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Effects of background concentrations of *Aureococcus anophagefferens* (brown tide) on growth and feeding in the bivalve *Mercenaria mercenaria*

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ABSTRACT: Much research on the toxic alga *Aureococcus anophagefferens* (brown tide) has focused on its effects on bivalve suspension feeding, but less is known about how brown tide influences bivalve growth. This study examined the extent to which background levels, defined as concentrations too low for toxicity to inhibit bivalve feeding, of *A. anophagefferens* influenced the growth and feeding physiology of northern quahogs *Mercenaria mercenaria* compared to other phytoplankton common to Long Island, New York, waters. Juvenile quahogs were fed either unialgal cultures of *A. anophagefferens*, non brown tide species, or diets mixed with background levels of brown tide. Absorption efficiency (AE) was determined using the ^{14}C:^{51}Cr dual-tracer method, and growth was determined by overall biomass change. Results showed that unialgal diets resulting in the highest AE, specifically *Isochrysis galbana* and *Thalassiosira pseudonana*, were associated with rapid *M. mercenaria* growth. Conversely, *Nitzschia closterium* resulted in a comparatively low AE and a loss in quahog biomass. Diets mixed with brown tide resulted in a significantly lower AE than the corresponding unialgal diet for all phytoplankton species except *N. closterium*. Additionally, mixed diets exerted a small negative influence on quahog growth compared to unialgal diets. These observations suggest that quahogs may suffer subtle, chronic effects when *A. anophagefferens* is present in the field at background levels. Moreover, the different responses of *M. mercenaria* to each experimental diet have broad implications for understanding how phytoplankton community composition may influence bivalve growth patterns in the field.

KEY WORDS: Bivalve growth · Phytoplankton · Long Island · Great South Bay · Absorption efficiency · Hard clam

INTRODUCTION

Occurrences of harmful algal blooms (HABs) have been increasing in frequency over recent decades (Smayda 1990). These blooms can have detrimental consequences for coastal organisms, including bivalves. Much harmful algal research has focused upon bloom initiation and persistence. Less is known about how HABs affect bivalve growth and feeding, which becomes complex considering that bivalve growth may be influenced by several phytoplankton species that co-occur with harmful algae, each offering a range of nutritional benefits depending upon availability. The degree to which this variability in plankton community composition influences bivalve growth is poorly understood.

Blooms of the pico-sized (2 µm) 'brown tide' pelagophyte *Aureococcus anophagefferens* Hargraves et Sieburth have recurred in several embayments across Long Island, New York, since 1985 (Nuzzi & Waters 1989, Bricelj & Lonsdale 1997). Blooms of *A. anophagefferens* are largely responsible for the decimation of Long Island's bay scallop industry (Bricelj et al. 1987), and they cause mortality and reduction in clearance rates of mussels (Tracey 1988). Brown tide also has detrimental consequences for the northern quahog

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(hard clam) *Mercenaria mercenaria* (L.). Laboratory studies have shown that clearance rates of *M. mercenaria* cease above a concentration of 3.5×10^4 cells ml^{-1} when fed *A. anophagefferens* strains CCMP 1707 and 1708 (Bricelj et al. 2001). Greenfield & Lonsdale (2002) found high mortalities and a slight but statistically non-significant decrease in whole animal biomass of juvenile *M. mercenaria* coincident with brown tide levels exceeding 10^6 cells ml^{-1} in the field. The concentration of this bloom was 2 orders of magnitude above the aforementioned feeding inhibition threshold, so brown tide likely caused quahog starvation. Exposure of cellular exudates of *A. anophagefferens* to excised gill tissue of certain bivalves, including *M. mercenaria*, causes ciliary motion to cease (Gainey & Shumway 1991), suggesting that 1 toxic effect of brown tide is via contact.

Brown tide clearly has detrimental consequences for quahogs, but *Aureococcus anophagefferens* is often not the dominant phytoplankton species and may be present in nature at abundances below that shown by Bricelj et al. (2001) to result in cessation of quahog feeding. Virtually nothing is known about how these low concentrations of *A. anophagefferens* ($<3.5 \times 10^4$ cells ml^{-1}), henceforth referred to as background levels, influence quahog growth and feeding physiology. *Mercenaria mercenaria* ingests *A. anophagefferens* at background levels along with other phytoplankton. Assuming a diverse assemblage consists of species with varying nutritional value to *M. mercenaria*, it is important to determine how these mixtures affect growth. This study examines how background levels of brown tide influence growth and feeding in *M. mercenaria* when presented in a diet mixed with non brown tide species common to Long Island waters.

The rationale for the current study is based upon field studies by Greenfield (2002) that showed growth of juvenile quahogs varied between 2 embayments on Long Island, New York, each with distinct plankton communities. Quahogs consistently grew well in an embayment where centric diatoms were abundant. By comparison, quahogs grew slowly within Great South Bay, where brown tide has been a recurrent problem. Slow quahog growth occurred even during times when *Aureococcus anophagefferens* was at background levels and the plankton community was numerically dominated by the pennate diatom *Nitzschia closterium*. An exception to the generally slow quahog growth in Great South Bay was a brief period of accelerated growth following a brown tide, when flagellate concentrations were high (Greenfield & Lonsdale 2002).

The importance of various phytoplankton species for quahog growth has been studied extensively. Unialgal diets of the cryptomonad flagellate *Isochrysis galbana* consistently result in good quahog growth and high carbon absorption efficiency (AE) (Epifano et al. 1975, Bricelj et al. 1984, Bass et al. 1990, Wikfors et al. 1992). Centric diatoms also promote rapid quahog growth (Walne 1970, Epifano 1979, Laing et al. 1987, Wikfors et al. 1992). Conversely, diets of some dinoflagellates and cyanobacteria result in slow quahog growth and low AEs (Bricelj et al. 1984, Lesser & Shumway 1993, Wikfors & Smolowitz 1993, Grizzle et al. 2001).

Much brown tide research has focused on cell toxicity, but less is understood about the nutritional value of *Aureococcus anophagefferens*. The AEs of scallops and mussels fed brown tide at levels of 1 to 7×10^4 cells ml^{-1} are high (Bricelj & Kuenster 1989), and the fatty acid composition of *A. anophagefferens* is similar to that of phytoplankton species that are nutritionally beneficial to bivalves (Bricelj et al. 1989). *Mercenaria mercenaria* exhibits positive growth when fed equal volumes of non-toxic *A. anophagefferens* and *Isochrysis galbana* (Bricelj 2000). These studies indicate that *A. anophagefferens* does not necessarily constitute a low-quality diet in the absence of toxin.

Aureococcus anophagefferens often co-occurs with other phytoplankton species in Long Island bays at levels below 3.5×10^4 cells ml^{-1}. These background brown tide levels may contribute to the slow quahog growth typical of Long Island's south shore (Greenfield 2002). The current study addresses the effects of background levels of *A. anophagefferens* on growth and feeding in *Mercenaria mercenaria*.

MATERIALS AND METHODS

Animal collection and acclimation. Juvenile *Mercenaria mercenaria* (5 to 10 mm shell length) were supplied by Frank M. Flowers and Sons, a shellfish hatchery located in Oyster Bay, New York. Quahogs were raised in predator-exclusion mesh bags within lantern nets at Flax Pond (S = 27‰), a temperate salt marsh on Long Island Sound during summer of 2000, until the experiment began. Individuals were randomly collected from the mesh bags and transported to the laboratory. Animals were then separated into 2 groups, 1 group per experimental treatment, and placed individually in 200 ml beakers containing aerated seawater (S = 27‰, T = 23°C) for 1 wk. Twenty individuals were acclimated for absorption efficiency experiments and 40 were acclimated for growth experiments.

Quahogs were fed experimental diets during acclimation. Algal concentrations were normalized to the dry weight of 1×10^5 cells ml^{-1} of *Isochrysis galbana* (9.12×10^{-4} mg ml^{-1}) because this diet promotes good growth and high absorption efficiency (AE) without pseudofeces production (Bricelj et al. 1984, Wikfors et al. 1992). Algal dry weights (mg) were measured as

follows: 2 to 4 ml of a known cell concentration, determined by haemocytometer, of log-phase cultured algae were filtered onto pre-combusted and weighed 25 mm GF/C filters, rinsed 3 times with an isotonic solution of ammonium formate to remove seawater, dried at 60°C for 24 h, then reweighed. The dry weight per cell was adjusted to the final ration of 9.12×10^{-4} mg ml^{-1} (Table 1).

Algae were representative of phytoplankton commonly found in Long Island waters (Greenfield 2002). Species included the flagellate *Isochrysis galbana*, the centric diatom *Thalassiosira pseudonana*, the pennate diatom *Nitzschia closterium*, the dinoflagellate *Prorocentrum minimum*, and the pelagophyte *Aureococcus anophagefferens* (CCMP 1708, a 'toxic' strain according to Bricelj et al. 2001). With the exception of *A. anophagefferens*, algae were cultured in f/2 media (Guillard & Ryther 1962) using 0.45 µm filtered Stony Brook Harbor seawater (27 ‰) in a 14:10 light:dark cycle and harvested at log phase. *A. anophagefferens* was cultured in modified f/2 medium containing 5.0×10^{-6} M citric acid, 9.0×10^{-6} M Fe, 1.0×10^{-8} M Se, and 3.6×10^{-5} M glycerophosphate. The first treatment (unialgal) was *I. galbana*, *T. pseudonana*, *N. closterium* or *P. minimum*. A second treatment (mixture) contained each algal species spiked with a background level of *A. anophagefferens* (3×10^4 cells ml^{-1}). The amount of non brown tide algae was corrected for the dry weight of *A. anophagefferens* (1.36×10^{-4} mg ml^{-1}) to ensure dry weights remained constant. A third treatment (background brown tide) of only 3×10^4 cells ml^{-1} *A. anophagefferens* isolated effects of background brown tide.

Absorption efficiency experiments. ***Labeling and culturing of algae:*** Absorption efficiency (AE) was determined using the ^{14}C:^{51}Cr labeling technique (Calow & Fletcher 1972, Lopez & Cheng 1983) for algae, modified from Bricelj et al. (1984). For mixed diets, the non-brown tide species was labeled. Cultures were harvested at log phase, transferred to 100 ml of f/2 media containing 37 kBq per 10 ml $NaH^{14}CO_3$, and the pH was adjusted to 7.5. Algae were grown for 5 d at 22°C, under a 14:10 h light:dark cycle, until cells were uniformly labeled. On the fifth day, algae were labeled with 370 kBq per 10 ml $^{51}CrCl_3$. Since ^{51}Cr was stored in 0.1N HCl, the ^{14}C-labeled algae was diluted 1:10 with filtered seawater, ^{51}Cr was added to the diluted culture then neutralized with 0.5 N NaOH. Under these conditions, ^{51}Cr adsorbs to cell surfaces without penetrating biological membranes (Lopez & Cheng 1983, Lopez et al. 1989). Subsequently, the 100 ml ^{14}C:^{51}Cr suspension was concentrated onto a Poretics 1 µm polycarbonate membrane filter at 4 mm Hg and resuspended in 10 ml filtered seawater. A 1 ml sample was taken to determine ^{14}C:^{51}Cr in the food. Analysis of ^{14}C:^{51}Cr in the seawater showed negligible activity suggesting that most ^{51}Cr adsorbed to cell surfaces.

Feeding and depuration: Quahogs were fed labeled algae by placing animals (n = 12) in beakers containing

Table 1. Experimental diets fed to *Mercenaria mercenaria* consisting of a unialgal diet, a mixture containing 3×10^4 cells ml^{-1} of *Aureococcus anophagefferens*, and a unialgal diet consisting of 3×10^4 cells ml^{-1} of *A. anophagefferens* (background brown tide) for a final ration of 9.12×10^{-4} mg ml^{-1}. BT: brown tide

Experiment	Treatment	Phytoplankton species	Cell morphology length/ width (µm)	Dry weight (mg cell^{-1})	Ration per treatment per species (cells ml^{-1})	Total ration per treatment (cells ml^{-1})
Aureococcus anophagefferens	Unialgal (high BT) Background BT	*A. anophagefferens* *A. anophagefferens*	Spherical 2–3	4.54×10^{-9}	2.01×10^5 3.00×10^4	2.01×10^5 3.00×10^4
Isochrysis galbana	Unialgal Mixture	*I. galbana* *I. galbana* + *A. anophagefferens*	Elipsoid 4–6/2–4	9.12×10^{-9}	1.00×10^5 8.51×10^4 3.00×10^4	1.00×10^5 1.15×10^5
Thalassiosira pseudonana	Unialgal Mixture	*T. pseudonana* *T. pseudonana* + *A. anophagefferens*	Cylindrical 4–6/4–5	1.01×10^{-8}	9.02×10^4 7.67×10^4 3.00×10^4	9.02×10^4 1.07×10^5
Prorocentrum minimum	Unialgal Mixture	*P. minimum* *P. minimum* + *A. anophagefferens*	Elipsoid 13–16/10–12	4.75×10^{-7}	1.92×10^3 1.63×10^3 3.00×10^4	1.92×10^3 3.16×10^4
Nitzschia closterium	Unialgal Mixture	*N. closterium* *N. closterium* + *A. anophagefferens*	Elongate 80–100/3–5	1.82×10^{-7}	5.02×10^3 4.27×10^3 3.00×10^4	5.02×10^3 3.43×10^4

50 ml of the experimental diet (Table 1). Phytoplankton were kept in suspension by periodic stirring. After 1 h (t_1), animals were removed, rinsed with filtered seawater, and 3 quahogs per treatment were frozen for determination of ingestion and initial ^{14}C:^{51}Cr. Remaining quahogs were placed into separate beakers containing 50 ml of seawater and unlabeled (cold) *Isochrysis galbana*. Cold *I. galbana* was added to the depuration chambers every 6 to 8 h so quahogs were constantly feeding.

Feces were collected immediately after quahogs consumed labeled algae and periodically afterwards. Feces collection was terminated after 48 h because 90% of ^{14}C for *Isochrysis galbana* is depurated in <28 h and ^{14}C recovery is negligible after 40 h (Bricelj et al. 1984). Feces were collected by filtering the 50 ml depuration water onto a 0.8 µm Nuclepore filter, rinsing 3 times with cold-filtered seawater, and placing filters inside 4 ml scintillation vials for subsequent analysis. Since feces were microscopic in size, filtration ensured most pellets were recovered.

^{51}Cr absorption: ^{51}Cr is assumed to be inert (Calow & Fletcher 1972). A portion may still be absorbed, possibly during the glandular phase of digestion (Decho & Luoma 1991). Quahogs were monitored during depuration by placing whole animals into gamma tubes and counting the ^{51}Cr activity. During the first 48 h, quahogs were counted simultaneously with fecal collection. Animals were counted every 1 to 2 d subsequently for a total duration of 1 wk. The majority of ^{51}Cr depurates within 1 d (Bricelj et al. 1984, Wang & Fisher 1996), so this method reflects the absorbed ^{51}Cr. ^{51}Cr activity (denoted $^{51}Cr^*$) was calculated such that:

$$^{51}Cr^* = \frac{100}{(100 - {}^{51}CrAE)} \times {}^{51}Cr\,(quahog) \quad (1)$$

where ^{51}Cr (quahog) is the sum of fecal ^{51}Cr activity (counts min^{-1}: cpm) collected over the 48 h depuration period and $^{51}CrAE$ is $\%^{51}Cr$ remaining in the quahog on Day 5 after accounting for radioactive decay ($t_{1/2}$ = 27.72 d). Preliminary calculations showed that 7.41% ± 3.51 of ^{51}Cr ingested was absorbed, so it was important to include this correction.

Sample preparation and counting: Counting was similar to Lopez & Chang (1983). Feces were solubilized using 500 ml of Packard tissue and gel solubilizer (0.5 M), counted for ^{51}Cr for 300 s on a Wallac LKB 1282 Compugamma CS counter, then stored overnight to ensure complete digestion. Subsequently, 3 ml of Packard Ultima Gold XR LSC cocktail was added, samples were vortexed for 30 s and settled for 1 to 2 h. Reference samples contained solubilizer and scintillation cocktail only. Samples were counted for ^{14}C activity for 600 s on a Wallac LKB 1217 Rackbeta liquid scintillation counter. A truncated channel (12 to 56 kilo electron volts [KeV]) reduced the overlap in the energy spectra between ^{14}C and ^{51}Cr.

AE calculations: Absorption efficiency (AE) was calculated for individual quahogs as ^{14}C retained:^{14}C ingested. Only quahogs that fed during exposure to labeled algae were included for analysis. AE was calculated according to Eq. (2) (Calow & Fletcher 1972) such that:

$$AE = \left[1 - \left(fecal\,{}^{14}C:{}^{51}Cr^*\right)\right] / \left(particulate\,{}^{14}C:{}^{51}CR\right) \times 100 \quad (2)$$

where fecal ^{14}C:$^{51}Cr^*$ is the ratio of ^{14}C activity (disintegrations min^{-1}: dpm) to ^{51}Cr activity (cpm, see Eq. 1) in feces, and particulate ^{14}C:^{51}Cr is the ratio of ^{14}C activity (dpm) to ^{51}Cr activity (cpm) in food.

Statistics: Parametric analyses (2-tailed Student's *t*-tests, $\alpha = 0.05$) determined whether *Aureococcus anophagefferens* influenced AE of algae. Before analysis, the *F*-max test was used to test for homogeneity of variances. Gut passage time (GPT) was determined for the 'half time recovery' of ^{14}C-labeled food (Kofoed et al. 1989). Since bivalves exhibit 2 digestion phases (Widdows et al. 1979), a linear model best described the intestinal (initial) phase.

Growth experiments. ***Feeding chambers:*** Juvenile (10 to 15 mm) *Mercenaria mercenaria* were acclimated as described previously and placed (n = 5) into plastic chambers onto a stainless-steel mesh elevated 4 cm from the base (Fig. 1). Each chamber had an outflow so that water volume was 1 l. A plastic lid covered each chamber to limit evaporation, which also had a small

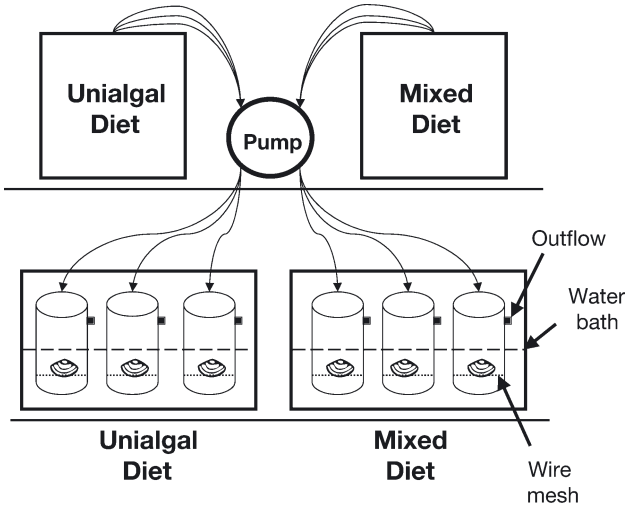

Fig. 1. Schematic design of growth experiments. Reservoirs filled with each experimental diet dispensed to feeding chambers containing clams *Mercenaria mercenaria* (n = 5) using a peristaltic pump. Separate air tubes were also supplied to each chamber (not shown)

hole for feeding and aeration tubes. Aeration also ensured mixing of the suspension. Chambers rested inside an aquarium tank, in triplicate, 1 tank per treatment. Overflow water was removed and discarded as necessary. Diets for growth experiments were similar to those used for AE experiments (Table 1). One additional treatment (unialgal) of *Aureococcus anophagefferens* (9.12×10^{-4} mg ml^{-1}) represented a high brown-tide level (2.01×10^5 cells ml^{-1}) because field studies conducted after termination of AE experiments showed quahog growth when brown tide was 1 to 2×10^5 cells ml^{-1} (Greenfield & Lonsdale 2002).

Mercenaria mercenaria were continuously fed for 21 d by dispensing log-phase algae from reservoirs, 1 reservoir per treatment, into chambers using a peristaltic pump (Fig. 1). Each day, reservoirs were emptied, rinsed, and changed to ensure diets remained constant throughout the experimental period and to account for growth of cultures and algae within reservoirs. Algae were counted daily with a haemocytometer.

Growth of Mercenaria mercenaria: Growth of *M. mercenaria* was measured for whole animal ash-free dry weight (AFDW). Initial weight was determined by randomly selecting quahogs (n = 5), drying whole animals for 48 h at 60°C, then ashing at 450°C until organic matter was combusted (4 to 6 h). Remaining quahogs were randomly assigned to 2 groups (unialgal and mixed diets) and placed (n = 5) inside feeding chambers. Instantaneous growth rates (μ) were calculted for AFDW according to Eq. (3), such that:

$$\mu = (\ln x_2 - \ln x_1)/(t_{21} - t_0) \quad (3)$$

where μ is the instantaneous growth rate (d^{-1}), x_2 and x_1 are the final and initial whole animal weights, and t_{21} and t_0 represent the final (Day 21) and initial (Day 0) dates.

Clearance, absorption, and ingestion: Quahog clearance rates (CR) were determined assuming 100% particle retention efficiency for non brown tide species (>5 μm). Bivalve retention efficiency decreases for particles <5 μm (Riisgård 1988), and Bricelj et al. (2001) found retention of *Mercenaria mercenaria* fed *Aureococcus anophagefferens* (2 to 3 μm) averaged 80.4% at 3.5×10^4 to 2×10^5 cells ml^{-1}, so CR for *A. anophagefferens* was adjusted accordingly. A 5 mm quahog filters natural particles at 0.1 l h^{-1} at 25°C (Malouf & Bricelj 1989). The pump was set at this flow rate per quahog unless it required further calibration.

Clearance rates were determined at the end of each growth experiment because physiological processes scale to body size (Bayne & Newell 1983, Peters 1983, Riisgård 1988). Water samples (n = 3) of 1 ml were taken from each replicate (t_0), quahogs fed for 1 h so that <30% of the initial phytoplankton concentration was removed, and chambers were resampled (t_f). Food was kept in suspension by aeration. CR was calculated according to:

$$CR = M/(n \times t) \times \ln (C_i/C_f) \quad (4)$$

by Coughlan (1969), where M = chamber volume (l), C_i = initial concentration (cells l^{-1}), C_f = final concentration, and n = number of individuals.

Individual ingestion rates (IR, mg h^{-1}) were calculated according to:

$$IR = CR \times p \quad (5)$$

where p is the particle concentration (mg l^{-1}). Absorption rate (AR, mg h^{-1}) was calculated according to

$$AR = IR \times (AE/100) \quad (6)$$

Values for mixed diets were based upon CRs of individual phytoplankton species to determine AR for each alga. CR values did not vary much if calculated from the total mixture. AR values represent dry weight, but carbon absorption can be approximated because carbon contributes ~40% of total dry weight (Finlay & Uhlig 1981, Smetacek 1981, Fenchel & Finlay 1983), and CN analysis showed that the %C-content of log-phase algae varied little between species.

Pseudofeces production may decrease bivalve ingestion (Bricelj & Malouf 1984, Bacon et al. 1998). Pseudofeces production was assumed to be negligible for these experiments because *Mercenaria mercenaria* produce low amounts of pseudofeces compared to other bivalves (Bricelj & Malouf 1984), and juvenile quahogs do not produce pseudofeces at the selected algal ration (Bass et al. 1990, Wikfors et al. 1992). Experiments determined whether quahogs produced pseudofeces from each diet. Adult (45 to 55 mm) quahogs were placed individually into 600 ml beakers, acclimated to 450 ml of each treatment (9.12×10^{-4} mg ml^{-1}) for 24 h, then pulse-fed diets over 3 h at intervals that approximated their clearance rates (Eq. 4). Fecal matter was subsequently removed using a glass pipet, viewed under a dissecting microscope, and the presence/absence of feces and pseudofeces was noted. Feces were visually characterized by compact dark structures, and pseudofeces were identified as fluffy, light-colored material.

Statistics: Tissue growth was determined by comparing the initial to final AFDW values using 2-tailed Students *t*-tests (α = 0.05) (Sokal & Rohlf 1995). A Wilcoxon's signed-rank test was done between the unialgal and mixed diets to determine whether *Aureococcus anophagefferens* influenced growth. Since 5 experiments were done, a significance level of α = 0.10 was used for the Wilcoxon's signed-rank test.

RESULTS

Absorption efficiency experiments

The ^{14}C absorption efficiency of *Mercenaria mercenaria* was highest for *Isochrysis galbana* (83.6% ± 5.2) and lowest for *Prorocentrum minimum* (mixture) (45.4% ± 1.7, Fig. 2). For unialgal diets, AE was highest for *I. galbana* followed closely by *Thalassiosira pseudonana* (79.9% ± 7.2), and lowest AEs were for *Nitzschia closterium* (61.7% ± 6.3) and background levels of *A. anophagefferens* (52.5% ± 4.8). For mixtures, all diets except *N. closterium* resulted in a significantly (*t*-test, $p < 0.05$) lower mean AE than the corresponding unialgal diet.

Most (>80%) of the ^{51}Cr was recovered in the feces within the first 6 h of the experiment, whereas quahogs retained ^{14}C for a longer time period (Fig. 3). The gut passage time for ^{14}C-labeled cultures differed for each algal species (Table 2). *Thalassiosira pseudonana* exhibited the longest GPT (7.17 h) for 50% of the labeled matter, while *Prorocentrum minimum* (mixture) exhibited the shortest GPT (1.46 h). GPT for unialgal diets were generally slower than mixtures, except *Nitzschia closterium*, which had comparable GPTs. These observations occurred during the linear phase of digestion, and likely represented intestinal digestion (Widdows et al. 1979).

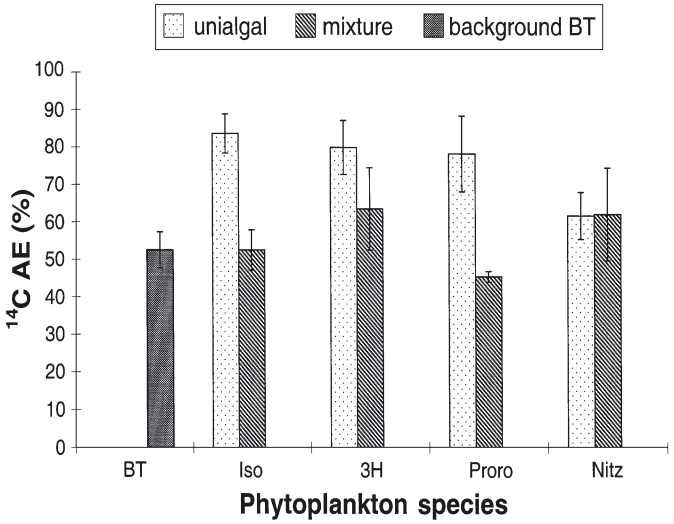

Fig. 2. *Mercenaria mercenaria.* Mean (±SD) carbon absorption efficiencies (%) for *M. mercenaria* fed a unialgal diet, a mixture containing 3×10^4 cells ml^{-1} of *Aureococcus anophagefferens*, and a background (3×10^4 cells ml^{-1}) level of *A. anophagefferens* (brown tide). Other algal species include *Isochrysis galbana* (Iso), *Thalassiosira pseudonana* (3H), *Prorocentrum minimum* (Proro), and *Nitzschia closterium* (Nitz)

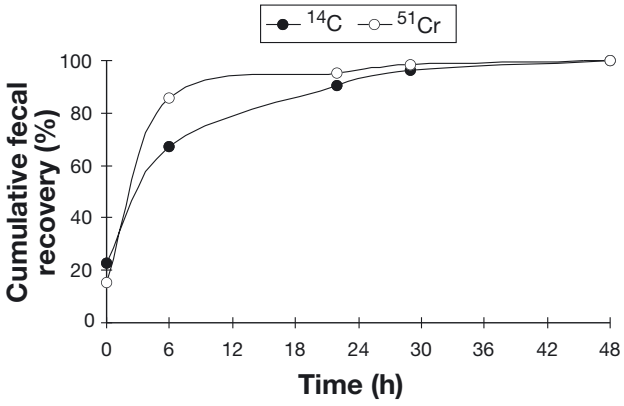

Fig. 3. *Mercenaria mercenaria.* Cumulative fecal recovery (%) for *M. mercenaria* fed *Thalassiosira pseudonana* in ^{14}C (dpm) and ^{51}Cr (cpm) over a 48 h depuration period. Recovery at the end of 48 h was assumed to be 100%

Growth experiments

Growth of *Mercenaria mercenaria*

At the end of each experiment, AFDW of *Mercenaria mercenaria* was lower for mixtures than unialgal diets (Fig. 4). AFDW increased for both treatments of *Isochrysis galbana*, *Thalassiosira pseudonana*, and *Prorocentrum minimum* compared to t_0, and the increase in AFDW for the unialgal diet was statistically significant (*t*-test, $p < 0.05$) for each experiment. For *I. galbana*, this corresponded to an instantaneous growth rate of 0.029 d^{-1} (Table 3), and quahogs fed *T. pseudonana* exhibited a similar μ (0.030 d^{-1}). AFDW decreased for *M. mercenaria* fed *Aureococcus anophagefferens* and *Nitzschia closterium*, but this decrease was not significant. For mixtures, a significant increase in AFDW compared to t_0 was found only with *I. galbana* and

Table 2. *Mercenaria mercenaria*. Time for 50% of different ^{14}C-labeled algal species to pass through the gut of *M. mercenaria*. Values were determined for the intestinal phase according to a linear model

Species	50% recovery time (h)	
	Unialgal diet	Mixture
Aureococcus anophagefferens	5.16	
Isochrysis galbana	5.65	3.90
Thalassiosira pseudonana	7.17	5.42
Prorocentrum minimum	3.28	1.46
Nitzschia closterium	5.34	5.38

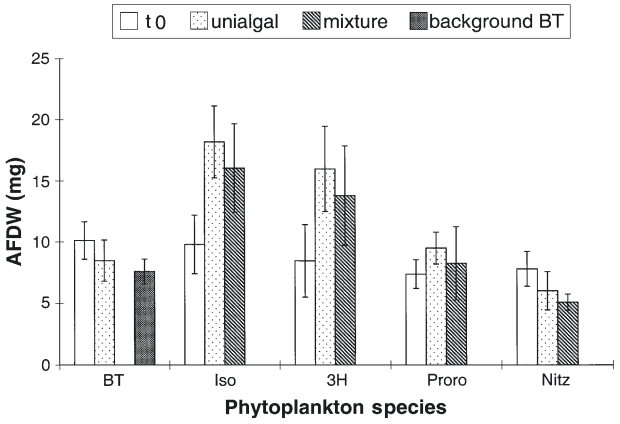

Fig. 4. *Mercenaria mercenaria.* Mean (±SD) initial and final ash-free dry weight (AFDW) over 21 d growth experiments compared to t_0 (initial AFDW). Diets as in Fig. 2

T. pseudonana, while a significant decrease was found for background levels of *A. anophagefferens* and *N. closterium* (mixture). The trend for mixed diets to result in slightly lower AFDW than the corresponding unialgal diet after 21 d was significant (Wilcoxon's signed rank test, $p < 0.10$). Instantaneous growth rates for mixtures were lower than the corresponding unialgal diet for all experiments, and the lowest growth rate was the mixture of *N. closterium* (-0.020 d^{-1}).

Clearance, absorption, and ingestion

Clearance rates varied considerably between algal species (Fig. 5). For unialgal diets, mean CR was highest for *Isochrysis galbana* (0.362 l h^{-1}) and *Thalassiosira pseudonana* (0.353 l h^{-1}) but lowest for *Nitzschia closterium* (0.037 l h^{-1}). CR generally increased between unialgal diets and mixtures, with the exception of *T. pseudonana*, which exhibited a slight, but statistically not significant (*t*-test, $p > 0.05$), decrease. The difference in CR between the unialgal (high concentration) and background levels of *Aureococcus anophagefferens* was statistically significant (*t*-test, $p < 0.05$), as were the differences between unialgal and mixed suspensions of *N. closterium.* Trends for IR were comparable to CR, with the exception of *A. anophagefferens*, which resulted in a decrease of IR between the unialgal (high concentration) and background levels (data not shown).

Absorption rate was greatest for the unialgal diets of *Isochrysis galbana* (27.570×10^{-5} mg h^{-1} ± 0.400 SE) and *Thalassiosira pseudonana* (25.725×10^{-5} mg h^{-1} ± 0.815), but least for *Nitzschia closterium* (2.082×10^{-5} mg h^{-1} ± 0.708, Fig. 6). Low AR was observed for background levels of *Aureococcus anophagefferens* (4.499×10^{-5} mg h^{-1} ± 1.500). A decrease in AR was found between unialgal diets and mixtures for all algae except *N. closterium*, which increased from 2.082×10^{-5} mg h^{-1} ± 0.708 to 8.629×10^{-5} mg h^{-1} ± 2.934. Quahogs did not produce pseudofeces when fed unialgal diets of *I. galbana* and *T. pseudonana*, but some individuals produced a combination of feces and pseudofeces when fed *P. minimum* and *N. closterium*. Some pseudofeces production occurred when *Mercenaria mercenaria* were fed diets containing *A. anophagefferens*.

Table 3. *Mercenaria mercenaria.* Instantaneous growth rates (μ) for ash-free dry weights (AFDW) of *M. mercenaria* grown for 21 d in laboratory experiments on a unialgal diet, a mixture containing 3×10^4 cells ml^{-1} of *Aureococcus anophagefferens*, and a diet consisting only of 3×10^4 cells ml^{-1} *A. anophagefferens* (background BT). Standard errors approximated by a first-order Taylor expansion series. BT: brown tide

Experiment	Treatment	μ (d^{-1})
Aureococcus anophagefferens	Unialgal (high BT)	−0.008 ± 0.151
	Background BT	−0.014 ± 0.593
Isochrysis galbana	Unialgal	0.029 ± 0.035
	Mixture	0.023 ± 0.053
Thalassiosira pseudonana	Unialgal	0.030 ± 0.055
	Mixture	0.023 ± 0.086
Prorocentrum minimum	Unialgal	0.012 ± 0.099
	Mixture	0.005 ± 0.449
Nitzschia closterium	Unialgal	−0.012 ± 0.178
	Mixture	−0.020 ± 0.083

DISCUSSION

This study showed that background levels of *Aureococcus anophagefferens* exert a minor negative influence on growth and absorption in *Mercenaria mer-*

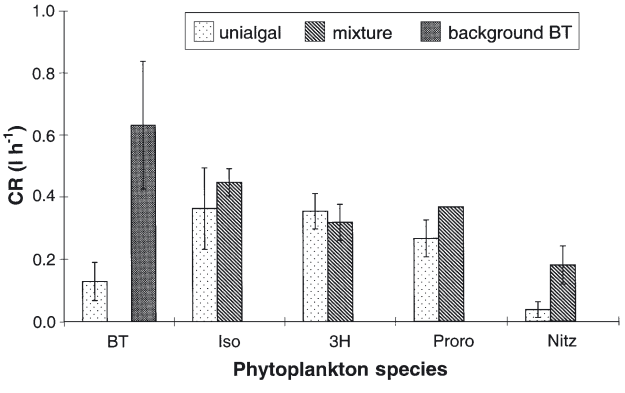

Fig. 5. *Mercenaria mercenaria.* Mean (±SE) clearance rates (l h^{-1}) determined at the end of the 21 d growth experiments. Diets as in Fig. 2

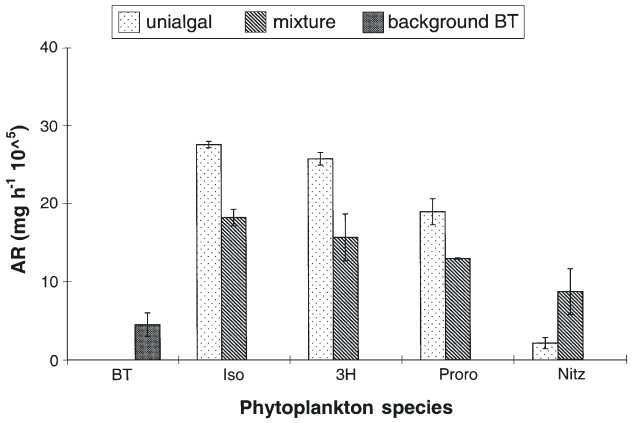

Fig. 6. *Mercenaria mercenaria.* Mean (±SE) absorption rates (mg dry wt $h^{-1} \times 10^5$). Diets as in Fig. 2

cenaria. The agreement between AR and growth patterns for experimental diets has implications for interpreting field data. The generally rapid quahog growth observed on Long Island's north shore compared to the south shore (Greenfield 2002) is likely partially attributed to the availability of centric diatoms on the north shore, whereas the slower growth on the south shore may be due to an abundance of *Nitzschia closterium*, a low-quality diet.

The benefit of *Isochrysis galbana* and *Thalassiosira pseudonana* for *Mercenaria mercenaria* found in the current study agrees with previous research. For example, the high AR of *M. mercenaria* fed *I. galbana* was attributed to a high AE within ranges represented by Bricelj & Malouf (1984), Bricelj et al. (1984), and Bass et al. (1990). Centric diatoms also support good quahog growth (Walne 1970, Epifano 1979, Laing et al. 1987). The AE for *T. pseudonana* was similar to that of *Mytilus edulis* fed *T. pseudonana* (Wang & Fisher 1996). The high absorption in the current study contributed to an AFDW increase of ~3% d^{-1} for quahogs fed both algal species. In context of the animal's carbon budget, a surplus is available after losses from respiration and metabolism, so that extra carbon contributed to 3% daily growth. Furthermore, the longer GPT of *T. pseudonana* and *I. galbana* compared to the other diets suggests that *M. mercenaria* retains these species during digestion to optimize nutritive gain.

Mercenaria mercenaria tended not to retain diets resulting in low AR and slow growth. Though quahogs exhibited positive AFDW increase when fed *Prorocentrum minimum*, growth was less than it was for *Isochrysis galbana* or *Thalassiosira pseudonana*. Since AE of *P. minimum* (unialgal diet) was equivalent to *T. pseudonana*, decreased CR probably explained the comparatively slower growth. These data support Wikfors & Smolowitz (1993), who found that diets containing *P. minimum* resulted in lower *M. mercenaria* growth than diets of *Isochrysis* sp. alone.

Of the non brown tide algal species, *Nitzschia closterium* provided the worst diet. Despite moderate AE, CR was negligible, suggesting that quahogs do not readily remove this species from the water column. Low CR was the primary factor contributing to low AR. The 1.2% daily loss in AFDW suggested that the metabolic demands for carbon outweighed the supply from *N. closterium*, so quahogs starved. *Nitzschia* sp. also results in slow growth of juvenile eastern oysters *Crassostrea virginica* (Ukeles & Wikfors 1988). The morphologically similar diatom *Phaeodactylum tricornutum* supports slow oyster growth (Ukeles & Wikfors 1982), and Wang & Fisher (1996) reported low (33.3 to 38.2%) AE for *Mytilus edulis* fed *P. tricornutum* using the dual tracer method but higher values (54.2 to 57.6%) using a mass balance. Both *N. closterium* and *P. tricornutum* are elongated, spiny pennate diatoms, and spines are more pronounced on *N. closterium*. This suggests that quahogs may be unable to handle this particular morphology.

The loss in AFDW for *Mercenaria mercenaria* fed high and background brown-tide levels showed that quahogs cannot survive on a toxic strain of *Aureococcus anophagefferens* alone. Since nearly monospecific blooms of *A. anophagefferens* frequently recur in Long Island embayments (Nuzzi & Waters 1989, Bricelj & Lonsdale 1997), the inability of *M. mercenaria* to grow on brown tide alone has serious consequences for quahog survival. Despite high CR for background levels, defined as below the laboratory-determined feeding inhibition 'threshold' of 3.5×10^4 cells ml^{-1} for the same strain (CCMP 1708) of *A. anophagefferens* (Bricelj et al. 2001), low particle concentration led to low AR and subsequent biomass loss. *M. mercenaria* had a lower AE than the 90% for bay scallops and mussels fed brown tide (Bricelj & Kuenster 1989). The different AE values between the 2 studies are likely due to bivalve species because Bricelj & Kuenster (1989) used a comparable allotment (1 to 7×10^4 cells ml^{-1}) of *A. anophagefferens*. The low CR for the higher brown tide concentration was expected because this diet exceeded 3.5×10^4 cells ml^{-1} by 5-fold, so toxicity impeded clearance. However, CR never reached zero for *A. anophagefferens*, suggesting that quahogs may feed at abundances $>3.5 \times 10^4$ cells ml^{-1} (Greenfield & Lonsdale 2002) in the field.

The growth differences between treatments within experiments were not as great as would be expected based upon absorption. Though a significant trend was found for lower growth of mixed diets, differences were not statistically significant within experiments. This suggests that diets containing background levels of *Aureococcus anophagefferens* do not necessarily

result in slow growth. Except for *Nitzschia closterium*, the lower AR for mixtures was attributed to the lower AE of the non brown tide species. The intriguing question, therefore, is why would AE be lower for mixtures than for unialgal diets? Since diets were normalized for algal dry weight, the ration of the labeled species was reduced to account for brown tide. Absorption varies according to ration and percent organic matter (Bricelj & Malouf 1984, MacDonald et al. 1998). However, the brown tide ration used in the current study was low (<15% of the total diet), making this explanation unlikely. Alternatively, the rapid GPT for mixtures compared to unialgal diets suggests that *A. anophagefferens* might interfere with the AE of other phytoplankton species. This hypothesis has been suggested by Wikfors & Smolowitz (1993) for *Prorocentrum minimum* fed to *M. mercenaria*. Finally, inhibition of AE in mixtures may be transient, so quahogs recover allowing for slightly more growth during the 3 wk study than would be predicted from short-term AE experiments. Bricelj (2000) also showed that quahogs initially grow slowly when fed moderate (8×10^4 cells ml^{-1}) levels of *A. anophagefferens* mixed with *Isochrysis galbana*.

Nitzschia closterium (mixture) was the only diet supporting no growth that also had an increase in CR and AR relative to the unialgal diet. The elevated CR for the mixture is noteworthy because it occurred in the presence of toxic *Aureococcus anophagefferens*. Since the ration of *A. anophagefferens* was too low to prevent starvation, the decrease in AFDW compared to t_0 was probably a combination of the low dietary quality of *N. closterium* and low *A. anophagefferens* concentration.

Pseudofeces production generally occurred for diets containing *Aureococcus anophagefferens.* This implies that quahogs may reject brown tide. This may occur by recognizing cells of *A. anophagefferens* or their toxin, possibly via gill chemoreceptors. Little is known about gill sensory mechanisms, but oysters reject refractory over labile material (Ward & Levinton 1997), suggesting that sensory mechanisms may play a role in bivalve feeding, or that surface properties of particles are important.

Results from this study have implications for understanding the effects of phytoplankton community composition on quahog growth in nature. Embayments dominated by *Nitzschia closterium* and potentially other pennate diatoms may be associated with slow quahog growth, such as Long Island's Great South Bay (Greenfield 2002). Embayments where centric diatoms, such as *Thalassiosira pseudonana*, are abundant may be associated with comparatively rapid quahog growth. During a brown tide, toxicity will cause quahogs to cease feeding or feed minimally until the bloom abates. After a bloom, quahog feeding and growth will depend upon phytoplankton species composition. For example, high numbers (10^5 to 10^6 cells ml^{-1}) of nanoflagellates were found in Great South Bay after a brown tide, coincident with a *Mercenaria mercenaria* growth spurt (Greenfield & Lonsdale 2002). This elevated growth rate was likely attributed to nutritious flagellates becoming available for consumption. Since ingestion, absorption and growth are positively correlated for quahogs (Albentosa et al. 1993), *M. mercenaria* likely increases feeding when nutritious species become available.

Background levels of *Aureococcus anophagefferens* exert a minor negative effect on the growth of *Mercenaria mercenaria*. The relationship between growth, AR, and concentrations of *A. anophagefferens* exceeding 3×10^4 cells ml^{-1} remains poorly understood. When *A. anophagefferens* is present, even at background levels, quahogs may experience subtle, chronic effects on feeding behavior and growth. Thus, *M. mercenaria* growth in the presence of *A. anophagefferens* likely depends on cell toxicity, concentration of *A. anophagefferens*, water temperature, and availability of other phytoplankton.

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