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Potential impacts of brown tide, *Aureococcus anophagefferens*, on juvenile hard clams, *Mercenaria mercenaria*, in the Coastal Bays of Maryland, USA

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Abstract

The rate of growth of juvenile hard clams, *Mercenaria mercenaria*, was studied in the Coastal Bays of Maryland during an outbreak of the brown tide, *Aureococcus anophagefferens*. Brown tide dominated the plankton community during the month of June 2002, with cell densities at several sites reaching category 3 (>200,000 cells ml^{-1}) levels. Temperatures during the bloom were 18.6–27.5 °C. Nutrient conditions preceding and during the bloom were conducive for the proliferation of *A. anophagefferens*: while inorganic nitrogen and phosphorus were <1 μg at N or P l^{-1}, urea was elevated during bloom development. Organic nitrogen, phosphorus and carbon were in the range of levels observed in previous brown tide blooms and increased following the collapse of the bloom. Growth rates of juvenile clams were significantly lower during the period of the brown tide bloom than following its collapse. Growth rates of *M. mercenaria* were found to be negatively impacted at brown tide densities as low as 20,000 cells ml^{-1}, or category 1 levels. The low growth rates of *M. mercenaria* could not be explained by temperature, as the lowest growth rates were found when water temperatures were at levels previously found to be optimal for growth.

Keywords: *Aureococcus anophagefferens*; Brown tide; HAB; Harmful algal bloom; Hard clam; *Mercenaria mercenaria*; Maryland Coastal Bays

1. Introduction

The brown tide pelagophyte *Aureococcus anophagefferens* has been associated with considerable ecosystem damage along the coasts of Long Island and more recently New Jersey. Blooms of *A. anophagefferens* have been linked with the starvation and mortality of bay scallops in Long Island (Cosper et al., 1987; Tettelbach and Wenczel, 1993), losses of seagrass (Dennison et al., 1989) and reductions in reproductive potential and growth in many shellfish (mussels, hard clams and scallops) (Tracey, 1988; Gallagher et al., 1989; Wikfors and Smolowitz, 1995; Bricelj and Lonsdale, 1997; Bricelj, 1999). Brown tide is a poor food for bivalves and can be potentially toxic (Bricelj et al., 2001). A survey of *A. anophagefferens*

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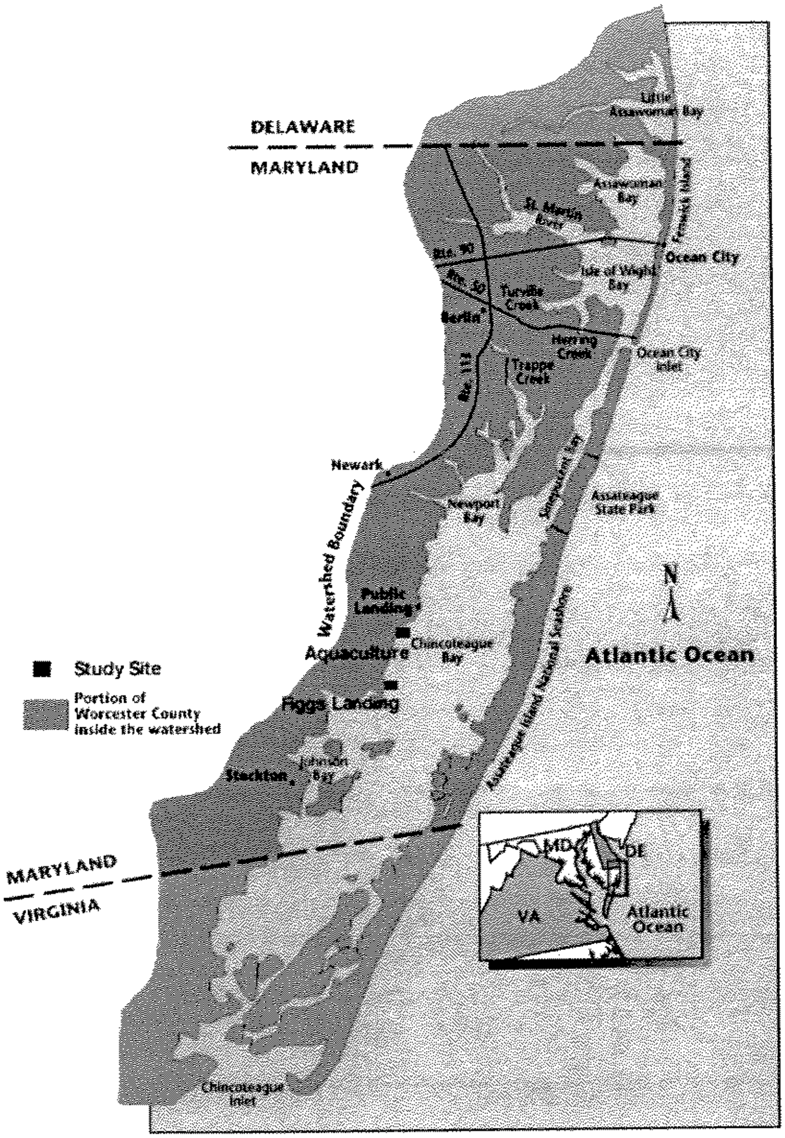

Fig. 1. The Maryland Coastal Bays. The study sites at the aquaculture facility and Figgs Landing are indicated by solid squares.

distribution along the eastern seaboard conducted in August 1990 (Anderson et al., 1993) did not detect presence of brown tide in Maryland, but both pigment data and direct counts have indicated that *A. anophagefferens* have likely been present in Maryland's Coastal Bays since at least 1993 (Trice et al., 2004). The Maryland Department of Natural Resources and the Assateague Island National Park Service have been monitoring the Coastal Bays for brown tide blooms since 1999. These monitoring efforts have shown that category 3 (>200,000 cells ml^{-1}; e.g. Gastrich and Wazniak, 2002) blooms develop regularly in the Newport Bay and Northern Chincoteague Bay regions of the Coastal Bays (Fig. 1; Wazniak, 2003). Blooms usually occur in late May to mid-June with peak concentrations lasting approximately two weeks.

Although category 3 blooms have been documented in the past several years in Maryland, the impacts to the Coastal Bays' bivalve populations (scallops and hard clams in particular) are unclear. Bay scallops first started to reappear in the Coastal Bays during the mid-1990s after an apparent absence of over 60 years (Tarnowski and Bussell, 2002). The impacts of brown tide on bay scallops has been well documented and managers have become concerned that the fledgling Coastal Bays scallop population may potentially be adversely affected (Bricelj et al., 1987; Gallagher et al., 1989; Cosper et al., 1987; Bricelj and Lonsdale, 1997). Furthermore, hard clams in the Coastal Bays currently have very low abundances ($0.25\,m^2$) compared to both many other coastal embayments on the east coast and to historic abundances in the 1950s–1970s (1.0 clams m^{-2}; Wells, 1957). Limited data on hard clam abundances since the 1970s make it difficult to determine when they began to decline in the Coastal Bays and thus what the causes may have been. Many studies have shown that category 3 blooms result in severe ecological impacts including scallop mortalities, shellfish recruitment failures, cessation of hard clam growth and shading of seagrasses (Gastrich and Wazniak, 2002). Anecdotal evidence of poor growth of shellfish in the Coastal Bays during a brown tide prompted the hypothesis that brown tide may be directly impacting shellfish growth. The purpose of this field study was thus to assess the effect of brown tide on juvenile hard clams in the Maryland Coastal Bays during 2002.

2. Materials and methods

The study ran from late April through July 2002. Juvenile clams (initial mean shell height $\mathcal{D}$ 16.67 mm), originating from a commercial hatchery in Virginia, were placed at three locations in the Coastal Bays and their growth was monitored weekly. The study sites included a commercial clam aquaculture site, a site just off the aquaculture facility near the water intake and a control site at Figgs Landing (Fig. 1). Clams in the aquaculture facility were placed in standard upwelling trays. Clams at the bay sites were deployed in modified Taylor floats. At each sampling time, a subsample ($n\ \mathcal{D}$ 100–142) of clams was measured for shell height and volume. Clam height was measured as the maximal dorsoventral dimension perpendicular to the hinge (Carriker, 2001). Clam volume was calculated by dividing the number of clams in the subsample by the change in water volume after the subsample of clams were added to a graduated cylinder. Growth rates (day^{-1}) were calculated as the difference in shell height between each sampling interval divided by the number of interval days.

Surface samples for brown tide enumeration were collected twice a week at the aquaculture facility and weekly at the other two sites. Samples were fixed immediately with 1% gluteraldhyde and held on ice. Epifluorescence microscopy was later (within 14 days) used to determine *A. anophagefferens* cell counts using the polyclonal technique (Anderson et al., 1993). Samples of the general phytoplankton community composition were taken weekly at the aquaculture site but more sporadically at the other sites. Samples were fixed in Lugol's iodine and stored in dark containers. Phytoplankton were identified to genus level and enumerated under light microscopy.

Environmental parameters were also measured at each sampling time. Temperature and salinity were recorded using either a Hydrolab or thermometer and hydrometer (accuracy was to the nearest tenths place). Whole water samples were collected and stored in the dark on ice until transferred to the laboratory (within 4 h) where they were immediately filtered for nutrient analysis. Samples were then stored frozen for subsequent analysis within several weeks. Inorganic nutrients (NO_3^-, NO_2^-, $NH_4^{\mathcal{C}}$, PO_4^{3-}) were analyzed using standard autoanalyzer methods. Samples for total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were analyzed using persulfate oxidation (Valderrama, 1981). Concentrations of dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were calculated by subtracting the inorganic nutrient concentrations from the respective TDN or TDP concentration. Dissolved organic (DOC) carbon samples were analyzed on a Shimadzu TOC 5000. Urea was measured using the urease method (Parsons et al., 1984).

3. Results

3.1. Brown tide and community composition

Brown tide blooms at the aquaculture facility reached category 3 densities (peak concentration of 376,512 cells ml^{-1}; Fig 2A) and dominated the phytoplankton community from May 31 through June 21.

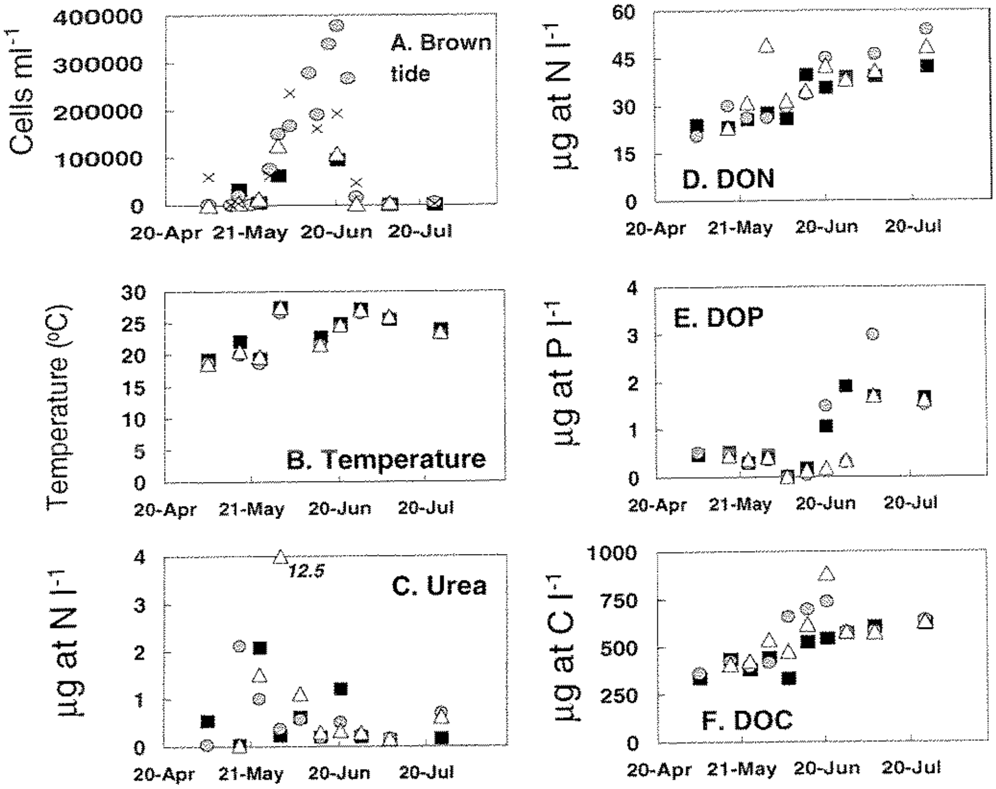

Fig. 2. Environmental conditions during the period of study. (A) Abundance of *Aureococcus anophagefferens*; (B) ambient water temperature; (C) urea nitrogen; (D) dissolved organic nitrogen; (E) dissolved organic carbon; and (F) dissolved organic phosphorus. Data are from Figgs Landing (squares), the aquaculture facility (circles), just off the aquaculture facility (triangles) and for brown tide abundance only, public landing (X). Other nutrients are not shown as they were <1 µg at N or P l^{-1} through the duration of the study.

At all sites monitored, the bloom collapsed on about June 20. During the peak of the brown tide bloom, *A. anophagefferens* represented 85–95% of the phytoplankton community. In the weeks preceding and following the peak of the brown tide blooms, diatoms represented 16–49% of the assemblage and microflagellates represented 25–57%, of which, dinoflagellates were <2% and blue-green algae represented up to 13% of the assemblage before and 7% after, the brown tide bloom (Table 1).

3.2. Environmental conditions

Salinity varied from 29 to 34 throughout the study period (data not shown) and temperature fluctuated from 18.6 to 27.5 °C (Fig 2B).

Concentrations of DIN and DIP were generally below 1.0 µg at N or P l^{-1} and did not show significant variation over the duration of the study. Concentrations of urea were >1.0 µg at N l^{-1} in late May, but decreased steadily throughout the remainder of the study (Fig. 2C). In contrast, DON, DOP and DOC increased following the collapse of the brown tide bloom (Fig. 2D–F). Chlorophyll concentrations just north of the aquaculture site had peak concentrations (25 µg l^{-1}) in June during the Brown Tide bloom compared to pre and post bloom concentrations (1.9 µg l^{-1} in May and 16.8 µg l^{-1} in July).

3.3. Hard clam growth

From early May through late June, clam height increased an average of 2 mm, from 17 to 19 mm. From late June to late July, mean clam height increased from 3 to 22 mm (Fig. 3). Thus, juvenile clam growth rates averaged 0.05 mm day^{-1} in May and June, but

Table 1
Phytoplankton community composition during the hard clam growth study at the aquaculture facility in the Coastal Bays, Maryland

Date	Brown tide (%)	Diatom (%)	Micro-flagellate (%)	Dinoflagellate (%)	Blue-green (%)
05/14/02	3.19	49.01	45.88	1.92	0.00
05/17/02	50.96	19.12	19.69	0.08	10.15
05/21/02	17.00	18.85	57.23	1.97	4.94
05/24/02	34.79	16.50	35.27	0.17	13.27
05/28/02	83.94	8.93	6.57	0.16	0.41
05/31/02	91.36	4.85	3.35	0.43	0.00
06/04/02	93.34	2.50	3.42	0.12	0.62
06/07/02	95.07	1.89	2.64	0.11	0.29
06/11/02	93.84	2.26	2.95	0.01	0.95
06/14/02	95.55	1.51	2.39	0.14	0.41
06/21/02	94.39	1.69	3.40	0.27	0.24
06/25/02	49.50	21.94	24.61	0.89	3.06
06/28/02	7.09	37.03	47.04	1.35	7.49
07/10/02	16.67	25.28	57.33	0.72	0.00

increased to 0.1 mm day^{-1} from late June through July.

4. Discussion

The environmental conditions of early summer 2002 in Maryland Coastal Bays were well suited for growth of brown tide. Peak brown tide concentrations occurred at temperatures between 21.6 and 24.4 °C. The nutrient concentrations were similar to other sites where brown tide has proliferated: high organic nutrients and low inorganic nutrients (e.g. Berg et al., 1997; LaRoche et al., 1997; Lomas et al., 2001; Glibert et al., 2001; Gobler et al., 2002). Elevated concentrations of urea preceded the maximum brown

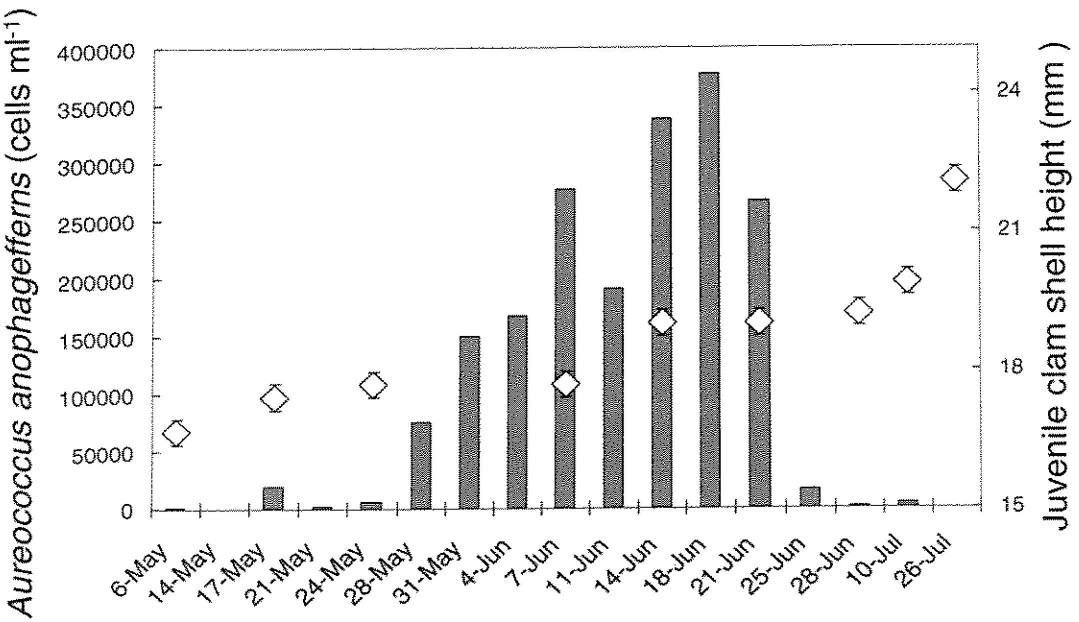

Fig. 3. Abundance of *Aureococcus anophagefferens* (bars) and mean shell height of juvenile clams (diamonds) as a function of duration of the study period for the aquaculture facility site only. Error bars are mean standard deviations; *nD* 100–142 for each average clam height recorded.

tide concentration. An analysis of long-term trends in urea concentrations in the Coastal Bays revealed that sharp elevations in concentration typically occur annually in late May/early June (Glibert et al., 2004). Urea has previously been shown to be a preferred growth nitrogen substrate (Dzurica et al., 1989; Lomas et al., 1996; Berg et al., 1997). Concentrations of DON and DOP during the bloom were very similar to values reported for blooms periods on Long Island (Lomas et al., 2001; Gobler et al., 2002). All organic nutrients (N, P and C) increased following the collapse of the bloom, suggesting either release of organic material from the bloom itself, or a decline in the demand for these nutrients with the decline in biomass. Furthermore, the ratios of DOC:DON were also higher (18–22) during the peak of the bloom than before or after (11–14), which is suggestive of depletion of DON during the bloom. These ratios are also consistent with previously reported bloom/non-bloom comparisons (Lomas et al., 2001; Glibert et al., 2001).

Growth rates of the juvenile clams were lower during the months of May and June than during the month of July. Several factors may have contributed to these variable growth rates, including temperature and brown tide abundance, either individually or synergistically. We explore both of these possibilities below.

Juvenile clam growth typically displays an inverted parabolic relationship with temperature with optimum growth range between 19 and 25 °C (maximum shell growth between 20 and 24 °C) and growth ceases below 9 °C and above 31 °C (Grizzle et al., 2001). Depressed rates of growth, therefore, may have been expected solely due to temperature during the latter stages of this study, when temperatures exceeded 25 °C. However, when growth rates are compared to ambient temperatures, it is apparent that the clams were growth inhibited at optimal growth temperatures and positive growth did not occur until temperatures exceeded 24 °C (Fig. 4). Indeed, over the optimum temperature range cited by Grizzle et al. (2001), the mean growth rate at the aquaculture facility was 0.05 0.06 mm day^{-1}. Thus, temperature alone cannot explain growth inhibition during mid-June when temperatures ranged from 21 to 24 °C.

Clam growth rate was inversely related to the average brown tide density for each time interval over which growth rate was measured (r^2 $\mathcal{D}$ 0.4; $P < 0.005$; Fig. 5). Juvenile clam growth rates declined rapidly with increasing brown tide abundance, with the onset of declining growth at low (20,000 cells ml^{-1}; e.g. category 1) abundances. This strongly agrees with laboratory results of the impacts on juvenile hard clams (Bricelj, 1999).

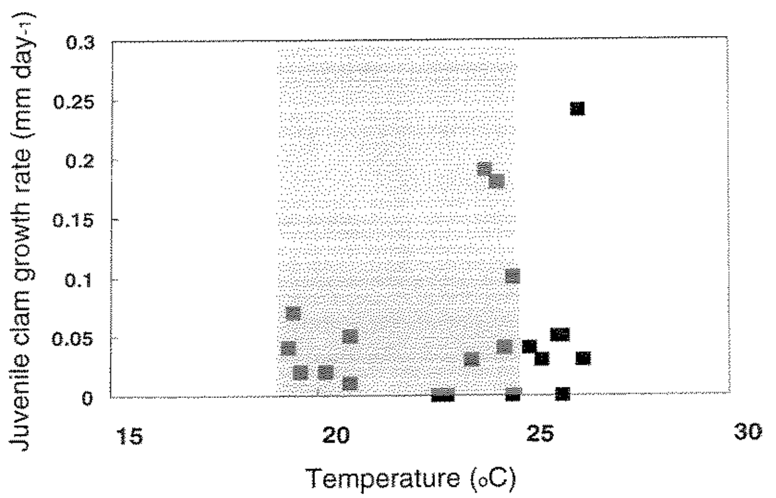

Fig. 4. Juvenile clam growth rates as a function of the average temperature for the interval over which growth rates were measured. Gray area represents the optimum temperature for juvenile hard clam growth according to Grizzle et al. (2001).

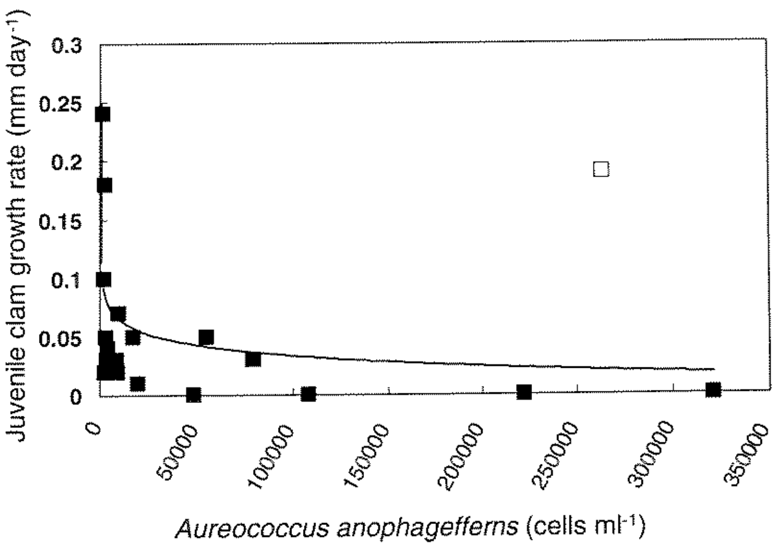

Fig. 5. Juvenile clam growth rates as a function of the average brown tide cell density for the interval over which growth rates were measured.

With one exception, there was no growth recorded at brown tide levels >150,000 cells ml^{-1}. The single point of elevated clam growth (0.19 mm day^{-1}) at brown tide levels of 180,000 cells ml^{-1} is a striking deviation from the declining trend of growth rate with brown tide cell density (Fig. 5). Upon closer examination of the environmental parameters at this time, it is apparent that this single measurement occurred when brown tide abundance temporarily declined; this occurred in mid-June. Brown tide abundances had increased to >270,000 cells ml^{-1} one week prior to this drop in growth, but within 3–4 days, had declined by nearly 30%, before increasing once again. Although any conclusion based on a single point is tenuous at best, this may be indicative of the ability of juvenile hard clams to recover rapidly from exposure to brown tide. Similar recovery was reported for scallops in Long Island (Bricelj et al., 1987). This potential is underscored by the increase in growth rates observed in July upon cessation of the brown tide bloom. Although the ultimate impact of a long-term depression in growth is mortality due to starvation, the sub-lethal impacts from a depression in hard clam growth are not completely known, from both a physiological and an ecological perspective.

This study has shown that category 3 brown tide blooms occur in Maryland's Coastal Bays, that the environmental conditions are conducive to these outbreaks and consistent with other areas where such outbreaks have occurred and that brown tide abundances as low as 20,000 cells ml^{-1} may have negative impacts on the growth of juvenile hard clams in the field. Further study is required to determine if brown tide is a factor restricting the growth and abundance of other stages of hard clams as well as other shellfish such as scallops in the Coastal Bays.

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