changes in food supply) occurring over the span of only a few days (Reeve et al. 1989).

Ctenophores (*Mnemiopsis leidyi*) and sea nettle medusae (*Chrysaora quinquecirrha*) used in the trials were collected on the morning of the first day of each trial. Ctenophores were collected by towing or hand-dipping 202 µm mesh nets. Sea nettle medusae were collected exclusively by hand dipping and were transferred from the nets into buckets by hand. Because individual *M. leidyi* were too delicate to measure without risking damage that could affect growth rates, their initial sizes were estimated from randomly selected

individuals that were not used in the experiments. In the lab, ctenophores were sorted into 15 groups, with an attempt made to visually sort ctenophores of similar sizes sequentially into the 15 holding vessels. Individuals in 3 groups randomly selected from the 15 were measured for biovolume by lightly blotting the ctenophores held in aquarium dip nets on paper towels and then placing them individually into graduated cylinders. In all but the first trial, ctenophores were initially sorted into 2 distinct size classes (small, with biovolumes generally less than 0.2 ml, and large, with biovolumes greater than 6 ml). The initial sizes of sea nettle medusae used in the experiments were measured directly by placing the medusae in shallow pans of water and measuring the bell diameter using a plastic ruler (Table 1).

Mesozooplankton used as food for gelatinous zooplankton were collected daily with 202  $\mu\text{m}$  nets from the Patuxent River using repeated short tows or from extra mesocosms filled with unfiltered Patuxent River

water and supplemented with N and P to encourage phytoplankton growth. Cod-end contents were poured gently through 1 cm mesh screens to remove small ctenophores and medusae and into 20 l buckets partially filled with air-saturated water. In the laboratory, the buckets were gently siphoned using 35  $\mu\text{m}$  filters to reduce the volume, and the contents were partitioned using a Wildco Folsom plankton splitter. Two replicate 30 ml aliquots were taken from each split for zooplankton enumeration, and splits were combined as appropriate to achieve the desired zooplankton density. The combined splits were then immediately added to the mesocosms. 87% of mesozooplankton in 2000 trials and 94% in 2001 trials were adult and copepodite stages of the calanoid copepod *Acartia tonsa*. The remainder of the mesozooplankton was generally comprised of the copepod *Eurytemora affinis*, barnacle nauplii and cyprids, and crab zoea.

Mesozooplankton densities in the mesocosms were estimated for *Mnemiopsis leidyi* trials as the average of 6 samples. The initial 24 l sample was

Table 1. Starting gelatinous zooplankton sizes and physical conditions in mesocosms averaged by trial and dissolved oxygen DO treatment. Values in parentheses are 1 SD. Variability was not estimated for starting ctenophore sizes since these were not directly measured for experimental animals. Starting sizes for all individual ctenophores were assumed to be the mean value (see text for details)

<b>Ctenophores</b>						
Year	Trial	Average start size (ml)		DO treatment ( $\text{mg l}^{-1}$ )	Average DO ( $\text{mg l}^{-1}$ )	Average temp. ( $^{\circ}\text{C}$ )
		Small	Large			
2000	1	0.2	–	1.5	1.66 (0.66)	25.1 (0.5)
				Air-saturated	6.84 (0.56)	25.1 (0.5)
	2	0.2	11.3	1.5	1.57 (0.24)	26.2 (0.6)
				Air-saturated	6.70 (0.34)	26.3 (0.6)
	3	0.18	8.3	1.5	1.46 (0.16)	24.7 (0.5)
				Air-saturated	6.13 (0.32)	24.7 (0.5)
2001	1	0.6	15.6	1.5	1.77 (0.26)	20.5 (0.7)
				2.5	2.48 (0.32)	20.5 (0.7)
				Air-saturated	7.15 (0.32)	20.5 (0.7)
	2	2.1	17.6	1.5	1.50 (0.17)	22.6 (0.8)
				2.5	2.45 (0.21)	22.5 (0.8)
				Air-saturated	7.28 (0.38)	22.6 (0.7)
<b>Sea nettles</b>						
Year	Trial	Average start size (mm)		DO treatment ( $\text{mg l}^{-1}$ )	Average DO ( $\text{mg l}^{-1}$ )	Average temp. ( $^{\circ}\text{C}$ )
2001	1	44.3 (6.1)		1.5	1.32 (0.27)	27.6 (0.9)
		44.7 (5.1)		2.5	2.09 (0.21)	27.5 (1.0)
		45.4 (5.6)		Air-saturated	6.20 (0.29)	27.5 (0.9)
	2	46.8 (7.1)		1.5	1.32 (0.14)	26.5 (0.6)
		46.7 (6.8)		2.5	2.20 (0.19)	26.6 (0.7)
		46.8 (6.7)		Air-saturated	6.10 (0.72)	26.5 (0.6)
	3	45.4 (6.0)		1.5	1.50 (0.09)	29.3 (0.7)
		45.0 (5.1)		2.5	2.53 (0.15)	29.3 (0.7)
		45.3 (4.7)		Air-saturated	6.30 (0.32)	29.3 (0.7)

taken 1 h after the first zooplankton addition but before ctenophores were added. Twelve liter samples were taken in the afternoons of the first and third day after the start of the experiment, prior to the daily addition of zooplankton. Two additional 12 l samples were taken on the second day of the experiment; one was taken prior to the daily addition of zooplankton and used to estimate the number of zooplankton needed in a particular mesocosm, while the second was taken 1 h after zooplankton addition to confirm densities achieved. A final 24 l sample was taken after ctenophores were removed from mesocosms on the 4th day of the experiment. In all cases, the plankton were collected by pumping water at approximately  $10 \text{ l min}^{-1}$  through a submerged 202  $\mu\text{m}$  mesh sieve and were preserved in 5% buffered formalin. For the sea nettle trials, only the initial and final mesozooplankton samples were taken because the pump damaged the tentacles of the medusae and interfered with subsequent feeding and growth. The reported average mesozooplankton densities in sea nettle trials are therefore the average of these 2 samples and the counts performed on the splits before addition. In some cases, neutral red stain was added to the zooplankton samples, which were allowed

to sit for 1 h prior to formalin preservation. This stain is taken up by live organisms, and the results of these stainings consistently showed that the bulk of the organisms collected were alive at the time of sampling and were probably not dead organisms suctioned from the bottom of the mesocosms.

**Ctenophore trials.** In June and July of 2000, we ran 3 *Mnemiopsis leidyi* growth trials. In the first trial, 10 small *M. leidyi* were added to each of 12 mesocosms, 6 with only air added to the headspace (referred to throughout as air-saturated) and 6 at a target DO concentration of  $1.5 \text{ mg l}^{-1}$  (Table 1). Mesozooplankton densities in each DO treatment ranged from  $<1$  zooplankter  $\text{l}^{-1}$  to about  $14 \text{ l}^{-1}$ . Dissolved oxygen treatment and mesozooplankton density were randomly assigned to the mesocosms with the caveat that equal numbers of mesocosms with the same DO treatment (i.e. three  $1.5 \text{ mg l}^{-1}$  and 3 air-saturated) were assigned to each of the 2 raceways surrounding the mesocosms. In the 2 subsequent trials 10 small and 5 large *M. leidyi* were added to each of 6 air-saturated DO and six  $1.5 \text{ mg l}^{-1}$  DO mesocosms. The highest zooplankton densities in these 2 trials were only  $11.8$  and  $5.8$  zooplankters  $\text{l}^{-1}$ , respectively, because of extremely low field densities.

At the end of the 4 d experimental period in each trial, ctenophores were removed from the mesocosms by gently pulling them toward the water surface using dip nets and then dipping beakers below the water surface. Small individuals were immediately measured for biovolume, while the large individuals were transferred to 10 l aquaria in the laboratory. These aquaria were filled with  $0.5 \mu\text{m}$  filtered river water that had been adjusted to the same DO level as the mesocosms from which the ctenophores were taken. Prior to the transfer, mesozooplankton had been added to the aquaria at the same density as in the mesocosms, and a second addition of zooplankton was added 4 h later. Four hours after this second mesozooplankton addition, the ctenophores were transferred to a second set of aquaria identical to the first except for the absence of mesozooplankton. The ctenophores were left in these aquaria for 16 h, after which they were removed and their biovolumes measured. The water in the aquaria was backfiltered through  $35 \mu\text{m}$  sieves, and the final, reduced volume (about 200 ml) was preserved with 10% Lugol's iodine. These water samples were subsequently examined for the presence of ctenophore eggs, which were counted in their entirety.

In May and June 2001, 2 additional *Mnemiopsis leidyi* trials were conducted using both small and large ctenophores. These trials differed from those conducted in 2000 in that there were 3 target DO treatments:  $1.5 \text{ mg l}^{-1}$ ,  $2.5 \text{ mg l}^{-1}$ , and air-saturated

(Table 1), each tested at 4 different mesozooplankton densities. High field densities allowed us to use higher maximum mesozooplankton densities ( $43.6$  and  $50.8$  zooplankters  $\text{l}^{-1}$  in the first and second trials, respectively) in the 2001 trials than in 2000. *M. leidyi* egg production was estimated using 3 replicate 2 ml aliquots taken from the preserved samples using Stempel pipets, a change necessitated by the much greater egg production that occurred in the 2001 trials.

The zooplankton sampling schedule allowed us to estimate the disappearance rate of mesozooplankton on Days 1 and 3 of the ctenophore trials in which both large and small ctenophores were used. Mesozooplankton disappearance during this time period could result from ctenophore feeding or natural mortality. However, our neutral red stainings indicated that there were few dead but uneaten mesozooplankton present in the tanks even at the low DO concentration and that the bulk of the disappearance was likely be due to direct ingestion by ctenophores. Because zooplankton samples were only taken on the first and last days of the trials for sea nettles (below), we could not estimate sea nettle feeding in a similar way.

**Sea nettle trials.** Three trials were performed in June, July, and August of 2001 in which 6 sea nettle medusae were added to each of the experimental mesocosms. All of these trials included the same 3 DO treatments as the 2001 *Mnemiopsis leidyi* trials, i.e.  $1.5$  and  $2.5 \text{ mg l}^{-1}$  DO and air-saturated (Table 1). Floating barriers constructed of  $6.35 \text{ mm}$  mesh Vexar excluded the medusae from the volume of water directly contacted by the paddlewheels and prevented medusae tentacles from becoming stuck to or damaged by the paddlewheel as it turned.

**Data analysis.** All analyses were performed using the SAS statistical software, Version 8 (SAS Institute). Because of the large difference in mesozooplankton abundance between the 2000 and 2001 trials, analyses were performed separately on data from the 2 years, with trials within a given summer treated as replicates. The growth of small and large *Mnemiopsis leidyi*, egg production by large *M. leidyi*, and sea nettle growth were analyzed using mixed-model ANCOVA (when no interactions with the covariate were statistically significant) or ANOVA with DO treatment as the main-effect, mesozooplankton abundance as a covariate (ANCOVA) or continuous main-effect variable (ANOVA), and trial as random blocks. In models in which the DO by mesozooplankton abundance interaction term was not significant (indicating an equality of slopes for the main effects levels), the interaction term was dropped from the model. For models in which the interaction was significant, differences between the DO treatment levels

were estimated at the minimum, mean, and maximum mesozooplankton densities using the methods suggested by Littell et al. (1996). Statistical analyses of growth data presented here use percent growth (in terms of biovolume for *M. leidy* and bell diameter for sea nettles) as the response variable, but in all cases, analyses performed using instantaneous growth rate as the response variable yielded similar results. Data were examined for normality and homoscedasticity and were left untransformed for statistical analyses with the exception of egg production data for which variance grouping was used to adjust for unequal variances (Littell et al. 1996).

## RESULTS

### Small ctenophore growth

Small *Mnemiopsis leidy* in the 2000 trials grew rapidly over the 4 d trial periods, with the highest growth being a 468% increase in biovolume in 1 high DO tank at an average mesozooplankton density of 15.3 ind. l<sup>-1</sup> (Fig. 1a). Ctenophores increased in size in all tanks except for 3 in which mesozooplankton densities averaged < 0.64 ind. l<sup>-1</sup> and ctenophore size decreased. The direct effect of DO on ctenophore growth in ANOVAs was not significant (df = 1, 24.4;  $F = 2.57$ ;  $p = 0.122$ ) although growth was positively related to mesozooplankton density (df = 1, 26;  $F = 152.42$ ;  $p < 0.0001$ ), and the test for an interaction between DO treatment and mesozooplankton density was significant (df = 1, 24.4;  $F = 19.60$ ;  $p = 0.0002$ ). While DO did not significantly affect estimated growth at the minimum mesozooplankton density of 0.6 l<sup>-1</sup> (df = 24.1,  $t = -1.29$ ,  $p = 0.208$ ), ctenophores did grow significantly faster at high DO tank at the mean mesozooplankton density of 4.8 l<sup>-1</sup> (df = 23.9,  $t = 2.73$ ,  $p = 0.012$ ) and at the maximum mesozooplankton density of 15.3 l<sup>-1</sup> (df = 24.3,  $t = 5.11$ ,  $p < 0.0001$ ). Increasing food concentration thus tended to increase the relative differences in growth due to DO level (Fig. 1a).

Small *Mnemiopsis leidy* growth was also high in the 2001 trials with only 3 tanks showing an average decrease in body volume (all of which were low DO treatment tanks)(Fig. 1b). Although average mesozooplankton densities were much higher in the 2001 trials than in 2000 (21.8 ind. l<sup>-1</sup> and 4.8 ind. l<sup>-1</sup>, respectively), the highest growth achieved in any 2001 tank was 370% in a high DO tank with a mesozooplankton density of 47.6 l<sup>-1</sup>. Initial size of small ctenophores in the 2001 trials was larger than that in 2000 and temperatures were a few degrees cooler (Table 1), potentially contributing to differences in growth rate between years. Both DO treatment (df = 2, 19;  $F = 10.62$ ;  $p =$

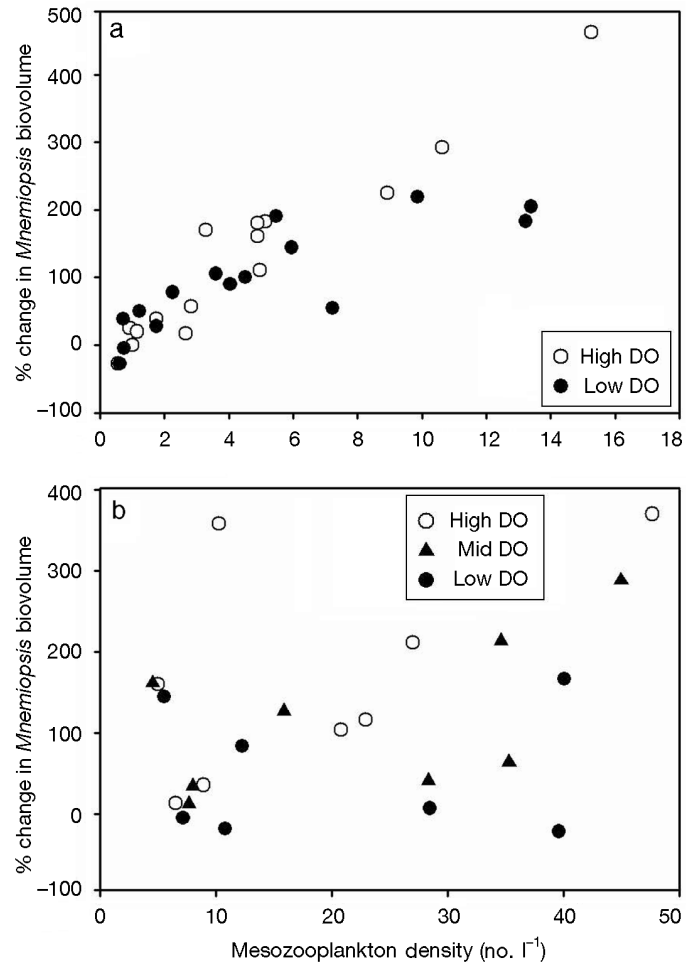


Fig. 1. *Mnemiopsis leidy*. Growth (in % change in biovolume) of small ctenophores in (a) trials in 2000 and (b) trials in 2001 at 1.5 mg l<sup>-1</sup> (low), 2.5 mg l<sup>-1</sup> (mid), and air-saturated (high) DO concentrations

0.0008) and mesozooplankton density (df = 1, 19;  $F = 5.07$ ,  $p = 0.036$ ) significantly affected growth, and the interaction between DO treatment and mesozooplankton density was not significant. All levels of DO treatments in the 2001 trials were significantly different from one another, with ctenophore growth increasing with increasing DO level (Table 2).

### Large ctenophore growth

Large *Mnemiopsis leidy* responded differently than small individuals in growth trials. In 2000, large ctenophores in all low DO tanks, and in high DO tanks with mesozooplankton prey densities  $\leq 2$  individuals l<sup>-1</sup> shrank (Fig. 2a). DO treatment had a marginally significant effect on the change in body size

Table 2. *Mnemiopsis leidyi* and *Chrysaora quinquecirrha*. Mean growth (as percent change in body size) in response to DO treatment. Data are mean  $\pm$  SE (number of mesocosm tanks) percent growth, and mean  $\pm$  SE instantaneous growth rate. The duration (in days) used for instantaneous growth calculations was based on the time animals were added to, and removed from, mesocosms, and does not include the time large ctenophores were held for egg production experiments. \*\*: means are significantly different at  $\alpha = 0.05$ . \*: means are significantly different at  $\alpha = 0.1$ . Means sharing a common letter are not significantly different using pairwise comparisons. Initial sizes are the ranges of average starting biovolumes (ml) (for *M. leidyi*) or bell diameter (mm) (for *C. quinquecirrha*) in the various trials. Mean growth rates of sea nettles were very close to zero in all DO treatments (especially at the intermediate DO concentration tested) reflecting the strong effect of prey density and negative growth at low prey density

Variable	Year	Initial sizes	Growth rate	High DO	Mid DO (2.5 mg l <sup>-1</sup> )	Low DO (1.5 mg l <sup>-1</sup> )
Small <i>Mnemiopsis leidyi</i> growth	2000	0.18 – 0.2	Percent	128.1 $\pm$ 34.5 (15)		97.0 $\pm$ 20.0 (15)
			Instantaneous	0.19 $\pm$ 0.04		0.17 $\pm$ 0.03
Small <i>Mnemiopsis leidyi</i> growth	2001	0.6 – 2.1	Percent	171.1 $\pm$ 47.7 (8) <sup>a</sup>	117.9 $\pm$ 34.6 (8) <sup>b</sup>	55.9 $\pm$ 26.6 (8) <sup>c,**</sup>
			Instantaneous	0.26 $\pm$ 0.05	0.20 $\pm$ 0.04	0.09 $\pm$ 0.05
Large <i>Mnemiopsis leidyi</i> growth	2000	8.3 – 11.3	Percent	1.3 $\pm$ 8.3 (9)		-29.8 $\pm$ 5.2 (9) <sup>*</sup>
			Instantaneous	0.004 $\pm$ 0.02		-0.11 $\pm$ 0.02
Large <i>Mnemiopsis leidyi</i> growth	2001	15.6 – 17.6	Percent	13.6 $\pm$ 4.1 (8) <sup>a</sup>	-14.1 $\pm$ 3.2 (8) <sup>b</sup>	-32.6 $\pm$ 4.2 (8) <sup>c,**</sup>
			Instantaneous	0.04 $\pm$ 0.01	-0.05 $\pm$ 0.01	-0.12 $\pm$ 0.02
<i>Chrysaora quinquecirrha</i> growth	2001	44.8 – 46.7	Percent	4.1 $\pm$ 3.3 (12)	0.2 $\pm$ 3.8 (11)	2.8 $\pm$ 3.1 (12)
			Instantaneous	0.0004 $\pm$ 0.0004	-0.00006 $\pm$ 0.00005	0.0003 $\pm$ 0.0003

(df = 1,13;  $F = 4.39$ ;  $p = 0.056$ ) and mesozooplankton density again had a highly significant effect on growth (df = 1, 13.1;  $F = 41.78$ ;  $p < 0.0001$ ), with ctenophores in higher mesozooplankton density tanks tending to grow more or lose less body volume. The interaction between DO treatment and mesozooplankton density was also highly significant (df = 1,13;  $F = 11.54$ ;  $p = 0.005$ ) indicating unequal slopes for the 2 DO treatments. Estimates of the difference in growth between high DO tanks and low DO tanks were significant at the minimum (df = 13,  $t = 2.74$ ,  $p = 0.017$ ), mean (df = 13,  $t = 7.45$ ,  $p < 0.0001$ ) and maximum (df = 13,  $t = 5.38$ ,  $p < 0.0001$ ) mesozooplankton densities used in these trials, suggesting that DO treatment does have an impact on growth within this range of prey densities.

Large *Mnemiopsis leidyi* in 2001 trials responded similarly to those in 2000 in that all low DO tanks experienced negative changes in average body size even though mesozooplankton densities were much higher than those used in 2000 (Fig. 2b). The mean response was for positive growth, however, in the high DO tanks (Table 1). DO treatment had a significant effect on body size change (df = 2,19;  $F = 57.73$ ;  $p < 0.0001$ ), with ctenophores in higher DO level tanks growing significantly more (or shrinking less) than those in lower DO tanks (Table 2). All 3 DO treatments differed significantly from each other. These trials also differed from the 2000 trials in that mesozooplankton density had no significant effect on growth (df = 1,19.1;  $F = 0.85$ ;  $p = 0.37$ ) perhaps reflecting the larger size of ctenophores tested in 2001. There was no significant interaction between DO treatment and mesozooplankton density.

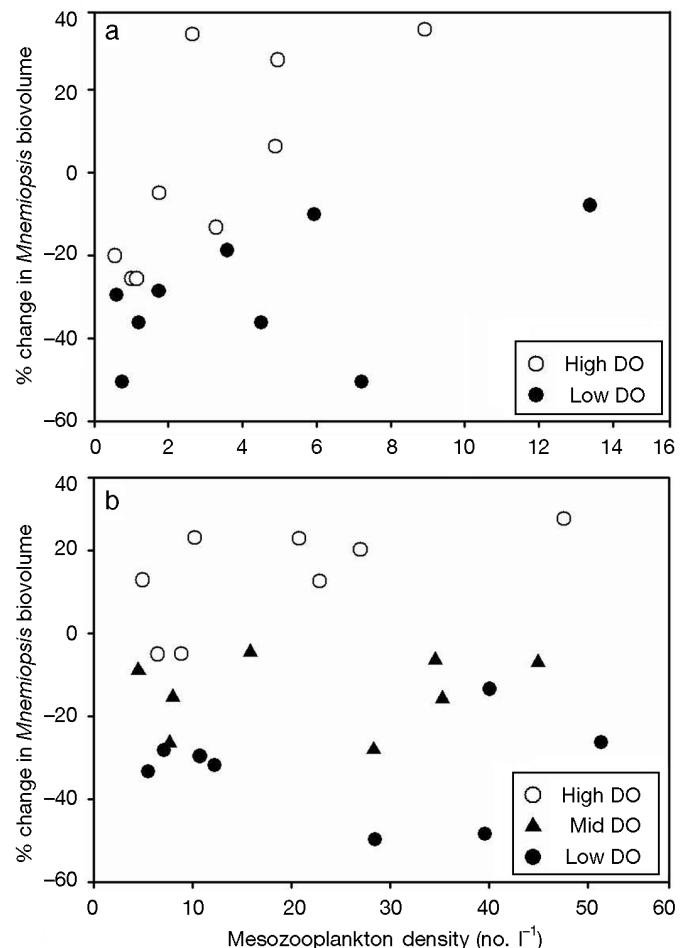


Fig. 2. *Mnemiopsis leidyi*. Growth (in % change in biovolume) of large ctenophores in (a) trials in 2000 and (b) trials in 2001 at 1.5 mg l<sup>-1</sup> (low), 2.5 mg l<sup>-1</sup> (mid), and air-saturated (high) DO concentrations

### Ctenophore egg production

Egg production by large *Mnemiopsis leidyi* in the 2000 trials was strongly affected by the mesozooplankton density experienced during the trial ( $df = 1, 7.1$ ;  $F = 6.58$ ,  $p = 0.037$ ), and it appears that this was especially true for ctenophores that had experienced a high DO environment throughout the trial (Fig. 3a). Egg production by low DO ctenophores was uniformly low at all mesozooplankton densities (Fig. 3a). Although the ANCOVA analysis suggested that DO treatment did not significantly affect egg production ( $df = 1, 7.1$ ;  $F = 0.03$ ;  $p = 0.874$ ), there was a marginally significant interaction between mesozooplankton density and DO treatment ( $df = 1, 7.0$ ;  $F = 6.11$ ;  $p = 0.043$ ). When the differences in egg production were estimated across the range of mesozooplankton densities experience by the ctenophores, there were significant differences when densities were at or above the mean density (Table 3).

The patterns of egg production in 2001 resembled those of 2000, with higher prey densities resulting in generally higher overall egg production ( $df = 1, 17$ ,  $F = 3.43$ ,  $p = 0.081$ ) (Fig. 3b). However, in the 2001 trials, there was also a direct positive effect of DO on egg production ( $df = 2, 17$ ,  $F = 9.75$ ,  $p = 0.002$ ). Egg production was highest at high DO, intermediate at  $2.5 \text{ mg l}^{-1}$ , and lowest at  $1.5 \text{ mg l}^{-1}$ , with all treatments statistically distinct. There was a marginally significant interaction between the mesozooplankton density and the DO environment ( $df = 2, 17$ ;  $F = 3.49$ ;  $p = 0.054$ ). Tests of the estimated differences in egg production suggest that low DO ctenophores produced significantly fewer eggs than either mid or high DO individuals at the minimum, mean and maximum prey densities (all  $p < 0.02$ ). The estimated difference between high and mid DO treatment ctenophores was not significant at the minimum mesozooplankton density tested ( $p = 0.413$ ) but became so at higher densities (all  $p < 0.02$ ).

Because mesozooplankton densities affected growth rates, and ctenophore size is known to affect egg production (at least under high DO concentrations), we also examined the potential effects of final ctenophore biovolume on egg production (Fig. 4a,b). Both simple regressions and stepwise models (with final ctenophore size, the average DO concentrations measured in each mesocosm, mesozooplankton prey densities and trial provided as potential explanatory variables) indicated that ctenophore size was positively and significantly related to egg production (measured either as the total eggs produced or eggs produced  $\text{ml}^{-1}$  ctenophore). In stepwise regressions ctenophore size explained the greatest percent of the variance with

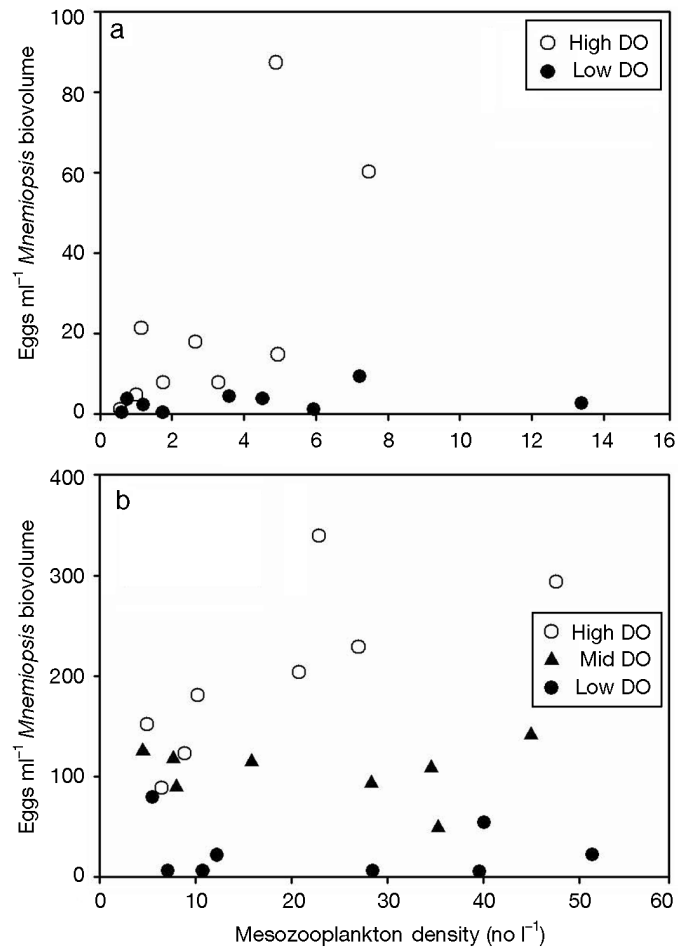


Fig. 3. *Mnemiopsis leidyi*. Egg production of large ctenophores compared to the availability of mesozooplankton prey in (a) trials in 2000 and (b) trials in 2001 at  $1.5 \text{ mg l}^{-1}$  (low),  $2.5 \text{ mg l}^{-1}$  (mid), and air-saturated (high) DO concentrations.

partial  $R^2$  values of 0.66 ( $p < 0.0001$ ) and 0.71 ( $p < 0.0001$ ) in 2000 and 2001, respectively.

Determining the effect of DO on egg production beyond its effect on final ctenophore size was confounded by the minimal to absent overlap in the range of final sizes among DO treatments (Fig. 4a,b) and significant DO by ctenophore size interactions. Mixed model

Table 3. Results of estimated differences in large ctenophore egg production during the trials in 2000 over the range of mesozooplankton densities at 3 dissolved oxygen levels. Estimated differences are back-transformed from  $\log_{10}$ -transformations

O <sub>2</sub> comparison	Mesozooplankton density (ind. l <sup>-1</sup> )	Estimated difference	df	t	p
High–low	0.6	2.1	13	0.20	0.848
High–low	3.8	25.8	13	4.82	0.0048
High–low	13.4	96.0	13	4.03	0.0025



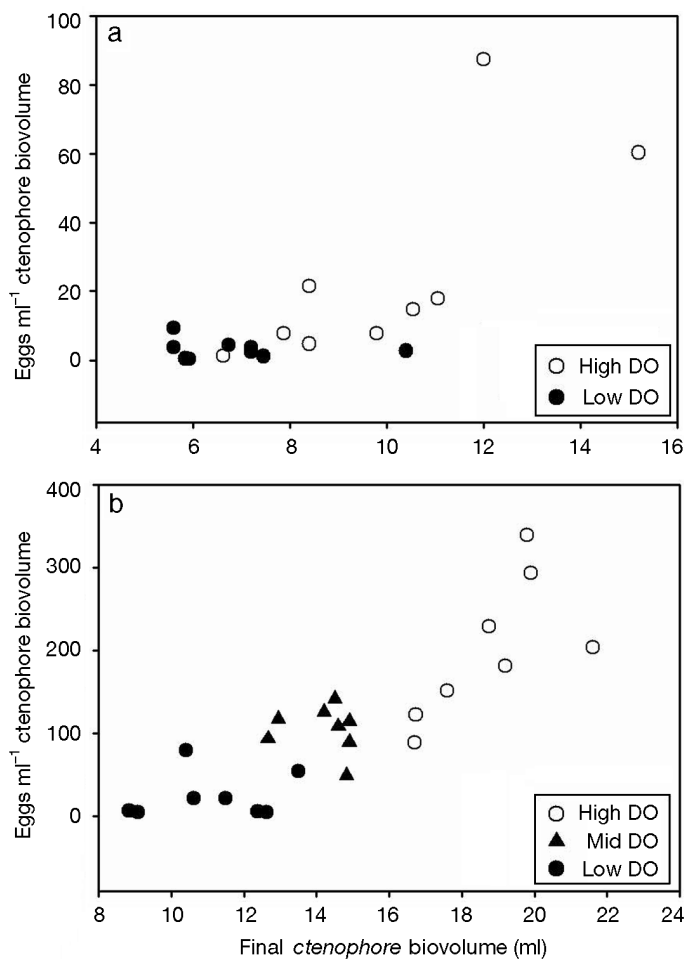


Fig. 4. *Mnemiopsis leidyi*. Egg production of large ctenophores compared to the final sizes of ctenophores in (a) trials in 2000 and (b) trials in 2001 at 1.5 mg l<sup>-1</sup> (low), 2.5 mg l<sup>-1</sup> (mid), and air-saturated (high) DO concentrations

ANOVAs indicated significant effects of DO treatment (df = 1, 7.26;  $F = 5.04$ ;  $p = 0.058$ ), final ctenophore size (df = 1, 7.32;  $F = 7.66$ ;  $p = 0.028$ ) and the interaction between DO and ctenophore size (df = 1, 7.57;  $F = 9.66$ ;  $p = 0.016$ ) on the number of eggs produced per ml of ctenophore during the 2000 trials, and significant effects of DO treatment (df = 2, 17;  $F = 3.90$ ;  $p = 0.040$ ) and the interaction between ctenophore size and DO (df = 2, 17;  $F = 3.99$ ;  $p = 0.038$ ) in 2001. The effect of final ctenophore size on eggs produced per ml of ctenophore in 2001 was not significant (df = 1, 17;  $F = 1.16$ ;  $p = 0.297$ ).

### Ctenophore feeding

DO treatment did not significantly affect estimated clearance rates on either the initial day (df = 1, 15;  $F = 0.71$ ,  $p = 0.413$ ) or Day 3 (df = 1, 14;  $F = 0.65$ ;  $p = 0.435$ ) of trials 2 and 3 in 2000 (Table 4). Clearance rates were

Table 4. *Mnemiopsis leidyi*. Clearance rates of ctenophores by DO treatment. Data are percent reduction per day in mesozooplankton density from initial value. Means are least square means with standard errors given in parentheses. \*: means are significantly different at  $\alpha = 0.10$ . Means sharing a common letter are not significantly different using pairwise comparisons. Year 2000 means are calculated using data from Trials 2 and 3 only (where both small and large ctenophores were present). Statistical results of estimated differences in the year 2001 initial day rates are given in Table 5

Year	Day	Clearance rate (% reduction d <sup>-1</sup> )		
		High DO	Mid DO (2.5 mg l <sup>-1</sup> )	Low DO (1.5 mg l <sup>-1</sup> )
2000	Initial	42.5 (7.8)	–	51.8 (7.8)
2000	3	38.1 (16.1)	–	27.7 (16.0)
2001	Initial	63.8 (12.3)	8.9 (12.3)	13.7 (11.8)
2001	3	36.8 (8.2) <sup>a,b</sup>	42.8 (7.7) <sup>a</sup>	19.6 (7.7) <sup>b</sup>

also independent of the initial mesozooplankton density during both time periods (initial period: df = 1, 15;  $F = 0.14$ ;  $p = 0.716$ ; Day 3: df = 1, 14.7;  $F = 1.31$ ,  $p = 0.270$ ) (Fig. 5a,b). There was no significant interaction between DO treatment and mesozooplankton density during either period.

In contrast to the 2000 trials, ctenophore clearance rates during the initial period in the 2001 trials were significantly affected by DO treatment (df = 2, 17;  $F = 6.07$ ;  $p = 0.010$ ), although there was a marginally significant interaction between DO treatment and initial mesozooplankton density (df = 2, 17;  $F = 3.55$ ;  $p = 0.052$ ) (Fig. 6a). Estimates of the differences in clearance rate at the minimum, mean, and maximum mesozooplankton densities indicate that reduction of DO concentrations to 2.5 mg l<sup>-1</sup> or lower did independently have a significant negative effect, at least at the minimum and mean mesozooplankton densities used in these trials (Table 5).

Clearance rates on Day 3 of the 2001 trials were marginally affected by DO treatment (df = 2, 18.1;  $F = 2.68$ ;  $p = 0.096$ ) with ctenophores in 2.5 mg l<sup>-1</sup> mesocosms clearing the water column at higher rates than those in the lowest DO treatment (Table 4). Clearance rates during this time period were not significantly affected by the initial mesozooplankton density (df = 1, 19;  $F = 0.01$ ;  $p = 0.911$ ) and there was no interaction between DO treatment and initial mesozooplankton density (Fig. 6b). The negative changes in mesozooplankton density observed at the lowest initial levels in both years are presumed to be due to measurement error.

### Sea nettle growth

As with large ctenophores, many of the sea nettle tanks showed a negative change in average bell diam-

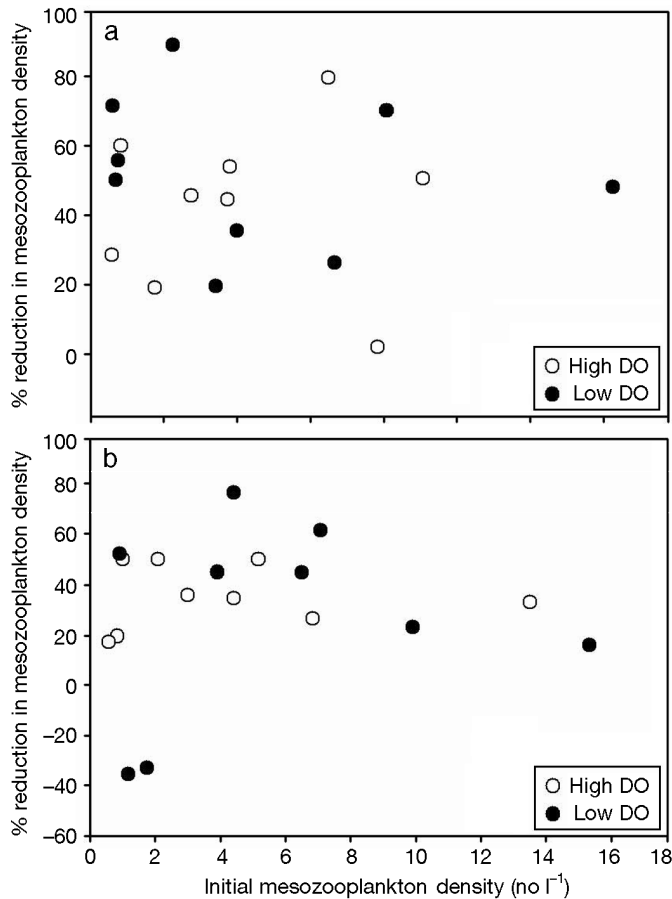


Fig. 5. Percent reduction in mesozooplankton density between successive samples at 1.5 mg l<sup>-1</sup> (low) and air-saturated (high) DO concentrations. (a) Day 1 and (b) Day 3 samples during ctenophore trials in 2000

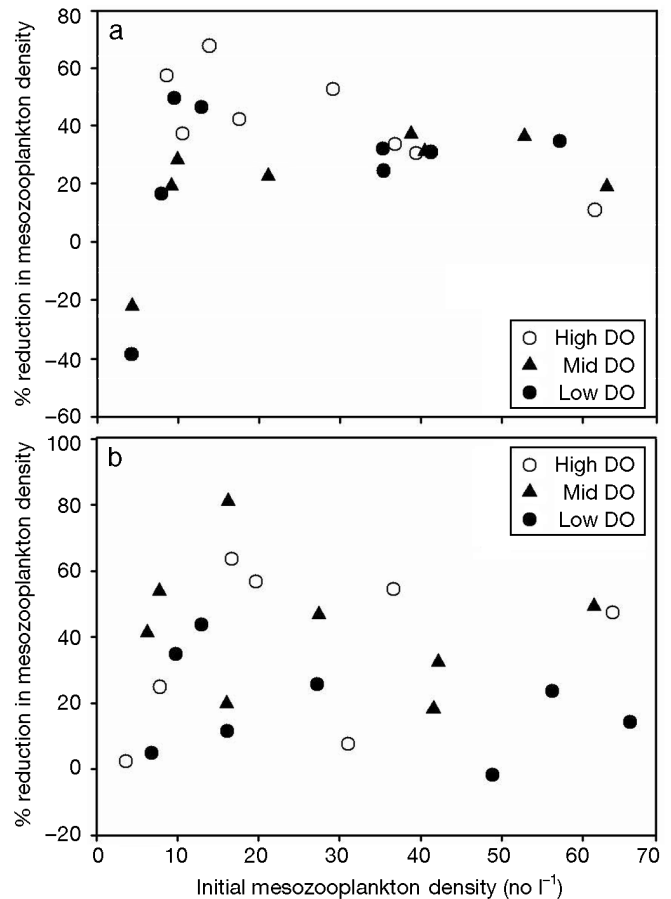


Fig. 6. Percent reduction in mesozooplankton density between successive samples at 1.5 mg l<sup>-1</sup> (low), 2.5 mg l<sup>-1</sup> (mid), and air-saturated (high) DO concentrations. (a) Day 1 and (b) Day 3 samples of ctenophore trials in 2001

eter, especially in low mesozooplankton density tanks (Fig. 7), and the overall average growth was generally low at all DO levels (Table 2). There was no significant effect of DO treatment on growth (df = 2, 29; *F* = 0.14;

Table 5. Results of estimated differences in clearance rates (in percent reduction per day) by the total ctenophore biovolume at 3 DO levels during the initial days of the 2001 trials. Mesozooplankton densities represent the minimum, overall mean, and maximum starting densities in the mesocosms

O <sub>2</sub> comparison	Mesozooplankton density (ind. l <sup>-1</sup> )	Estimated difference	df	<i>t</i>	<i>p</i>
High–mid	4.3	49.7	17.0	3.20	0.005
High–mid	27.5	21.2	17.0	2.23	0.039
High–mid	62.9	42.5	17.4	1.21	0.244
High–low	4.3	44.8	17.0	2.93	0.009
High–low	27.5	15.7	17.0	1.65	0.117
High–low	62.9	39.4	17.3	2.03	0.006
Mid–low	4.3	-14.9	17.0	0.33	0.746
Mid–low	27.5	-9.5	17.0	0.58	0.571
Mid–low	62.9	-35.1	17.3	2.03	0.058

*p* = 0.871), although there was a highly significant effect of mesozooplankton density (df = 1, 29; *F* = 16.34; *p* < 0.0004), with average growth tending to increase with increasing mesozooplankton density (Fig. 7). There was no significant interaction between mesozooplankton density and DO treatment (df = 2, 27; *F* = 0.37; *p* = 0.691)

For these analyses, medusae that had entered the Vexar cages and potentially contacted the paddlewheels were not included in the calculation of the final average bell diameter change. This problem was only encountered in the first of the 3 trials and generally necessitated removing only 1 or 2 sea nettles per mesocosm from the body size calculations. Analyses run using the final sizes of all sea nettles yielded similar results.

### DISCUSSION

The results of our experiments indicated a clear difference in the way that the ctenophore *Mnemiopsis*

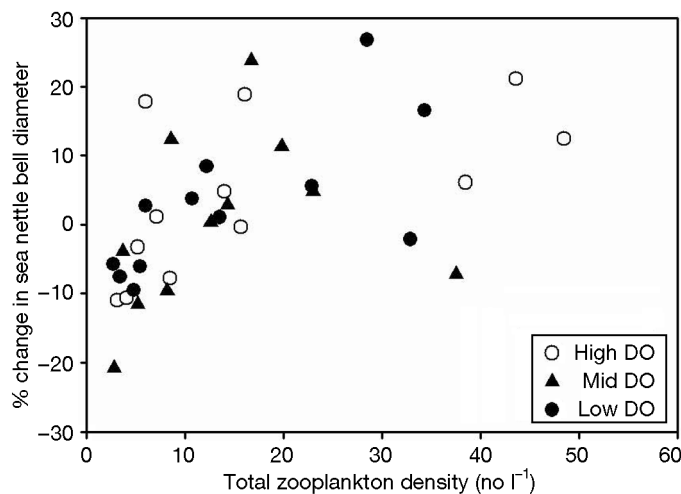


Fig. 7. *Chrysaora quinquecirrha*. Growth (in % change in bell diameter) at 1.5 mg l<sup>-1</sup> (low), 2.5 mg l<sup>-1</sup> (mid), and air-saturated DO (high) concentrations in trials in 2001

*leidy* and the scyphomedusan jellyfish *Chrysaora quinquecirrha* respond to low dissolved oxygen levels. These differences were counter to what would be predicted *a priori* based on experiments that show that ctenophores have higher survival rates at low DO and field data indicating that ctenophores preferentially utilize moderately hypoxic bottom waters while sea nettles do not. Dissolved oxygen levels  $\leq 2.5$  mg l<sup>-1</sup> significantly reduced the growth of small *M. leidy*, and the effect of low DO on growth became more pronounced as mesozooplankton densities increased (Fig. 1a,b; Table 2). Large ctenophores were similarly affected by changes in DO (Fig. 2a,b; Table 2), although growth was unaffected by changes in mesozooplankton density once these densities were greater than about 10 ind. l<sup>-1</sup> (2001 trials: Fig. 2b). The growth effects experienced by large ctenophores, however, were more dramatic than those experienced by smaller individuals. Small individuals were generally able to maintain positive growth at all combinations of mesozooplankton density and oxygen concentration (Fig. 1a,b), but the growth of large individuals was negative in all reduced DO tanks regardless of food concentration (Fig. 2a,b). Even with a high percentage of mitochondria towards the surface in ctenophores (Childress & Seibel 1998), larger size would present greater diffusion distances to be overcome in providing oxygen for metabolism, which may explain, at least in part, the greater effects of reduced oxygen on growth in large animals. Laboratory experiments indicate that large individuals of *C. quinquecirrha* and the ctenophore *Beroe ovata* have lower survival rates at low DO than do smaller conspecifics (Breitburg et al 2003); similar experiments have not been performed with *M. leidy*.

It is not clear whether DO directly affected *Mnemiopsis leidy* egg production or whether the effect was only indirect through the influence of DO on growth rate (Figs. 3 & 4). By the end of the growth experiment, when reproduction was measured, ctenophores in all high DO mesocosms averaged larger in size than those in any of the mid or low DO treatment mesocosms. Because of the lack of overlap in sizes among treatments, we could not evaluate the significant DO  $\times$  ctenophore biovolume interaction to determine whether similar sized ctenophores produced fewer eggs under low DO than under high DO conditions.

The effects of low DO on growth and reproduction are likely to be exacerbated in the field due to the reduced numbers of mesozooplankton present in hypoxic bottom waters. While all possible combinations of DO level and mesozooplankton density were used in these experiments, mesozooplankton in the field are free to migrate and avoid areas of hypoxia. Thus, gelatinous zooplankton populations may only rarely experience the specific combination of low oxygen levels and high food densities and would most commonly be simultaneously negatively impacted by low food densities whenever they are impacted by hypoxia. This appears to be particularly true when bottom water DO levels fall below 2 mg l<sup>-1</sup> (Roman et al. 1993, Keister et al. 2000).

Sea nettles presented a dramatic contrast to ctenophores in their growth patterns under reduced DO conditions. Dissolved oxygen levels as low as 1.5 mg l<sup>-1</sup> had no significant effects on the growth of *Chrysaora quinquecirrha* medusae at any mesozooplankton density (Fig. 7, Table 2). In contrast, mesozooplankton density did have a significant effect of growth, with the medusae experiencing positive growth only above densities of about 10 l<sup>-1</sup> (Fig. 7). While sexual reproduction was not measured in these experiments, the lack of effect of DO reduction on growth and the fact that asexual strobilation occurs in polyps at DO levels as low as 0.5 mg l<sup>-1</sup> (Condon et al. 2001) suggest that DO effects on sexual reproduction might be minimal, at least within the range of DO concentrations tested here. While we did not test the interaction of jellyfish size and reduced oxygen effects on growth, the increased diffusion distance present in larger medusae may result in growth changes in jellyfish larger than those used in our trials.

The growth effects observed in *Mnemiopsis leidy* under reduced DO are most likely not due to altered feeding behaviors. While reduced growth was observed under reduced DO conditions in both years (Fig. 1a,b, Fig. 2a,b), DO treatment only affected presumptive ctenophore clearance rates in the 2001 trials (Fig. 5a,b Fig. 6a,b; Tables 4 & 5). Decker et al. (2004) report that clearance rates of *M. leidy* averaging

2.9 ml in volume (intermediate between our small and large-size ctenophores) are unaffected by DO concentrations as low as  $1.0 \text{ mg l}^{-1}$  over 1 h periods. However, individuals averaging 22.5 ml (larger than those used in this study) had very slightly higher clearance rates at 1, 2 and  $3 \text{ mg l}^{-1}$  than in air-saturated DO conditions. The larger ctenophores used in our 2001 trials (Table 1) may thus be within a size range in which clearance rate becomes affected by environmental DO concentration.

The type 1 clearance rate response (in which the percentage of available prey consumed remains constant across a range of prey densities) observed in these experiments has previously been reported for *Mnemiopsis leidyi* over a much wider range of mesozooplankton food densities than used here (Reeve et al. 1978). However, while clearance rate is independent of food density, ingestion and assimilation rates, and the consequent growth efficiencies, have been shown to be sensitive to food density, as *M. leidyi* will tend to egest undigested food when feeding under constant high food densities (Kremer & Reeve 1989, Reeve et al. 1989). Thus we would expect to see the leveling off of growth at high food concentrations observed in these experiments (Figs. 1b, 2b).

The overall growth rates observed under high DO conditions here also agree well with those previously reported. Reeve et al. (1978, 1989) have suggested that small lobate ctenophores may double their size daily when feeding at high mesozooplankton densities, but growth rates tend to decrease with increasing ctenophore size (Reeve & Baker 1975). In our experiments, small ctenophores grew as much as 468% over a 4 d period under the highest food concentrations (Fig. 1a,b), while large individuals displayed much lower growth (Fig. 2a,b).

*Mnemiopsis leidyi* reproduction was similar in our trials under high DO conditions to that observed by others. Large individuals raised under higher food concentration than used here are capable of producing up to 9900 eggs per day (Baker & Reeve 1974), while a 10 ml individual would have produced about 3000 eggs in our trials at mesozooplankton densities of  $45 \text{ l}^{-1}$  (Fig. 3b). Despite a low carbon investment per egg, egg production in ctenophores is very sensitive to changes in food availability (Reeve et al. 1989), so the decrease in egg production observed with decreasing mesozooplankton density under high DO conditions (Fig. 3a,b) was not unexpected. The energetic cost paid during exposure to reduced DO conditions may similarly affect egg production to an even greater degree. Most large ctenophores grown under DO concentrations of  $1.5 \text{ mg l}^{-1}$  essentially shut down egg production even under high mesozooplankton densities (Fig. 3, Fig. 4, Table 3).

Our results suggest that, in spite of the tolerance of *Mnemiopsis leidyi* to low oxygen concentrations, hypoxia may negatively influence this species' population growth by reducing egg production. *M. leidyi* resumes egg production following periods of low food availability within 2 to 4 d (Reeve et al. 1989). If recovery from exposure to low oxygen is also rapid, short-term hypoxia exposure may not dramatically affect ctenophore populations over the course of an entire season. Persistent or widespread hypoxia, combined with an apparently active selection of hypoxic habitats by *M. leidyi* (Keister et al. 2000, Breitbart et al. 2003), could, however, result in reduced population size.

Three alternate explanations for the use of hypoxic bottom waters by *Mnemiopsis leidyi* in the face of reduced growth and reproduction merit further examination. The first is that ctenophores utilize this low prey, physiologically stressful habitat to avoid predation by sea nettles. Field data from the Patuxent River do not provide support for this hypothesis in that ctenophores preferentially utilize hypoxic bottom waters regardless of sea nettle densities (Keister et al. 2000, Breitbart et al. 2003). However, this bottom-oriented behavior may be one that has been selected for over evolutionary time and is not triggered by proximate cues produced by sea nettles. A geographic comparison among estuaries that vary in bottom DO and the presence of sea nettles could provide a test of this hypothesis. A second possibility is that ctenophores spend a substantial portion of their time in bottom waters to promote retention within tributaries. Directed field sampling and transport models could provide a test of whether export from tributaries is minimized by ctenophore behavior, but these predictions would need to be combined with population models to predict the net advantage or disadvantage of reducing export given the high cost of hypoxia to growth and reproduction. Finally, use of the bottom layer may reduce predation on *M. leidyi*, including eggs and larvae, by other predators not yet identified as important controls on ctenophore populations.

Given the lack of impact on growth shown in our experiments (Fig. 7), the direct effects of hypoxia on sea nettle population dynamics are likely to be minimal. The expected indirect effects driven by changes in ctenophore population dynamics are, however, much less certain. The net effect of hypoxia on ctenophores may be negative (via reduced reproduction) or positive (via increased refuge availability). These changes may, in turn, affect sea nettle population dynamics in several possible ways. A reduced and spatially segregated ctenophore population would simultaneously reduce the availability of ctenophores as prey items for sea nettles while reducing competition for mesozooplankton prey. The use of spatially explicit

models may help to estimate the net effects of hypoxia on ctenophore population dynamics and the consequent impacts on sea nettles.

Our expectations of reduced sea nettle growth but unaffected ctenophore growth under hypoxia are directly opposite from the results obtained in this study. *Mnemiopsis leidyi* in hypoxic areas pay a double penalty: reduced food availability as mesozooplankton move into more oxic areas (Keister et al. 2000, Breitburg et al. 2003) and reduced growth and reproduction given the food available. *Chrysaora quinquecirrha* conversely are advantaged in both aspects: they apparently track mesozooplankton and fish larvae prey into areas with higher DO concentrations, and do not pay a direct growth cost of exposure to moderate levels of hypoxia.

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#### LITERATURE CITED

- Adelson JM, Helz GR, Miller CV (2001) Reconstructing the rise of recent coastal anoxia; molybdenum in Chesapeake bay sediments. *Geochem Cosmochim Acta* 65:237–252
- Baird D, Ulanowicz RE (1989) The seasonal dynamics of the Chesapeake bay ecosystem. *Ecol Monogr* 59:329–364
- Baker LD, Reeve MR (1974) Laboratory culture of the lobate ctenophore *Mnemiopsis mccradyi* with notes on feeding and fecundity. *Mar Biol* 26:57–62
- Breitburg DL (1990) Nearshore hypoxia in the Chesapeake bay: Patterns and relationships among physical factors. *Estuar Coast Shelf Sci* 30:593–609
- Breitburg DL (1994) Behavioral responses of fish larvae to low dissolved oxygen concentrations in a stratified water column. *Mar Biol* 120:615–625
- Breitburg DL (2002) Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries. *Estuaries* 25:767–781
- Breitburg DL, Loher T, Pacey CA, Gerstein A (1997) Varying effects of low dissolved oxygen on trophic interactions in an estuarine food web. *Ecol Monogr* 67:489–507
- Breitburg DL, Adamack A, Rose KA, Kolesar SE, Decker MB, Purcell JE, Keister JE, Cowan JH (2003) The pattern and influence of low dissolved oxygen in the Patuxent River, a seasonally hypoxic estuary. *Estuaries* 26:280–297
- Chesney EJ, Houde ED (1989) Laboratory studies on the effects of hypoxic waters on the survival of eggs and yolk-sac larvae of the bay anchovy, *Anchoa mitchilli*. In: Houde ED, Chesney EJ, Newberger TA, Vazquez AV, Zastrow CE, Morin LG, Harvey HR, Gooch JW (eds) Population biology of bay anchovy in mid-Chesapeake bay. Final report to Maryland Sea Grant Ref No. (UM-CEES) CBL 89–141. Maryland Sea Grant, Solomons, MD, p 184–191
- Childress JJ, Seibel BA (1998) Life at stable low oxygen: adaptations of animals to oceanic oxygen minimum layers. *J Exp Biol* 201:1223–1232
- Condon RH, Decker MB, Purcell JE (2001) Effects of low dissolved oxygen on survival and asexual reproduction of scyphozoan polyps (*Chrysaora quinquecirrha*). *Hydrobiologia* 451:89–95
- Cooper SR, Brush GS (1991) Long-term history of Chesapeake bay anoxia. *Science* 254:992–996
- Cowan JH Jr, Houde ED (1993) Relative predation potentials of scyphomedusae, ctenophores and planktivorous fish on ichthyoplankton in Chesapeake bay. *Mar Ecol Prog Ser* 95: 55–65
- Cronin TM, Vann CD (2003) The sedimentary record of climatic and anthropogenic influence on the Patuxent River and Chesapeake bay ecosystems. *Estuaries* 26:196–209
- Decker MB, Breitburg DL, Marcus NH (2003) Geographical differences in behavioral responses to hypoxia: local adaptation to an anthropogenic stressor? *Ecol App* 13: 1104–1109
- Decker MB, Breitburg DL, Purcell JE (2004) Effects of low dissolved oxygen on predation by the ctenophore, *Mnemiopsis leidyi*, on zooplankton. *Mar Ecol Prog Ser* 280:163–172.
- D'Elia CF, Boynton WR, Sanders JG (2003) A watershed perspective on nutrient enrichment, science, and policy in the Patuxent River, Maryland: 1960–2000. *Estuaries* 26: 171–185
- Graham WM (2001) Numerical increases and distributional shifts of *Chrysaora quinquecirrha* (Desor) and *Aurelia aurelia* (Linné) (Cnidaria: Scyphozoa) in the northern Gulf of Mexico. *Hydrobiologia* 451:97–111
- Greco PA, Steirhoff KL (2002) A device for simultaneously controlling multiple treatment levels of dissolved oxygen in laboratory experiments. *J Exp Mar Biol Ecol* 280:53–62
- Hagy JD, Boynton WR, Keefe CW, Wood KV (2004) Hypoxia in Chesapeake Bay, 1950–2001: Long-term change in relation to nutrient loading and river flow. *Estuaries* 27: 634–658
- Keister JE, Houde ED, Breitburg DL (2000) Effects of bottom-layer hypoxia on abundances and depth distributions of organisms in Patuxent River, Chesapeake bay. *Mar Ecol Prog Ser* 205:43–59
- Kremer P (1994) Patterns of abundance for *Mnemiopsis* in U.S. coastal waters: a comparative overview. *ICES J Mar Sci* 51:347–354
- Kremer P, Reeve MR (1989) Growth dynamics of a ctenophore (*Mnemiopsis*) in relation to variable food supply. II. Carbon budgets and growth model. *J Plankton Res* 11: 553–574
- Littell RC, Milliken GA, Stroup WW, Wolfinger DD (1996) SAS System for Mixed Models. BBU Press, Cary, NC
- Miller DC, Poucher SL, Coiro L (2002) Determination of lethal dissolved oxygen levels for selected marine and estuarine fishes, crustaceans, and bivalve. *Mar Biol* 140:287–296
- Miller RJ (1974) Distribution and biomass of an estuarine ctenophore population, *Mnemiopsis leidyi* (A. Agassiz). *Chesapeake Sci* 15:1–8
- Purcell JE, Cowan JH (1995) Predation by the scyphomedusan *Chrysaora quinquecirrha* on *Mnemiopsis leidyi* ctenophores. *Mar Ecol Prog Ser* 129:63–70
- Purcell JE, Nemazie DA, Dorsey SE, Houde ED, Gamble JC (1994a) Predation mortality of bay anchovy *Anchoa mitchelli* eggs and larvae due to scyphomedusae and ctenophores in Chesapeake bay. *Mar Ecol Prog Ser* 114:47–58
- Purcell JE, White JR, Roman MR (1994b) Predation by gelatinous zooplankton and resource limitation as potential con-

- trols of *Acartia tonsa* copepod populations in Chesapeake bay. *Limnol Oceanogr* 39:263–278
- Purcell JE, Breitburg DL, Decker MB, Graham WM, Youngbluth MJ, Rastoff K (2001a) Pelagic cnidarians and ctenophores in low dissolved oxygen environments. In: Rabalais NN, Turner RE (eds) Coastal hypoxia: consequences for living resources and ecosystems. *Coastal and Estuarine Studies* 58, American Geophysical Union, Washington DC, p 77–100
- Purcell JE, Shiganova T, Decker MB, Houde ED (2001b) The ctenophore *Mnemiopsis* in native and exotic habitats: U.S. estuaries versus the Black Sea basin. *Hydrobiologia* 451: 145–176.
- Reeve MR, Baker LD (1975) Production of 2 planktonic carnivores (chaetognath and ctenophore) in south Florida inshore waters. *US Nat Mar Fish Ser Bull* 73:238–248
- Reeve MR, Walter MA, Ikeda T (1978) Laboratory studies of ingestion and food utilization in lobate and tentaculate ctenophores. *Limnol Oceanogr* 23:740–751
- Reeve MR, Syms MA, Kremer P (1989) Growth dynamics of a ctenophore (*Mnemiopsis*) in relation to variable food supply. I. Carbon biomass, feeding, egg production, growth and assimilation efficiency. *J Plankton Res* 11:535–552
- Roman MR, Gauzens AL, Rhinehart WK, White JR (1993) Effects of low oxygen waters on Chesapeake bay zooplankton. *Limnol Oceanogr* 38:1603–1614
- Sanford LP, Sellner KG, Breitburg DL (1990) Covariability of dissolved oxygen with physical processes in the summertime Chesapeake bay. *J Mar Res* 48:567–590
- Stalder LC, Marcus NH (1997) Zooplankton responses to hypoxia: behavioral patterns and survival of 3 species of calanoid copepods. *Mar Biol* 127:599–607
- Sullivan BK, Van Keuren D, Clancy M (2001) Timing and size of blooms of the ctenophore *Mnemiopsis leidyi* in relation to temperature in Narragansett bay, RI. *Hydrobiologia* 451:113–120
- Taft JL, Taylor WR, Hartwig EO, Loftus R (1980) Seasonal oxygen depletion in Chesapeake bay. *Estuaries* 3:242–47
- Thuesen EV, Childress JJ (1994) Oxygen consumption rates and metabolic enzyme activities of oceanic California medusae in relation to body size and depth. *Biol Bull (Woods Hole)* 187:84–98

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