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# Effect of green macroalgal mats on burial depth of soft-shelled clams *Mya arenaria*

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ABSTRACT: Green macroalgal mats are becoming prevalent in many parts of the world, including on important clam-harvesting beaches in SW New Brunswick, Canada. Such mats (*Enteromorpha* sp. and *Cladophora* sp.) may be affecting populations of soft-shelled clams *Mya arenaria* (L.). We investigated the effect of these mats on burial depth of soft-shelled clams in the field (2 impacted sites with high algal cover and 2 reference sites with no algal mats) and laboratory. At impacted sites, burial depth was significantly shallower for clams under macroalgal mats than for those in areas clear of algae. In the comparison of areas clear of algae at impacted sites to the reference sites, clam burial depth was not significantly different; rather, burial depth varied between sites independent of site type. Field measurements of benthic respiration and total sulfides were significantly higher in areas in which algal mats were present than in areas in which they were absent. In an 8 d laboratory experiment, clams (4 per aquarium) were placed in sand (10 cm deep) and covered with 0, 2 or 6 cm of macroalgae. Clam burial depth quickly decreased under algae and remained significantly shallower under 2 and 6 cm of algae than in the control (no algae). Near the end of the experiment, we removed the algae, and burial depth quickly increased. At the end of the experiment, clam body mass and dissolved oxygen at the sediment surface did not differ significantly between treatments, although both variables showed a decreasing trend with increasing algal mat cover. Dissolved organic carbon in pore water at the end of the experiment was significantly higher in the 6 cm algal treatment than in the control and 2 cm algal aquaria. The presence of algal mats clearly affects burial depth of soft-shelled clams. This in turn may have impacts on predator–clam interactions and on the surrounding environment.

KEY WORDS: Bay of Fundy · Eutrophication · Filamentous green algae · Infauna · Behavior



## INTRODUCTION

Coastal eutrophication, the over-enrichment of coastal zones with inorganic and organic nutrients, is becoming a global phenomenon (Rosenberg 1985, Loo & Rosenberg 1989, Strain & Yeats 1999, Cloern 2001). Increases in anthropogenic activities near coastal zones (e.g. housing developments, aquaculture, agriculture) and atmospheric deposition augment nutrient levels, such as nitrogen and phosphorus, in coastal waters. The impact of this enrichment depends on the capacity at which the environment can receive and absorb these excess nutrients (Strain & Yeats 1999, Cloern 2001). A common feature of additional nutrient loading in many locations is an increase in the formation of dense green macroalgal mats composed mainly of *Enteromorpha* sp. and *Ulva* sp. (Reise et al. 1989, Lavery & McComb 1991, Kolbe et al. 1995, Auffrey 2003).

Green macroalgal mats have been documented to decrease invertebrate abundance, diversity and growth (Nicholls et al. 1981, Soulsby et al. 1982, Everett 1994, Norkko & Bonsdorff 1996a,b,c, Thiel et al. 1998), and affect behavior by causing vertical migration in benthic infauna (Norkko & Bonsdorff 1996a,b,c, Norkko et al. 2000, Österling & Pihl 2001).

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Impacts on bivalves have been attributed to changes in sediment chemistry, water chemistry at the sediment–water interface (e.g. hypoxia), and the physical environment (e.g. decreased water flow) caused by dense macroalgal mats (Nicholls et al. 1981, Hull 1987, Bonsdorff 1992, Isaksson & Pihl 1992, Everett 1994, Escartin & Aubrey 1995, Norkko & Bonsdorff 1996a,b,c, Norkko et al. 2000). Hypoxic conditions, presumably created by dense algal mats, result in organisms altering their behavior (e.g. migration), physiological condition (e.g. increased respiration), feeding abilities, and reproductive output (Diaz & Rosenberg 1995 and references therein).

Soft-shelled clams *Mya arenaria* (L.) are bivalves distributed along the western North Atlantic coast from Labrador to South Carolina, the European coast, and the Pacific coast from Alaska to California (Strasser 1999). They inhabit muddy to gravel substrates from the intertidal zone down to 200 m in depth, but are most abundant in intertidal and shallow subtidal areas (Strasser 1999). Following a planktotrophic larval development stage, they settle on the bottom, burrow in the sediment and permanently inhabit that area unless conditions deteriorate or the clams are washed away. As a soft-shelled clam matures, its siphon elongates allowing the animal to burrow deeper into the sediment (Zwarts & Wanink 1989). Hypoxic conditions at the water–sediment interface have been shown to affect the behavior of soft-shelled clams both in the field and laboratory (Jørgensen 1980, Rosenberg et al. 1991, Taylor & Eggleston 2000). Therefore, the presence of algal mats on mudflats where clams occur, and their associated changes to environmental conditions, may also be affecting behavior.

The present study investigated clam behavior, specifically burial depth, in the presence and absence of green macroalgal mats in the field and laboratory. We predicted that clam burial depth would be shallower in the presence of green macroalgal mats than in their absence because of the altered environmental conditions caused by these mats (Nicholls et al. 1981, Hull 1987, Bonsdorff 1992, Isaksson & Pihl 1992, Everett 1994, Escartin & Aubrey 1995, Norkko & Bonsdorff 1996a,b,c, Norkko et al. 2000). We measured clam burial depth in field sites with high algal mat cover, both in areas covered with and free of algal mats. We also measured burial depth in field sites with no algal mat cover. Finally, we performed a laboratory experiment on the effect of different algal mat thicknesses on clam burial depth, clam body mass and abiotic variables.

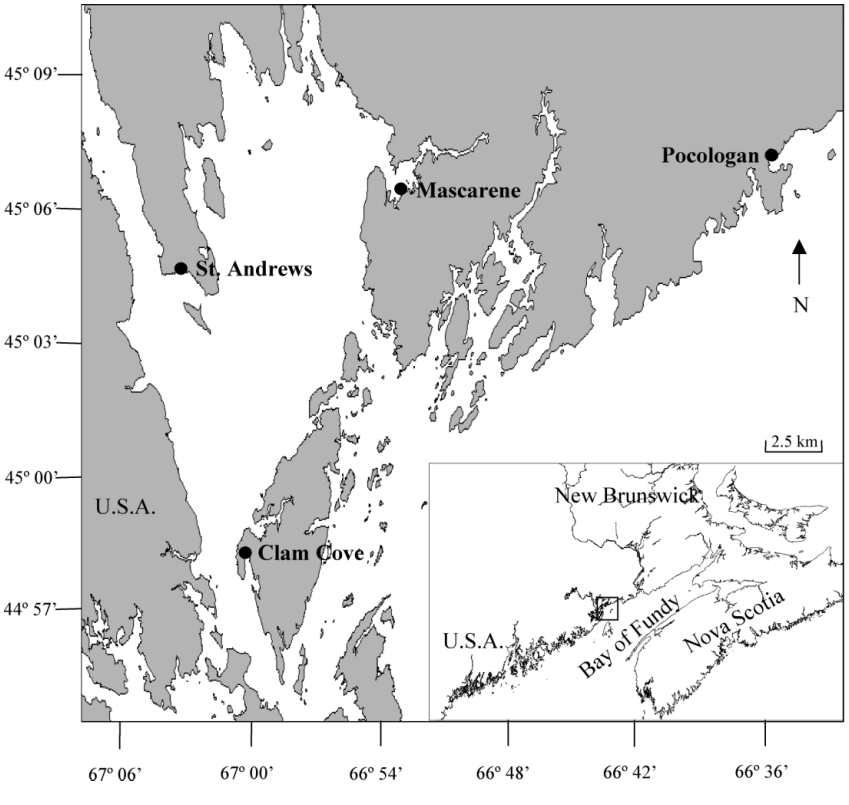


Fig. 1. The study area in SW New Brunswick, Canada, showing our 4 sites (2 impacted sites and 2 reference sites)

## MATERIALS AND METHODS

**Field sampling.** Burial depth of soft-shelled clams *Mya arenaria* (L.) was measured at 4 sites in SW New Brunswick (Fig. 1) on 24 to 26 June and 2 to 7 August 2001. The sites included 2 impacted mudflats with high green macroalgal cover (up to 40% cover of mostly *Enteromorpha* sp. and <5% *Cladophora* sp., Auffrey 2003), St. Andrews Blockhouse and Clam Cove on Deer Island, and 2 reference mudflats with no macroalgal cover, Pocologan and Mascarene. At the impacted sites, clam burial depth was measured in areas covered with macroalgal mats and in clear areas.

To measure clam burial depth, 2 to 4 trenches (30 cm deep) were dug in haphazardly chosen locations at the impacted sites (in both areas covered and free of mats) and at the reference sites. The walls of the trenches were gently scraped away with a trowel and, upon reaching a clam, burial depth was recorded by measuring the distance between the posterior edge of the clam

and sediment surface with a plastic ruler to the nearest mm. For a given month and area, 30 to 60 clams were sampled.

Sediment cores for total oxygen uptake (respiration) measurements were collected on 18 and 19 July 2001 in Mascarene (n = 6) and Clam Cove (n = 4 for each of the areas with and without algal mats), respectively. Sample collection and laboratory analysis were as described in Hargrave et al. (1983). Concurrently, 5 ml cores were collected from the upper 2 cm of the intertidal sediment using cut-off 5 ml syringes for measurements of total sulfides (n = 6 for each of Mascarene and Clam Cove in an area with and an area without algal mats). These samples were capped, labeled with common identifier labels, and stored on ice for up to 24 h until analysis. The syringe was then ejected into a 100 ml biotight jar and the sediment was diluted with 5 ml of sulfide anti-oxidant buffer solution (SAOB). Measurements of sulfide ($S^{2-}$) were obtained as described in Hargrave et al. (1995).

**Laboratory experiment.** The effect of different algal thicknesses on burial depth of soft-shelled clams was investigated in a laboratory experiment performed between 31 July and 8 August 2001 at the St. Andrews Biological Station. Aquaria (10 l, 14.3 cm [width] × 29.5 cm [height] × 23.8 cm [length]), each containing 4 large juvenile clams (2.9 ± 0.8 g fresh mass, 26.8 ± 2.5 cm shell length, 16.9 ± 1.7 cm shell height [mean ± SD]) buried in 10 cm of sand, had 1 of 3 algal thicknesses (mostly *Enteromorpha* sp. and <5% *Cladophora* sp.) placed on the sediment: 0 cm (control), 2 cm (215.9 ± 0.2 g wet mass [mean ± SD]) or 6 cm (645.6 ± 0.4 g wet mass). There were 3 replicate aquaria per algal treatment (total of 9 aquaria). Burial depth of each clam was measured daily. Clam survival was checked at the end of the experiment. Dissolved organic carbon in the sediment and dissolved oxygen at the sediment–algal (or sediment–water) interface were measured at the beginning and end of the experiment.

Clams were collected at the Blockhouse, St. Andrews, on 24 July 2001 and maintained in fresh continuously flowing seawater until transplanted in experimental aquaria. Green macroalgae were collected at the Blockhouse on 30 July 2001, rinsed in seawater to remove small invertebrates and placed in a tank with running seawater until the start of the experiment. Sand was collected at the Blockhouse, sieved with a 2 mm mesh in freshwater to remove large-sized particles and placed in the experimental aquaria (10 cm deep). Fresh seawater flowed over the sediment in the aquaria for 24 h before the addition of clams.

Semi-diurnal tides were simulated in the aquaria during the experiment. The aquaria were set up as follows (Fig. 2). Two 0.4 cm holes were drilled on 1 side of each aquarium, 10.5 cm from the bottom; one hole was open to allow outflow of seawater at the sediment surface and the other hole was plugged with silicone (and used to sample dissolved oxygen with a syringe, see below). Three 0.4 cm overflow holes were drilled on another side of each aquarium, 1.2 cm from the top. A solenoid valve, connected to the main seawater pipe, was plugged into an electric timer to control the opening and closing of the valve. Seawater flowed into each aquarium for 6 h, starting at 09:00 and 21:00 h (and was off at 15:00 and 03:00 h). Upon closing of the valve, the seawater would empty out. The inflow was set at 72.0 to 75.6 l $h^{-1}$, and outflow at the sediment surface was 72.4 ± 5.2 l $h^{-1}$ (mean ± SD) at the simulated high tide. The inflow was aimed at the back wall of each aquarium to create a gentle flow of water during high tides and avoid direct flow onto the sediment or algal layer. The outflow holes were cleaned every day with a dissecting needle to allow proper flow.

Salinity and temperature of the water were measured each day and were consistent throughout the experiment (temperature = 13.3 ± 0.4°C [mean ± SD]; salinity = 31.4 ± 0.30‰). A growth light (Sylvania Gro-lux F40/GRO/AQ/RP) was placed over the experimental aquaria and daily photoperiod was set at 14 h light:10 h dark (on at 07:00 h, off at 21:00 h) to simulate natural lighting conditions at the time of the experiment.

To monitor individual clams during the experiment, we affixed on the shell of each a plastic numbered tag (4 × 10 mm) and a 40 cm long monofilament line (nylon line, 20 kg) using cyanoacrylate glue, 1 d prior to planting in experimental aquaria. After gluing, the

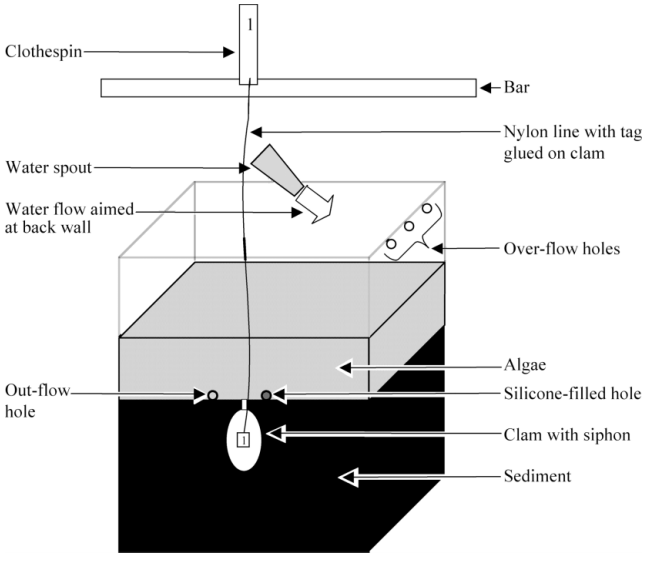


Fig. 2. An experimental aquarium. Dimensions of aquarium are 14.3 cm (width) × 29.5 cm (height) × 23.8 cm (length). Sediment (10 cm) was placed in the aquarium and was covered with either 0, 2 or 6 cm of algae

length of the line was cut so that the length between the posterior end of the clam and tip of the line measured 35 cm. After placing the clams in the experimental aquaria, the line was secured (leaving some slack in the line) with a numbered clothespin, matching the clam number, to a bar above the aquarium (Fig. 2). The clams, randomly assigned to the experimental treatments, were allowed to acclimate and burrow for 24 h prior to the addition of algae. Clam shell length did not vary between treatment levels ($F_{2,29}$ = 1.07, p = 0.36). During the experiment, burial depth was measured at the simulated low tide by unfastening the clothespin and gently placing the line against a long, thin wooden dowel, vertically placed on the sediment surface, adjacent to the clam being examined. The top of the line was marked on the vertical dowel with a lead pencil. We then measured the distance between the bottom of the dowel and the mark, and subtracted this value from 35 cm to obtain burial depth for a particular clam.

Dissolved organic carbon (DOC) was measured at the beginning of the experiment (prior to addition of algae) in 3 randomly (using a random number table) chosen aquaria, and at the end of the experiment in all 9 aquaria. Nalgene bottles (50 ml) were filled halfway with sediment and topped off with seawater from the aquaria. The bottles were lightly shaken and placed overnight in a refrigerator to allow particle settlement. The cooled supernatant was drawn in a sterile syringe and filtered through a pre-ashed GF/F filter (at the St. Andrews Biological Station) into a clean bottle and frozen until analyzed (at the Bedford Institute of Oceanography), as described in Bugden et al. (2001).

Dissolved oxygen was measured at the beginning of the experiment (prior to addition of algae) in 3 randomly chosen aquaria and at the end of the experiment in all 9 aquaria, both at night (when algae are respiring only) and during the day (when algae are photosynthesizing and respiring). We extracted 180 ml of water at the sediment interface with a 60 ml syringe and 16-gauge needle (38 mm long) through the silicone-filled hole. The water was gently poured from the syringe into a conical flask by removing the needle and applying slow and steady pressure to the plunger. Contact with atmospheric oxygen and stirring or shaking of the flask were kept to a minimum. Winkler analysis for dissolved oxygen was done as described in Levy et al. (1977).

One day before the end of the experiment, the algae were removed and re-weighed to check for differences between initial and final algal cover (final 2 cm = 190.6 ± 7.5, 6 cm = 630.8 ± 67.9 g wet mass [mean ± SD]). Clams were left in the experimental aquaria for an additional 24 h to see if their burial depth would change after the algae had been removed. After completion of the experiment, body mass analyses were performed on the experimental clams.

To assess body mass, the clams were first placed in seawater for 24 h in order to allow depuration of the gut (Hawkins & Rowell 1987). Prior to dissecting, wet fresh mass (g), shell length (mm), and shell height (mm) were measured. The clams were then dissected with a spatula, and the tissues were blot-dried to remove excess water. The tissues were dried in a 60°C drying oven for 48 h and then re-weighed.

**Effect of nylon line.** Concurrent with the above laboratory experiment, a small laboratory experiment was conducted to assess the effect of the nylon line on clam burial depth. Each of 3 algal treatments (0, 2 and 6 cm algal thickness) was set up in an aquarium (total of 3 aquaria). Four clams were deployed per aquarium: 2 with nylon line and 2 without nylon line. Other experimental conditions (tidal cycle, temperature, salinity and lighting) were as in the above experiment. For clams affixed with a nylon line, burial depth was measured on a daily basis; for clams without a nylon line, burial depth was observed only at the end of the experiment.

**Statistical analysis.** Measurements of clam burial depth from the impacted sites (Clam Cove and St. Andrews) were analyzed for each site separately, using 2-way ANCOVA (analysis of covariance) with time (2 levels: June, August) and algal presence (2 levels: presence and absence of algal mat) as fixed factors, and clam shell length as the covariate. We could not use a 3-way ANCOVA with site, time and algal presence as factors because the assumption of homogeneity of regression slopes was not met. Assumption of normality was examined visually by inspection of the residuals. Homogeneity of variance and homogeneity of regression slopes were checked as described in Huitema (1980). To meet the assumptions of normality and homogeneity of variance, a reflexion transformation (Tabachnick & Fidell 1983) was applied to data from Clam Cove and a square-root transformation was applied to data from St. Andrews. Post hoc comparisons for the detection of a significant interaction were done using a simple effects test (Huitema 1980).

To compare field burial depths from the areas without algae at the impacted sites to those from the reference sites (Pocologan and Mascarene), we ran a nested ANCOVA with type of site (2 levels: reference, impacted-algae absent) and time (2 levels: June, August) as fixed factors, and site as a random factor nested within type. Clam shell length was the covariate. Assumptions of normality, homogeneity of variance, and homogeneity of regression slopes were checked as described above, and were met.

We analyzed measurements of respiration and total sulfides from the field as follows: (1) measurements

Table 1. *Mya arenaria*. ANCOVA examining the effects of algal mats (presence, absence) and month (June, August) on burial depth for each of the 2 impacted sites. Prior to analysis, a reflexion transformation ($\sqrt{K-X}$, where $K$ represents the maximum value of $X+1$, and $X$ the data) was applied to data from Clam Cove; a square-root transformation was applied to data from St. Andrews. For post hoc comparisons, treatment levels are listed in increasing magnitude of their mean; those sharing a common underline do not differ significantly, as determined by simple effects test. A = algal presence, NA = algal absence, Aug = August

| Site | Source of variation | df | MS | *F* | p | Post hoc comparison |
|---|---|---|---|---|---|---|
| Clam Cove | Month | 1 | 14.29 | 15.13 | <0.001 | |
| | Algae | 1 | 85.33 | 90.30 | <0.001 | |
| | Month × Algae | 1 | 1.81 | 1.91 | 0.17 | |
| | Clam length | 1 | 105.43 | 111.57 | <0.001 | |
| | Error | 115 | 0.95 | | | |
| St. Andrews | Month | 1 | 0.34 | 0.55 | 0.46 | |
| | Algae | 1 | 35.43 | 57.70 | <0.001 | |
| | Month × Algae | 1 | 2.75 | 4.48 | 0.037 | A: Aug June<br>NA: June Aug<br>June: A NA<br>Aug: A NA |
| | Clam length | 1 | 66.16 | 107.74 | <0.001 | |
| | Error | 115 | 0.61 | | | |

from areas with and without algal mats in Clam Cove were compared using a paired *t*-test, and (2) measurements from Mascarene and the area without algal mats in Clam Cove were compared using 2-sample *t*-tests (Zar 1999). The data for respiration met all assumptions, while the data for total sulfides were transformed using natural logarithm.

Clam burial depths observed in the laboratory experiment were analyzed using a nested, repeated-measures ANOVA with algal thickness (3 levels: 0, 2, 6 cm) as a fixed factor, aquarium (3 levels) as a nested random factor, and time (Days 1 to 7) as a repeated factor. Data from 1 aquarium in the 2 cm algal treatment were not used because the algal mat did not settle on the sediment during simulated high tide as in the other experimental aquaria (it floated). In addition, 2 clams died in the 6 cm algal treatment on Day 5; we included burial depths of these clams in our analysis for Days 1 to 4 only (during those 4 d their behavior was very similar to their conspecifics). The data met the assumptions of sphericity (Potvin et al. 1990), normality (inspected visually) and homogeneity of variance (verified using Cochran's *C*-test). Post hoc comparisons were performed using the Student-Newman-Keuls (SNK) test (Winer 1971).

Other measurements obtained at the beginning and at the end of the laboratory experiment were analyzed as follows. Initial oxygen and DOC measurements were compared to final measurements in the control aquaria with 2-sample *t*-tests (Zar 1999). Final clam burial depth (Day 8), clam body mass and DOC were all analyzed using a 1-way ANOVA with algal thickness as a fixed factor. The 2 dead clams in the 6 cm algal treatment were not included in the analysis of body mass. Dissolved oxygen was analyzed using a repeated-measures ANOVA with algal thickness as a fixed factor and time (2 levels: day, night) as the repeated factor. Assumptions of normality and homogeneity of variance were tested as indicated above and were met, except for oxygen measurements in the comparison between the beginning and the end of the experiment (these were transformed using natural logarithm).

## RESULTS

### Field sampling

At both impacted sites, clam burial depth was significantly shallower in the presence of algae than in their absence (Table 1, Fig. 3). We actually observed a few clams that were on the sediment surface under algae in June in Clam Cove. Clam burial depth was shallower here in June than in August. In St. Andrews, burial depth showed no significant difference between the 2 mo.

Comparison of the areas clear of algal mats in the impacted sites to the reference sites showed that clam burial depth was not significantly affected by mudflat type (Table 2, Figs. 3 & 4). Rather, it varied between sites independently of mudflat type. Clam burial depth tended to be shallower in June than in August, but this trend was non-significant.

Although we did not measure oxygen concentrations at the sediment–algal interface in the field, black, sulfurous-smelling surface sediments under macroalgal mats indicated hypoxic conditions. Comparison of total oxygen uptake under algal mats and in areas clear of algae in Clam Cove indicated that respiration

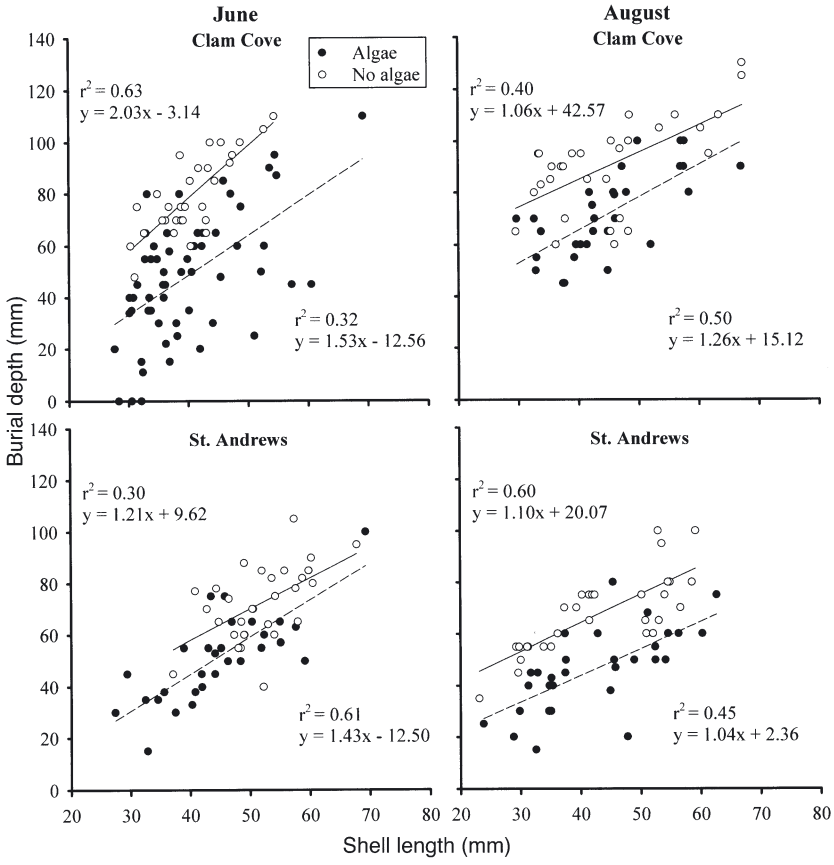


Fig. 3. *Mya arenaria*. Relationship between burial depth and shell length at impacted sites in the presence and absence of algal mats

in covered areas was higher than in algae-free areas (Fig. 5, $t_3$ = 4.12, p = 0.026). Total oxygen uptake in areas clear of algae in Clam Cove was significantly lower than that in the reference site (Mascarene) ($t_8$ = 2.72, p = 0.026).

In Clam Cove, measurements of total sulfides under algal mats were significantly higher than those in areas clear of algae (Fig. 5, $t_5$ = 4.43, p = 0.007). Total sulfides in areas clear of algae in Clam Cove were significantly higher than in Mascarene ($t_{10}$ = 3.23, p = 0.009).

### Laboratory experiment

The nylon line did not prevent the burrowing abilities of clams. In our small experiment that examined the effect of the nylon line, tethered and non-tethered clams migrated vertically in the 2 and 6 cm algal treatments, but remained burrowed in the control aquaria.

One day after the addition of algae to aquaria in our main experiment, clam burial depth decreased dramatically (Fig. 6). For most of the remainder of the experiment (Days 1 to 7), burial depth remained significantly shallower in the aquaria with algae than in the control aquaria (Table 3, Fig. 6). Although clams tended to be buried shallower in the 6 cm algal treatment level than in the 2 cm algal treatment level, this trend was not significant. On Day 7, 7 out of 8 clams and 10 out of 12 clams were on the sediment surface in the 2 cm algal treatment level and 6 cm algal treatment level, respectively (Fig. 6). Once all algae were removed (Day 8), clams quickly increased their burial depth (Fig. 6), with no significant difference in burial depth between treatments ($F_{2,23}$ = 1.869, p = 0.18).

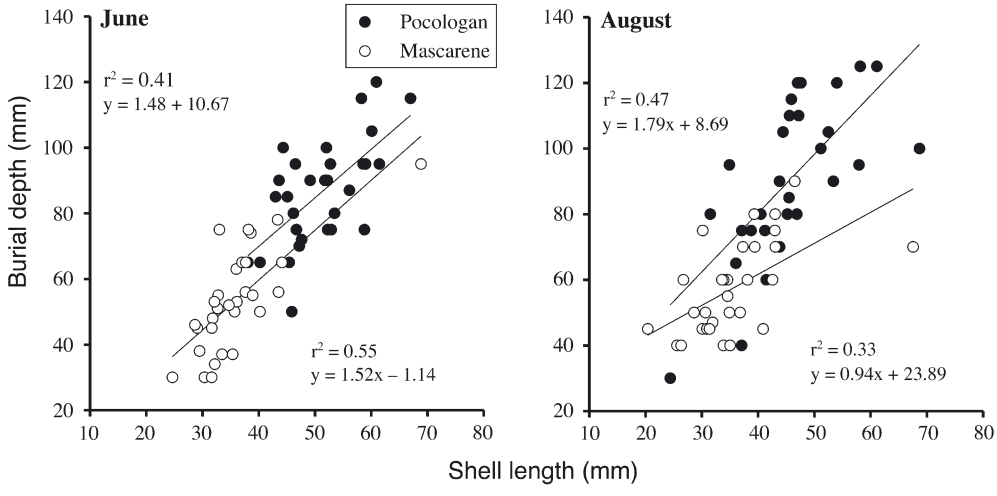


Fig. 4. *Mya arenaria*. Relationship between burial depth and shell length at reference sites (algal mats absent)

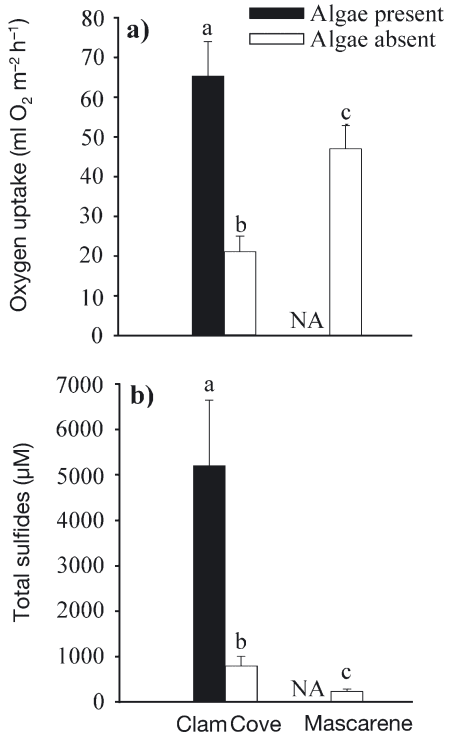


Fig. 5. (a) Mean benthic oxygen uptake and (b) total sulfides in Clam Cove (impacted site with areas covered with and clear of algal mats) and Mascarene (reference site). Error bar = 1 SE; n = 4 to 6. Bars with different letters differ significantly

Although clam dry body mass tended to decrease with increasing algal thickness (Fig. 7a), the trend was not significant ($F_{2, 27}$ = 1.828, p = 0.18).

Initial and final dissolved oxygen concentrations in the control aquaria did not differ significantly ($t_4$ = 1.40, p = 0.24). At the end of the experiment, dissolved oxygen tended to be lower in the 6 cm algal treatment than in the other algal treatment levels (Fig. 7b). In the presence of algae, dissolved oxygen also tended to be higher at night than in the day (Fig. 7b). However, none of these trends was significant (Algae: $F_{2, 5}$ = 4.10, p = 0.088; Time: $F_{1, 5}$ = 0.11, p = 0.76) because, we suspect, of a sampling artifact (as discussed in the 'Discussion' section). At the end of the experiment we observed black, sulfurous-smelling sediments in the 2 and 6 cm algal treatment levels, and brown, non-smelly sediments in the control aquaria.

Initial DOC did not differ significantly from final DOC in the control aquaria (Fig. 7c, $t_4$ = 2.70, p = 0.054). At the end of the experiment, DOC was significantly higher in the 6 cm algal treatment level than in the 2 cm algal treatment level and control aquaria ($F_{2, 5}$ = 10.92, p = 0.015).

Table 2. *Mya arenaria*. Nested ANCOVA examining the effect of mudflat type (impacted, reference) and month (June, August) on burial depth. Site is a random factor, nested in mudflat type (Clam Cove and St. Andrews for the impacted sites, and Pocologan and Mascarene for the reference sites). Note that for the impacted sites, we used data for areas clear of algal mats in this analysis

| Source of Variation | df | MS | *F* | p |
|---|---|---|---|---|
| Month | 1 | 2764.07 | 15.23 | 0.060 |
| Type | 1 | 112.35 | 0.011 | 0.93 |
| Site(Type) | 2 | 10360.64 | 63.73 | <0.001 |
| Month × Type | 1 | 54.49 | 0.30 | 0.64 |
| Month × Site(Type) | 2 | 181.43 | 1.12 | 0.33 |
| Clam length | 1 | 28863.70 | 177.55 | <0.001 |
| Error | 230 | 162.57 | | |

## DISCUSSION

As predicted, the burial depth of soft-shelled clams *Mya arenaria* was shallower in the presence of green macroalgal mats than in the absence, both in the field and laboratory. We also observed clams at the sediment surface under algal mats at 1 impacted site (Clam Cove) and in our laboratory experiment. Vertical migration to the sediment surface under macroalgal mats has been observed for bivalves such as *Cerastoderma* spp. and *Macoma balthica* in the laboratory and field (Norkko & Bonsdorff 1996a,b,c, Norkko et al. 2000, Österling & Pihl 2001), and for *Mya* sp. in an intertidal mudflat (Thiel et al. 1998). Vertical migration of bivalves into the overlying algal mats has also been documented

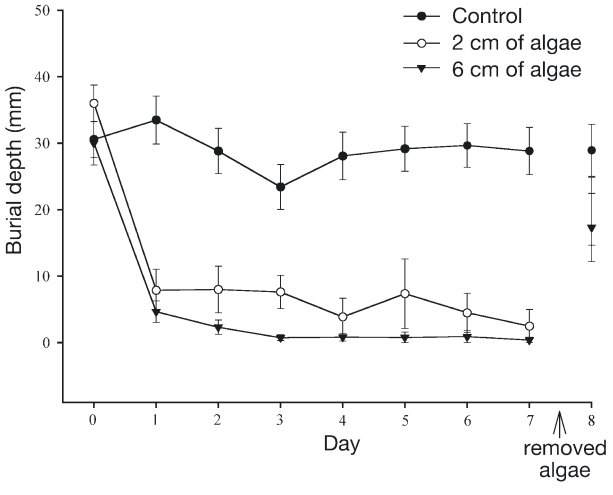


Fig. 6. *Mya arenaria*. Burial depth (mean ± SE) during an 8 d laboratory experiment which examined the effect of algal thickness (either 0, 2 or 6 cm). See Fig. 2 for aquarium set-up. Algae were removed from 2 cm and 6 cm algal treatment levels on Day 7 and final burial depth in all aquaria was taken on Day 8. n = 6 to 12

Table 3. *Mya arenaria*. Repeated-measures ANOVA examining the effect of algal thickness (0, 2, 6 cm) on burial depth during an 8 d laboratory experiment. For the post hoc comparisons (SNK), treatment levels are listed in increasing magnitude of their mean; those sharing a common underline do not differ significantly

| Source of variation | df | MS | *F* | p | Post hoc comparison |
|---|---|---|---|---|---|
| Algae[a] | 2 | 15697.39 | 31.80 | <0.001 | 6 2 0 |
| Aquarium(Algae) | 6 | 493.72 | 1.09 | 0.40 | |
| $Error_{between}$ | 21 | 453.99 | | | |
| Time[b] | 6 | 75.60 | 1.79 | 0.13 | |
| Time × Algae[b] | 12 | 24.16 | 0.57 | 0.85 | |
| Time × Aquarium(Algae) | 36 | 42.28 | | | |
| $Error_{within}$ | 126 | 16.73 | | | |

[a]Aquarium(Algae) is a random factor. The denominator for the *F*-ratio of Algae is Aquarium(Algae)
[b]The denominator for the *F*-ratio of these factors is Time × Aquarium(Algae)

(Norkko & Bonsdorff 1996a,b,c, Norkko et al. 2000, Österling & Pihl 2001). We observed only 1 clam in the algal mat during our laboratory experiment. The lack of movement into algal mats by *M. arenaria* could be explained by the lower mobility of these clams compared to other clam species, as well as the age of the clams used in our experiment. We used clams >20 mm; clams <20 mm are more agile because of the need to re-burrow if washed away (Matthiessen 1960, Hunt & Mullineaux 2002).

### Reasons underlying changes in burial depth

Hypoxic conditions cause organisms to alter their behavior and physiological condition (Diaz & Rosenberg 1995), and infaunal animals commonly respond to such conditions by emerging at the sediment surface (Jørgensen 1980). For example, *Mya arenaria*, in Limfjorden (Denmark) and in laboratory experiments, responded to low oxygen saturation (that was not induced by algal mats) by extending their siphon above the sediment surface or by completely emerging and lying on the sediment, thus allowing maximum siphon extension above the hypoxic layer at the sediment–water interface (Jørgensen 1980, Rosenberg et al. 1991, Taylor & Eggleston 2000).

In our laboratory experiment and during our field-sampling study, hypoxic conditions developed in the sediments in the presence of algae, as indicated by the blackness and sulfurous smell of the sediments. However, in our laboratory experiment, the oxygen concentration at the sediment–algal interface did not reach hypoxic values (which would be between 0.0 and 2.0 ml $l^{-1}$, Diaz & Rosenberg 1995), and the observed trend of lower oxygen concentration at the sediment surface in the presence of algae was not significant. These observations are likely to be due to the flowing seawater and simulated tidal cycles in our experiment. Also, since our experiment lasted 7 d, it is possible that oxygen levels under the algal mats did not have time to reach hypoxic values. Other studies documenting hypoxic levels at the sediment interface under algal mats did not have flowing water, thus likely enhancing oxygen depletion at the sediment interface (Norkko & Bonsdorff 1996c, Norkko et al. 2000, Österling & Pihl 2001). In addition, the volume of water collected at the sediment interface in our experiment was relatively large (180 ml); we may have collected aquarium water, high in oxygen, along with interface water, thus contaminating our samples.

We detected significantly higher DOC values in pore water under thick algal mats than under thin or absent algal mats in our laboratory experiment. Organic

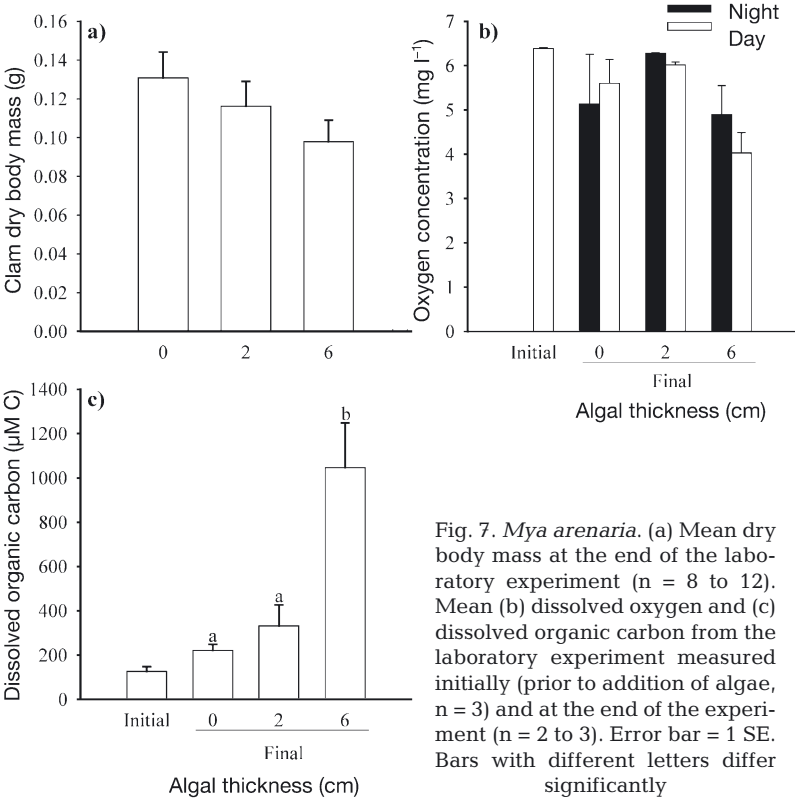


Fig. 7. *Mya arenaria*. (a) Mean dry body mass at the end of the laboratory experiment (n = 8 to 12). Mean (b) dissolved oxygen and (c) dissolved organic carbon from the laboratory experiment measured initially (prior to addition of algae, n = 3) and at the end of the experiment (n = 2 to 3). Error bar = 1 SE. Bars with different letters differ significantly

enrichment of sediments results from the decomposition of organic matter, as well as dissolved organic carbon exuded by algae (Valiela et al. 1997). Sediment enrichment increases benthic oxygen demand by bacteria and fauna associated with algal mats and underlying sediments, creating hypoxic conditions (Valiela et al. 1997). Benthic respiration measurements from our field study were approximately 3 times higher in areas covered with algae than those in clear areas, indicating higher oxygen uptake under algal mats.

Hypoxic conditions allow sulfate-reducing bacteria, usually deep in the sediment, to survive at the surface. This results in hydrogen sulfide production at the surface, which can be lethal to infaunal species (Jørgensen 1980, Nicholls et al. 1981, Everett 1994, Diaz & Rosenberg 1995, Norkko & Bonsdorff 1996a,b,c). Laboratory studies have shown that high hydrogen sulfide concentrations in the sediment intensify the negative effects of hypoxia (such as decreased survival, Diaz & Rosenberg 1995). In both our laboratory experiment and field observations, black, sulfurous-smelling sediments under algal mats indicated hydrogen sulfide production. In addition, values of total sulfides in the field were significantly higher under algal mats than in algae-free areas. Further, areas clear of algal mats at impacted sites might have been covered with algae in the past; therefore, these areas could still record higher levels of total sulfides than reference sites. Although our measurements for respiration and total sulfides were not taken at the same time as those for burial depth, black, sulfurous-smelling sediments under algae were observed throughout the period for the field study (Auffrey 2003).

Toxic exudates, released by macroalgal mats, may cause escape responses in infaunal animals (Norkko & Bonsdorff 1996a,c, Österling & Pihl 2001). Although green algae are thought to have the least amount of toxic exudates (Valiela et al. 1997), activated toxins resulting from predator damage have been found in them (van Alstyne et al. 2001). We were careful to remove any fauna from the algae used in the laboratory experiment. However, it is possible that damage done prior to the experiment could have caused some toxin production. Green macroalgal mats could also release non-predator-induced toxins**.** For example, *Ulva lactuca* releases a non-predator-induced toxin which, acting in combination with oxygen depletion, may cause mortality in marine invertebrates (Johnson & Welsh 1985).

The physical cover of algal mats may also cause vertical migration in infaunal species (Norkko & Bonsdorff 1996a,b,c, Österling & Pihl 2001). We did not test this hypothesis directly, but algal mats do impose a physical barrier between clams and the water column by reducing the water flow over the sediment surface (Escartin & Aubrey 1995). In our laboratory experiment, *Mya arenaria* quickly re-burrowed after algae removal-indicating that algae may impose a physical stress. By mimicking algal mat cover using nylon mesh, Raffaelli et al. (1991) found that the physical presence of the cover changed the behavior of crustacean infauna. Furthermore, benthic infauna (e.g. *Macoma balthica*) responded more quickly to hypoxia induced by algal mats than to hypoxia alone in the laboratory and in the field (Norkko & Bonsdorff 1996c).

In summary, hypoxic conditions, sulfides, other toxic substances, and physical cover may act in combination to cause an escape response in infaunal animals. Currently, hypoxia is thought to be the most important factor inducing vertical migration in infaunal animals (Norkko & Bonsdorff 1996a,b,c, Norkko et al. 2000, Österling & Pihl 2001). However, the relative importance of each of the possible factors and the interactions between them have yet to be determined.

### Implications of changes in burial depth

Changes in the burial depth of soft-shelled clams, brought about by the presence of macroalgal mats, may have complex effects on clam populations and on the surrounding environment. Soft-shelled clams avoid predators and extreme temperatures, and prevent being washed away, by burrowing deep in sediments (Blundon & Kennedy 1982b, Zwarts & Wanink 1989, Zaklan & Ydenberg 1997). Yet, infaunal suspension feeders, such as soft-shelled clams, obtain food and oxygen by pumping and filtering water, in quantities which are proportional to the rate of flow through the elastic siphon. For a given clam, this rate of flow is inversely proportional to the length of the siphon, because a longer siphon stretch results in a smaller siphonal radius (as per Poisseuille's equation in Vogel 1983). Thus, for a given environmental concentration of oxygen, a clam's uptake should be inversely proportional to burial depth. Decreased burial depth under algal mats increases siphonal radius, which should lead to increases in pumping rate and oxygen uptake in low-oxygen waters, such as those under the mats. However, there is a trade-off between the rate of oxygen uptake or suspension feeding and predator avoidance (Blundon & Kennedy 1982b, Zwarts & Wanink 1989, Zaklan & Ydenberg 1997). Risk of predation by benthic predators, such as crabs, increases with decreasing burial depth. In the presence of green macroalgal mats, predators may have easier access to abundant prey. Further, this increase in easily available prey may lead to increases in predator abundance. Indeed, the green crab *Carcinus maenas*, an important predator of soft-shelled clams (Glude 1955, Ropes 1968), has been observed to increase in numbers in the presence of macroalgal mats (Nicholls et al.

1981, Soulsby et al. 1982, L. M. Auphrey pers. obs.). However, the effect of algal mats on predator–prey interactions between crabs and clams may not be so simple. Clams under algal mats are stressed, and may not be a preferred prey for crabs. For example, blue crabs *Callinectes sapidus* have been observed to ignore clams lying on the substrate surface in the laboratory (Blundon & Kennedy 1982a). Crab predation may also be hindered by the physical structure of algal mats or by hypoxia induced by these mats (Taylor & Eggleston 2000).

Decreased burial depth of clams may also have effects on the benthic and pelagic environment. For example, suspension-feeding bivalves are important in benthic–pelagic coupling (Cloern 1982, Rosenberg & Loo 1988, Loo & Rosenberg 1989, Peterson et al. 1994) because they filter large amounts of water. Their filtering activity may reduce eutrophication and phytoplankton blooms (Officer et al. 1982, Newell 1988, Gili & Coma 1998). If reduced burial depth leads to increased pumping volume, then eutrophication and phytoplankton blooms may further be reduced (that is, if clam density is not also reduced by the agent causing the decreased burial depth). Another example is related to the role of soft-shelled clams in benthic chemistry. Changes in clam burial depth may lead to changes in the denitrification, sulfate reduction and depth of ventilation in sediments (Pelegrí & Blackburn 1995, Hansen et al. 1996).

Soft-shelled clams are an important economic resource in North America. However, in the Scotia-Fundy region of Canada, estimated commercial catches are declining (537 metric tonnes in 1989 to 153 metric tonnes in 2001, data from the Department of Fisheries and Oceans, Canada). Green macroalgal mats are affecting populations, already impacted by harvesting, diseases and predation by green crabs (Robinson & Rowell 1990, Barber et al. 2002, Auffrey 2003). Behavioral changes, such as shallower burial depth and even complete surfacing, will likely result in increased predation and further population declines.

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