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Effects of shear stress and hard clams on seston, microphytobenthos, and nitrogen dynamics in mesocosms with tidal resuspension

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ABSTRACT: To test the interacting effects of hard clams *Mercenaria mercenaria* and bottom shear stress on nutrient- and ecosystem dynamics, we performed a 4 wk experiment in six 1000 l shear turbulence resuspension mesocosms (STURM). Three tanks each contained 50 hard clams (RC set-up), and 3 tanks had no clams (R set-up). All tanks contained defaunated muddy sediment and estuarine water and had similar water column turbulence intensities (~1 cm s^{-1}), energy dissipation rates (~0.08 cm^2 s^{-3}), and tidal cycles (4 h mixing on and 2 h off). The same high instantaneous bottom stress (0.35 to 0.4 Pa) was applied to all tanks during the mixing-on cycles. Hard clams in interaction with high bottom shear stress initially destabilized the sediments and increased seston levels to ~200 mg l^{-1} in the RC tanks during the mixing-on cycles. Over time, seston concentrations declined in the RC tanks until they reached levels similar to the R tanks of ~60 mg l^{-1}. Bivalve feeding in the RC tanks significantly reduced phytoplankton biomass and shifted the phytoplankton community structure to Chlorophyceae/Prasinophytes. Nutrient (particulate phosphorus, nitrogen, and carbon, dissolved inorganic nitrogen, nitrate + nitrite, phosphate) concentrations were significantly enhanced in the RC tanks, mediated by high sediment resuspension and bivalve excretion. A brown tide organism, *Aureococcus anophagefferens,* bloomed in 2 of 3 RC tanks. Bivalve feeding and light limitation reduced microphytobenthos biomass in the RC tanks. Microphytobenthos biomass was low overall but significantly higher in the R tanks. Phytoplankton abundance, microphytobenthos biomass, seston concentrations, and nitrogen dynamics were significantly affected by interactions between hard clams and bottom shear stress.

KEY WORDS: *Mercenaria mercenaria* · Bivalve · Bottom shear · Experimental ecosystem · STURM · Destabilization · Benthic flux · Scaling

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INTRODUCTION

The commercially and ecologically important hard clam *Mercenaria mercenaria* (L.) is a suspension feeder abundant in shallow estuarine, and often turbid, waters on the east coast of North America (Stanley 1985, Bricelj et al. 1984). In these shallow habitats, tides, waves, and storms produce benthic shear-induced sediment resuspension at variable time scales that cause high turbidity (Schoellhamer 2002) with temporal changes in seston quantity and quality (Fegley et al. 1992, Prins et al. 1996). Mediated by changes in bottom shear stress, sediments are resuspended at different magnitudes and frequencies (Wright et al. 1997). Resuspension in low to moderately energetic tidal estuaries causes seston concentrations from 40 mg l^{-1} (Northern Chesapeake Bay; Sanford et al. 1991) to hundreds of mg l^{-1}, e.g.

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300 mg l^{-1} during storms in Tampa Bay (Schoellhamer 1995), 600 mg l^{-1} in the Hudson River (Heyes et al. 2004), and 1500 mg l^{-1} in San Francisco Bay (Schoellhamer 2002).

The critical erosional shear stress and subsequent resuspension are mediated by the sediment bed properties and are affected by the water flow and the organisms present (Davis 1993), and the bed properties can change over time. Macrofauna change sediment erodibility through adhesive-cohesive bonding among particles (Blanchard et al. 1997) and by influencing the sediment water content, which indirectly affects the critical shear stress for resuspension (Aberle et al. 2004). In addition, bio-resuspension directly affects sediment erodibility (Davis 1993). Polychaetes and amphipods alter the sediment water content and enhance or decrease sediment stability through the type and composition of their burrow structures (Meadows & Tait 1989), and the mucus secreted by organisms influences sediment cohesiveness (Blanchard et al. 1997) and thus sediment erodibility. The density of certain organisms, such as infaunal clams, has been shown to increase sediment erodibility in short-term flume experiments (Willows et al. 1998). The hard clam *Mercenaria mercenaria* can be abundant, with, for example, up to 400 ind. m^{-2} in a California lagoon (Murphy & Kremer 1985) or exceeding 60 ind. m^{-2} in the Providence River area (Saila et al. 1966 as cited by Doering et al. 1986).

Webs of cyanobacteria or polysaccharide matrices caused by diatom movements can stabilize sediment and reduce resuspension (Yallop et al. 1994). However, microphytobenthos can also destabilize sediment as the mat ages and bubbles form within the mat, rendering the mat more erodible (Porter et al. 2004b). In ecosystems, microphytobenthic biomass is mediated by both the light that reaches the bottom and bottom shear (MacIntyre et al. 1996, Porter et al. 2004a,b) as sediment resuspension affects light transmission to the sediment surface (Porter et al. 2010). Microphytobenthos can also reduce nutrient regeneration from the sediments (Sundbäck et al. 2000) and are eaten by macrofaunal filter and deposit feeders (Muschenheim & Newell 1992, Herman et al. 2000).

Bivalve suspension-feeders have been the subject of numerous studies and much management interest due to their ability to filter particles and clear the water column. There are numerous examples that show that bivalve suspension feeders reduce phytoplankton concentrations. For example, in previous mesocosm experiments, bivalve feeding resulted in lower phytoplankton biomass, higher light penetration through the water column and higher light levels at the sediment-water interface (Porter et al. 2004a,b) and enhanced microphytobenthos growth. In the present study, we examine the interplay between shear stresses above the sediment resuspension threshold and *Mercenaria mercenaria* and examine the effects on the ecosystem and on water quality. We address the following specific questions and hypotheses:

(1) How does a high density of hard clams and high bottom shear stress affect phytoplankton abundance and diversity in whole ecosystem experiments? Hypothesis 1: Hard clams decrease phytoplankton abundance and affect phytoplankton diversity despite high total suspended solid concentrations.

(2) How do hard clams and high bottom shear stress affect nutrient dynamics and the nitrogen budget in an ecosystem experiment? Hypothesis 2: Hard clams in combination with high shear stress do not affect the nitrogen dynamics.

(3) How do hard clams and high bottom shear stress affect microphytobenthos abundance in whole ecosystem experiments? Hypothesis 3. Hard clams and high bottom shear stress decrease microphytobenthos abundance due to both feeding and light limitation.

MATERIALS AND METHODS

Physical and biological scaling in the experimental ecosystems

We set up 6 cylindrical shear turbulence resuspension mesocosms (STURM) tanks, with 3 tanks containing 50 hard clams (RC) each and 3 tanks containing 0 hard clams (R). We measured the physical, chemical, and biological responses to high bottom shear stress and hard clams and calculated a nitrogen budget. The STURM tanks contained a single paddle that produced high instantaneous bottom shear stress to resuspend sediments and had realistic water column turbulence levels, without overmixing the water column. The STURM tanks are the successor design of large linked mesocosms reported by Porter et al. (2004a,b) and are further described by Sanford et al. (2009), Mason & Porter (2009) and Schneider et al. (2007; there called STORM tanks).

The paddle moved at 12.5 rpm with a forward-stop-backward-stop motion (9 s, 1.5 s stop, 8 s, 1.5 s stop) to avoid plug flow during the mixing 'on' phase. Mixing was 4 h on:2 h off in all systems to simulate tidal cycles throughout a 4 wk experiment. The

1000 l tanks were 1 m deep and had a 10 cm layer of muddy sediment.

Direct flow and turbulence (turbulence intensities and energy dissipation rates) were measured using an acoustic Doppler velocimeter (ADV) at predefined locations throughout the tank and at different mixing speeds. Turbulence intensity, as defined by Porter et al. (2010), and energy dissipation rates were determined following Sanford (1997). Volume-weighted average turbulence intensities as determined using Surfer (Golden Software) were ~1.08 cm s^{-1} (Fig. 1a,b), and volume weighted energy dissipation rates were ~0.08 cm^2 s^{-3} during mixing (Fig. 1c,d). Turbulence intensities were similar to previous experiments (Petersen et al. 1998, Porter et al. 2004a,b, 2010) and are typical of turbulence intensities in microtidal estuaries and continental shelf bottom boundary layers (Sanford 1997 and references cited therein). This mixing setting kept energy dissipation rates from being unrealistically high.

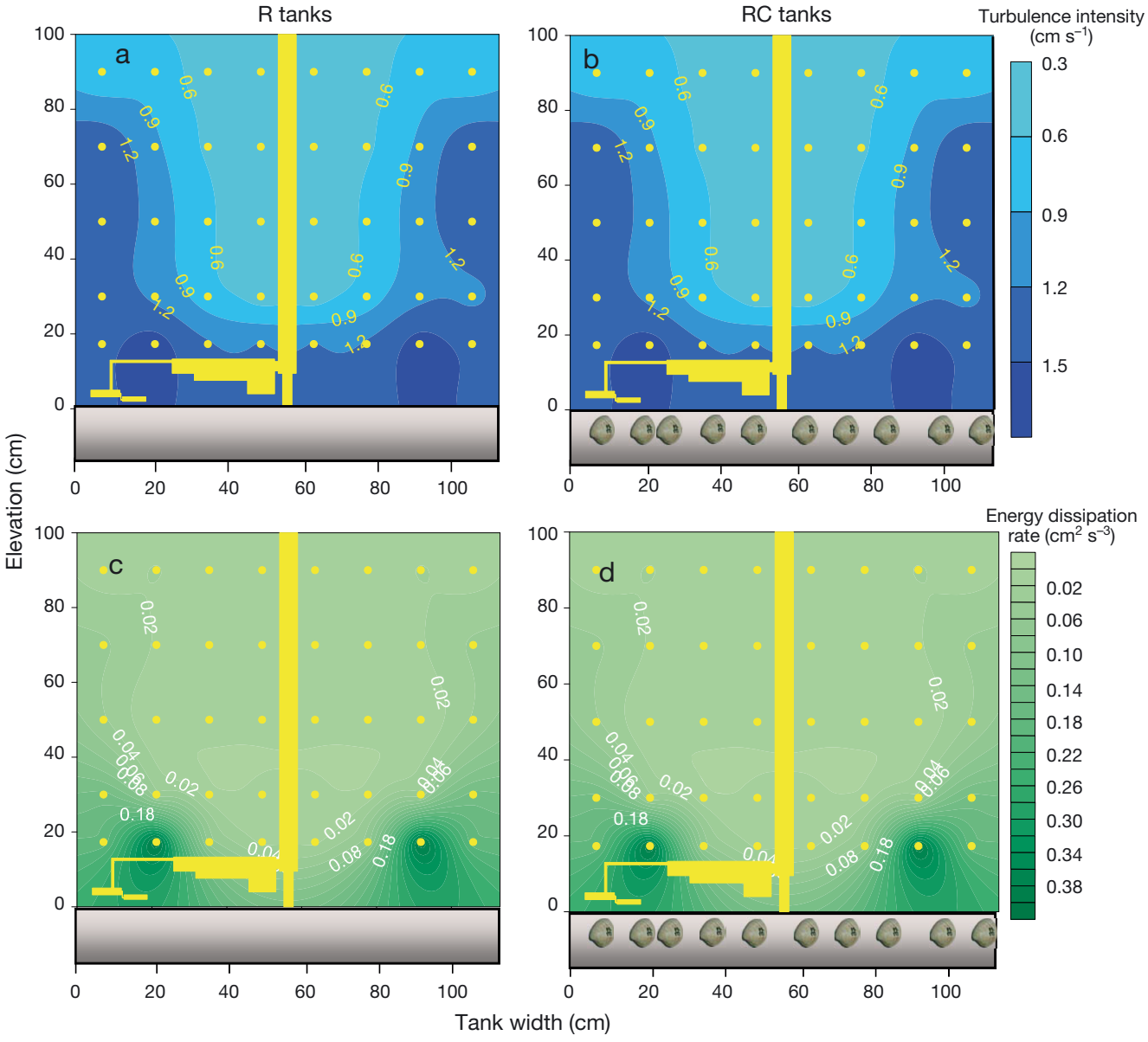


Fig. 1. Turbulence intensity in (a) 3 R tanks and (b) 3 RC tanks during a mixing 'on' phase. Paddles in the tanks indicated in yellow. Energy dissipation rate in the (c) R tanks and (d) RC tanks. The RC tanks contained fifty 40 mm long hard clams *Mercenaria mercenaria* (symbols along x-axis). ●: measurement locations. Tidal cycles (4 h mixing 'on', 2 h mixing 'off') were generated in all tanks

We determined shear stress at the bottom directly using hot film anemometry (Gust 1988). Shear (or 'friction') velocity u_* (cm s^{-1}) was defined as follows:

$$u_* = \sqrt{\frac{\tau_b}{\rho}} \quad (1)$$

where τ_b is bottom shear stress in dynes cm^{-2}, and ρ is the density of water in g cm^{-3}. The shear velocity was measured at 5 locations across the tank radius. Bottom shear stress (Pa) was calculated as $\tau_b = \frac{u_*^2 \rho}{10}$. At the chosen mixing setting, the maximum instantaneous bottom shear stress reached 0.35 to 0.4 Pa in the tanks (Fig. 2) and produced sediment resuspension, as mediated by bivalves during the mixing-on phases. The spikes in shear stress in Fig. 2 correspond to passage of the paddle directly over the hot film sensor. While the regularity of these spikes is probably not representative of typical turbulent boundary layer flows, similar short-lived bursts of turbulent shear stress have been shown to be present and responsible for sediment resuspension in laboratory flume experiments without paddles (Diplas et al. 2008).

We scaled the biomass of hard clams to high natural densities to filter the entire water column once every ~1.5 d using literature filtration rates of 32 mm long clams (Bricelj & Malouf 1984). Thus, we distributed a total of 50 individually numbered, preweighed, ~40.5 ± 2 mm long hard clams in each of 3 tanks, keeping the size distribution between tanks the same. Clam weight gain was determined after the experiment from 91 clams with still identifiable numbers.

Experimental setup

The mesocosms were prepared with defaunated sediment from Baltimore Harbor (39° 11′ 29″ N, 76° 31′ 10.5″ W) that was equilibrated to realistic biogeochemical porewater gradients over a 2 wk period in the dark as described by Porter et al. (2006, their Treatment HD-m), while the initial fill water was unfiltered. Daily water exchange during mixing-off times (10% exchanged) was with 19 g kg^{-1} filtered water. The collected sediment had a grain size composition of 0% gravel, 3.27% sand, 38.7% silt, and 58.03% clay by dry weight (Baker et al. 1997). The nitrogen content was 0.37%, and the carbon content was 3.93% (unpubl. data). Sediment was transported to Chesapeake Biological Laboratory (CBL) and defaunated as described previously (Porter et al. 2006; their Table 2, Treatment HD-m). After defaunation and chemical stabilization, fifty 40 ± 2 mm long hard clams were added to each of 3 tanks. Any clams that did not bury were replaced on the second day. The mesocosm experiment began 3 d later.

We measured light levels of ~227 µmol photons m^{-2} s^{-1} just below the water surface of the R tanks and RC tanks using an LI-192 Underwater Quantum sensor (LI-COR Biosciences) attached to a model LI-250 readout, which is sufficient to prevent light limitation (Porter et al. 2004a). Therefore, any light limitation within the tanks was due to the impact of resuspension or the presence of the hard clams.

Three days after the hard clams were added, all of the tanks were simultaneously slowly filled with unfiltered 15 g kg^{-1}salinity water from the Patuxent estuary, a tributary of Chesapeake Bay, containing the resident plankton community. Only megazooplankton > 3 cm were excluded. All tanks were filled with a combination of Patuxent River water and filtered 0.5 µm (absolute) 35 g kg^{-1} seawater to achieve the target salinity of 19 g kg^{-1}, since a salinity of 18 g kg^{-1} is the minimum recommended to avoid growth limitation for *Mercenaria mercenaria* (R. Grizzle pers. comm.). Mixing began with the programmed tidal cycles, and all tanks were synchronized. On Day 26, the experiment was terminated in the R tanks because the mixing system failed. The experiment was continued for an additional 5 d in the RC tanks to follow the trend of total suspended solids (TSS) concentrations that had been in a constant decline.

The water in each tank was removed daily at the end of the mid-day mixing 'off' phase to mimic tidal exchange and replaced with 0.5 µm absolute filtered Patuxent estuary water supplemented with instant ocean to achieve a final salinity of 19 g kg^{-1}. This water was prepared in an indoor facility 1 d before each water exchange to ensure all salts were dissolved. We cleaned tank walls of wall periphyton every 2 d

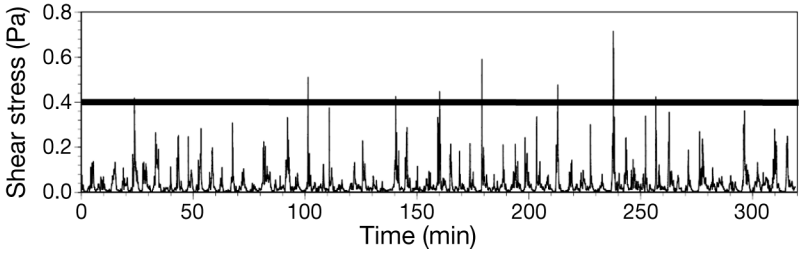


Fig. 2. Bottom shear stress used in all tanks measured with hot-film anemometry after Gust (1988)

to minimize wall growth (Chen et al. 1997) and left the wall material in the tank. On Day 1 of the experiment, we added a nutrient spike with ammonium (25 ml of a solution of 53.5 g l^{-1} NH_4Cl) and soluble reactive phosphorus (25 ml of a solution of 8.51 g l^{-1} KH_2PO_4), in Redfield proportions, to each 1000 l tank to stimulate a phytoplankton bloom as done previously (Porter et al. 2004b, 2010). Immediately after addition, the ammonium concentrations were 27 µmol l^{-1}, and the phosphate concentrations were 1.3 µmol l^{-1}. Silicate was abundant with 30 to 35 µmol l^{-1} at the start of the experiment, and no silicate additions were made. The ecosystem experiment was performed from 25 June 2002 to 25 July 2002 at the Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science in Solomons, MD.

Sampling regime and variables sampled

The biological and geochemical variables measured included water column chlorophyll *a* (chl *a*), phaeophytin, TSS, phytoplankton pigments, water column nutrients, i.e. ammonium, nitrate plus nitrite, dissolved inorganic nitrogen (DIN), phosphate, total nitrogen (TN), and total phosphorus (TP), hard clam live weight change, light profiles, and sediment chl *a* (see Appendix 1 for details of analytical methods). In addition, we measured particle size spectra during settling during a mixing 'off' phase in all tanks.

We measured turbidity continuously at 1 s intervals in each tank with optical backscatter (OBS-3, D&A Instruments) turbidity sensors located at mid-depth. The sensors were calibrated against mid-depth TSS samples. In post-processing, OBS data were averaged over 66 s intervals. Temperature was measured using Campbell T107 temperature probes connected to a Campbell CR10x data logger (Campbell Scientific) every 10 min as well as daily using a YSI 6600 instrument during mixing 'on' phases in the afternoon. The 6 tanks tracked each other closely, and the Campbell temperature data were analyzed in detail to determine the temperature variability in the systems.

Dissolved oxygen concentrations were measured during mixing 'on' phases in the afternoon at mid-depth using a YSI 6600 Sonde. Light penetration was measured every 2 d during the mixing 'on' and 'off' phases using a PVC cap painted black and white, similar to a Secchi disk, mounted onto a PVC rod and lowered into the water column until it disappeared from view.

We measured light levels twice a week during mixing 'on' phases using a LI-192 Underwater Quantum sensor (LI-COR Biosciences) light meter to measure the total downwelling photosynthetically available radiation (PAR, in the 400 to 700 nm waveband) at 0, 10, 25, 50, and 65 cm depth. Light attenuation coefficients and irradiance levels at the sediment surface were determined. In addition, we calculated mean geometric irradiance in the water column as $\exp\{0.5 \cdot [\ln(E_0) + \ln(E_{Sed})]\}$, where E_0 and E_{Sed} are irradiances at the surface of the water column and the sediment-water interface, respectively. The values obtained for mean geometric irradiance were similar to irradiance values measured at 50 cm depth.

We took 4 l water samples from mid-depth of each tank biweekly during mixing 'on' for particulates and solutes. Water was filtered through 47 mm Whatman GFF filters (0.7 µm nominal pore size) and rinsed with an isotonic solution of ammonium formate (Berg & Newell (1986) to remove salts. TSS, particulate inorganic matter (PIM), and particulate organic matter (POM) levels were determined following techniques by Berg & Newell (1986). Known volumes of water were filtered through 25 mm Whatman GFF filters for chl *a* and phaeophytin concentrations and for particulate nitrogen (PN), particulate phosphorus (PP), and particulate carbon (PC). The filtrate was captured and frozen for analysis for dissolved nutrients including ammonium (NH_4^+), nitrate + nitrite (NO_3^- + NO_2^-), dissolved phosphate (PO_4^{3-}), dissolved silicate (Si), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP). Exchange water (outflow and fill water) was also sampled for dissolved nutrients to track nutrient inputs or outputs from the 10% daily water exchange.

On Days 7, 17, and 24, 25 mm GFF filtered water was used to measure the phytoplankton pigment composition. Folded filters were stored in aluminum foil at −70°C until analysis using high performance liquid chromatography (HPLC; Van Heukelem & Thomas 2001). Pigments characteristic for phytoplankton in the Chesapeake Bay area, our source water, were used for identification: alloxanthin (Cryptophyceae), fucoxanthin (Chrysophyceae and Bacillariophyceae), lutein (Chlorophyceae and Prasinophyceae), peridinin (Dinophyceae), and zeaxanthin (Cyanobacteria) following Jeffrey & Vesk (1997), Marshall (1994), and Marshall et al. (2005). We also found the pigment 19′-butanoyloxyfucoxanthin (but-fuco), which is a marker pigment for brown tide organisms *Aureococcus anophagefferens* (Trice et al. 2004) when 19′-hexanoyloxyfucoxanthin is absent (e.g. compare pigments of Prymnesiophyceae

and Chrysophyceae in Table 2.3, p. 74–75, in Jeffrey & Vesk 1997) and when gyroxanthin diester is absent (see Table 50, p. 88–89, in Van Heukelem & Thomas 2005). Gyroxanthin diester was absent throughout the experiment (C. Thomas pers. comm.). 19′-Hexanoyloxyfucoxanthin was absent except for Day 7, when it was present only in all RC tanks. Thus, we used but-fuco to indicate the presence of the chrysophyte *Aureococcus anophagefferens* (Trice et al. 2004). Moreover, *Aureococcus anophagefferens* has been previously found in Chesapeake Bay water (Marshall et al. 2005), and we observed this species in mesocosm experiments with tidal resuspension (Porter et al. 2010). We normalized the pigment to chl *a* ratio to account for variance between the R and RC tanks. We filtered duplicate subsamples at random for quality control during each sampling. We also collected more frequent samples of TSS during 3 mixing 'off' periods to examine changes in the particulate properties over time during settling.

We measured sediment chl *a*, phaeophytin, PC, and PN concentrations in the surface sediment as well as the sediment water content at the midpoint of the experiment (on Day 15) and at the end of the experiment. Due to mechanical failure of the mixing system in the R tanks, sediment chl *a* was sampled on Day 26 in the R tanks, while it was sampled on Day 31 in the RC tanks. At the start of the ecosystem experiment (Day 0), these variables were measured in the benthic chambers set up in parallel to the mesocosm experiment in order to represent the sediment conditions at the start of the experiment. We used 2.5 cm diameter cutoff syringes with a 1-way valve (BE130-23BB, Instrumentation Industries), affixed to a pole as necessary, as coring devices to sample the tanks for sediment chl *a*, phaeophytin, PC, PN, and sediment water content from 3 tank quadrants per tank during the mixing 'on' phase while mixing was briefly turned off. Sediment chl *a* in the R tanks at the end of the experiment was sampled after mixing was off, and the sediment chl *a* concentrations were corrected for the settled chl *a* from the water column using previously obtained measurements. We sampled both the 0–0.5 and 0.5–1 cm sediment sections but combined them for data analysis. Sediment chl *a* and phaeophytin samples were frozen at –70°C pending analysis with HPLC (Van Heukelem & Thomas 2001). For all variables, the same analytical techniques as described by Porter et al. (2010) were followed (see Appendix 1).

To determine the abundance of any macroinfauna that may have settled or grown up over the 4 wk period of the experiment, we took 13.3 cm diameter sediment cores from the tanks at the end of the experiment. We washed the contents through a 0.5 mm diameter mesh, and no macrofauna was found.

Bivalves are known to repackage organic matter into biodeposits, and we expected the size structure and settling of the particles to be different in the R compared to RC tanks. We used a LISST-100C (laser *in situ* scattering and transmissometry) instrument to measure particle sizes during settling during 1 mixing 'off' phase in each tank. The LISST was deployed at mid depth during a mixing 'on' phase and recorded particle size distributions at 1 s intervals throughout the subsequent mixing 'off' phase to examine changes in particle sizes during settling of previously resuspended material. Settling experiments were performed in each of the 6 tanks sequentially on Days 8, 10, 13, and 14. Simultaneous TSS measurements at mid-depth were done using OBS3 instruments, each calibrated using a range of TSS concentrations taken over the course of the experiment. The LISST uses a laser diffraction method for sizing particles, resulting in estimates of particle volume concentration in 32 size classes arranged geometrically between 2.5 and 500 µm (Agrawal & Pottsmith 2004).

Zooplankton

We sampled mesozooplankton twice a week during mixing 'off' by pumping 40 l tank^{-1} at 22 l min^{-1} through a 63 µm Nitex screen using a diaphragm pump. The samples were washed into bottles, and mesozooplankton were preserved with buffered formaldehyde. We determined the dominant taxa and age groups on a dissecting microscope using direct counts. To estimate the dry weights (W, µg ind.$^{-1}$) of the individuals of different taxa, the number of individuals was multiplied by their taxa's respective weight characteristic (Table 2 in White & Roman 1992). The dominant taxa were copepod nauplii, *Acartia* spp. adults, polychaete larvae, and copepodites.

Zooplankton weight was converted to carbon (µg l^{-1}) for each taxon, and the taxa were combined for an estimate of combined mesozooplankton biomass to compare to phytoplankton abundance (in a common carbon unit) following White & Roman (1992, their Table 1: 'Carbon [µg C ind.$^{-1}$] = 0.32 W'). This was done to examine whether zooplankton affected phytoplankton biomass in addition to the effect of hard clams. A POC to chl *a* ratio of 51.68 (Porter et al. 2010) was used to convert phytoplankton biomass to

phytoplankton carbon. Linear regression analyses were performed using SAS (SAS Institute). In addition, we estimated the nitrogen content of the zooplankton community for an overall nitrogen budget.

Nitrogen budget

We also estimated nitrogen budgets in the R and RC tanks, based on data from Day 7 to the experiment end, which excluded the nutrient spike. Nutrient inputs and outputs with the exchange water were taken into consideration. Inputs for the RC tanks were 6 µg l^{-1} chl *a*, 20 g dry tissue weight clams, 10 µmol l^{-1} DIN, and 26 µmol l^{-1} DON. Inputs for the R tanks were 25 µg l^{-1} chl *a*, 2 µmol l^{-1} DIN, and 21 µmol l^{-1} DON. Zooplankton inputs for the RC tanks were 3 *Acartia* ind. l^{-1}, 4 copepodites l^{-1}, 22 polychaete larvae l^{-1}, and 30 copepod nauplii l^{-1}. Zooplankton inputs for the R tanks were 3 *Acartia* ind. l^{-1}, 1 copepodite l^{-1}, 22 polychaete larvae l^{-1}, and 29 copepod nauplii l^{-1}. Microphytobenthos inputs were 14 and 27 mg chl *a* m^2 in the RC and R tanks, respectively. Water-column chl *a* was converted to nitrogen equivalents (phytoplankton biomass nitrogen, µmol N $tank^{-1}$) using the ratio of POC to chl *a* of 51.68 and a C:N ratio of 5.138 (Porter et al. 2010). Sediment chl *a*, indicative of microphytobenthos abundance, was converted to nitrogen equivalents (µmol N $tank^{-1}$) in the same way. The N content of the zooplankton community was calculated from dry weights of the individuals of the different taxa, following White & Roman (1992) and measured abundances. The nitrogen content was determined as 7.5% of the mesozooplankton dry weight (5 to 10% of mesozooplankton dry weight is nitrogen; Parsons et al. 1984). Water-column concentrations of TDN were scaled up to the whole tank.

Statistical analyses

Statistical approaches are detailed by Porter et al. (2010). Most parameters were each averaged from Day 6 to Day 24 of the experiment for each tank (ca. 6 measurements over a 3 wk period). As in Porter et al. (2010), data from the first 5 d of the experiment were not included to avoid the immediate effects of the initial nutrient spike. Mesozooplankton abundance for each tank was averaged from Day 6 to 25. Only data from the mixing 'on' phases were included in statistical comparisons. Statistical *t*-tests were used for most water-column parameters. We used split plot analyses in time for sediment chl *a* over the duration of the experiment. We used linear regression analyses of mesozooplankton biomass and phytoplankton biomass to determine the relationship between these communities. Statistical analyses were performed using SAS (SAS Institute), and the *t*-tests were conducted using the Microsoft Excel Analysis tool pack. Significances of all analyses were defined at the $p \leq 0.05$ level.

RESULTS

Water-column processes

Water temperatures (means ± SD) ranged from 22.8 to 29.6°C in the RC tanks (26.37 ± 0.02°C) and from 22.7 to 29.2°C (25.96 ± 0.01°C) in the R tanks. Dissolved oxygen concentrations were significantly lower in the RC tanks compared to the R tanks (Fig. 3), with conditions being hypoxic in the RC tanks during the first week of the experiment.

TSS concentrations were significantly higher in the tanks with hard clams (RC: ~180 mg l^{-1} on Day 1; R: ~30 mg l^{-1}; Fig. 4). Throughout the experiment, TSS concentrations were ~50 mg l^{-1} in the R tanks but in the RC tanks continuously decreased from ~200 mg l^{-1} on Day 2 to ~60 mg l^{-1} on Day 20 and then remained at ~60 mg l^{-1} until the end of the experiment on Day 31. During mixing 'off' phases, TSS concentrations were ~5 to 15 mg l^{-1} in the RC and the R tanks. PC, PN, and PP concentrations were linearly related to TSS concentrations, and PC and PP concentrations were significantly enhanced in the RC tanks. TSS concentrations were significantly higher in the RC tanks than in the R tanks.

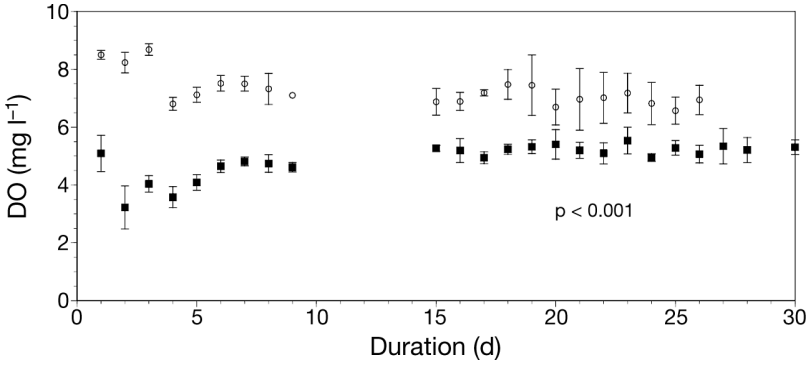


Fig. 3. Dissolved oxygen (DO) concentrations over the experiment. Means ± SD, n = 3 tanks for each system. Values < 5 mg l^{-1} indicate hypoxia. Statistical results indicated with p-value, statistical significance $p < 0.05$. ■: RC tanks, ○: R tanks

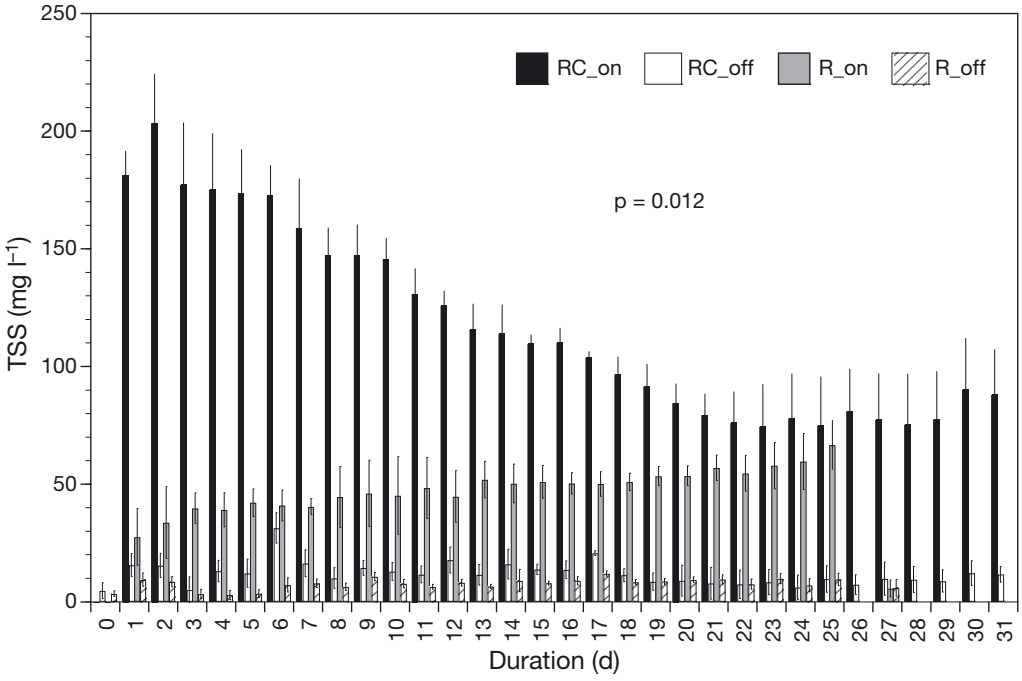
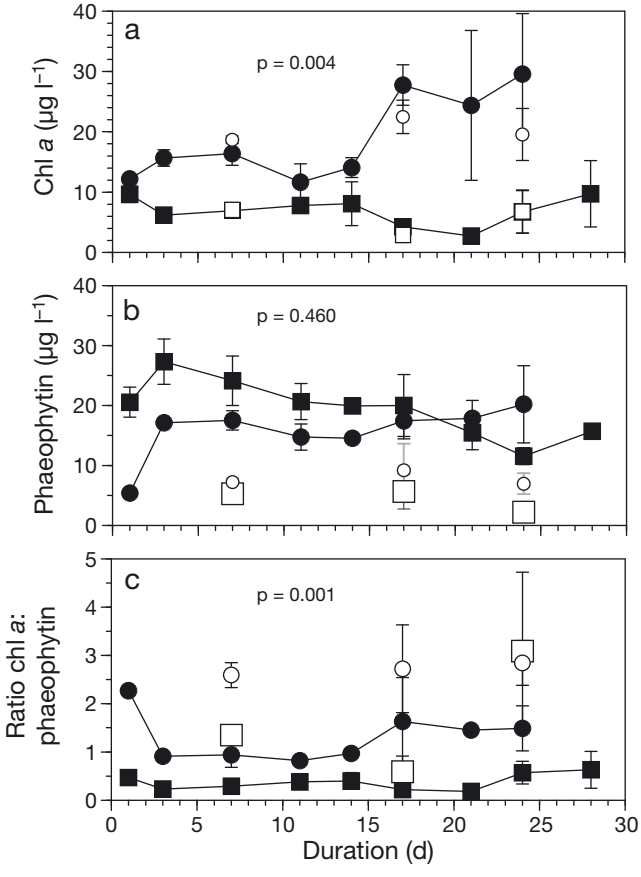


Fig. 4. (a) Total suspended seston (TSS) concentrations (mean ± SD, n = 3 tanks for each system and mixing phase) over time in the RC and the R tanks during the mixing 'on' phases and the mixing 'off' phases as measured using OBS3 sensors, calibrated with TSS samples. Samples on Day 0 were taken before mixing was started. Difference between R_on and RC_on statistically significant ($p < 0.05$)



Water-column chl *a* (11 to 30 µg l^{-1}) levels were significantly higher in the R tanks than in the RC tanks (2 to 10 µg l^{-1}), and while a phytoplankton bloom developed in the R tanks, this did not occur in the RC tanks (Fig. 5a). While a portion of the chl *a* settled and was resuspended with the mixing 'on' and 'off' cycles in the R tanks, it was concluded that chl *a* did not substantially resuspend and settle in the RC tanks (Fig. 5a) as chl *a* concentrations were the same during the mixing 'on' and 'off' phases. Clams likely fed on the microphytobenthos as it was resuspended. Phaeophytin concentrations differed significantly between the mixing 'on' and the mixing 'off' phases as degraded material was resuspended and deposited (Fig. 5b). The ratio of chl *a* to phaeophytin was 2- to 4-fold higher in the R compared to the RC tanks (Fig. 5c). Assuming that the ratios of chl *a*:phaeophytin would have been the same in the R and RC tanks in the absence of clam grazing, we can use this assumption to calculate the expected concentration

Fig. 5. (a) Chlorophyll *a* (chl *a*), (b) phaeophytin, and (c) ratio of chl *a* and phaeophytin concentrations (mean ± SD, n = 3 tanks for each system and mixing phase) over time in the R versus the RC tanks during the mixing 'on' and 'off' phases. Difference between values statistically significant ($p < 0.05$). ●: R_on, ■: RC_on, ○: R_off, □: RC_off

in the RC tanks: $RC_{Chla} = RC_{Phae} \cdot (R_{Chla}/R_{Phae})$, where RC_{Chla} removed is RC_{Chla} expected – RC_{Chla} actual. Thus, we estimate the amount of chl *a* removed by the clams as 15.25 mg chl *a* per tank daily from Day 7 to Day 24 of the experiment, with peak removal rates of 27 µg l^{-1} d^{-1} or 27 mg per tank on Day 17 of the experiment. This is 7 mg chl *a* or 373 mg carbon removed per clam over the experiment. About one third of the phytoplankton can be resuspended microphytobenthos (Fig. 5a), so 250 mg phytoplankton carbon were removed over the experiment. Assuming a 10% trophic transfer to biomass (Reece et al. 2011), this suggests that each clam should have gained 25 mg carbon over the experiment from feeding on phytoplankton alone.

Phytoplankton community structure was affected by resuspension and hard clams (Fig. 6). Lutein, indicative of Chlorophyceae and Prasinophyceae, was significantly higher in the RC tanks than in the R tanks (Fig. 6b). But-fuco, which is indicative of *Aure-*

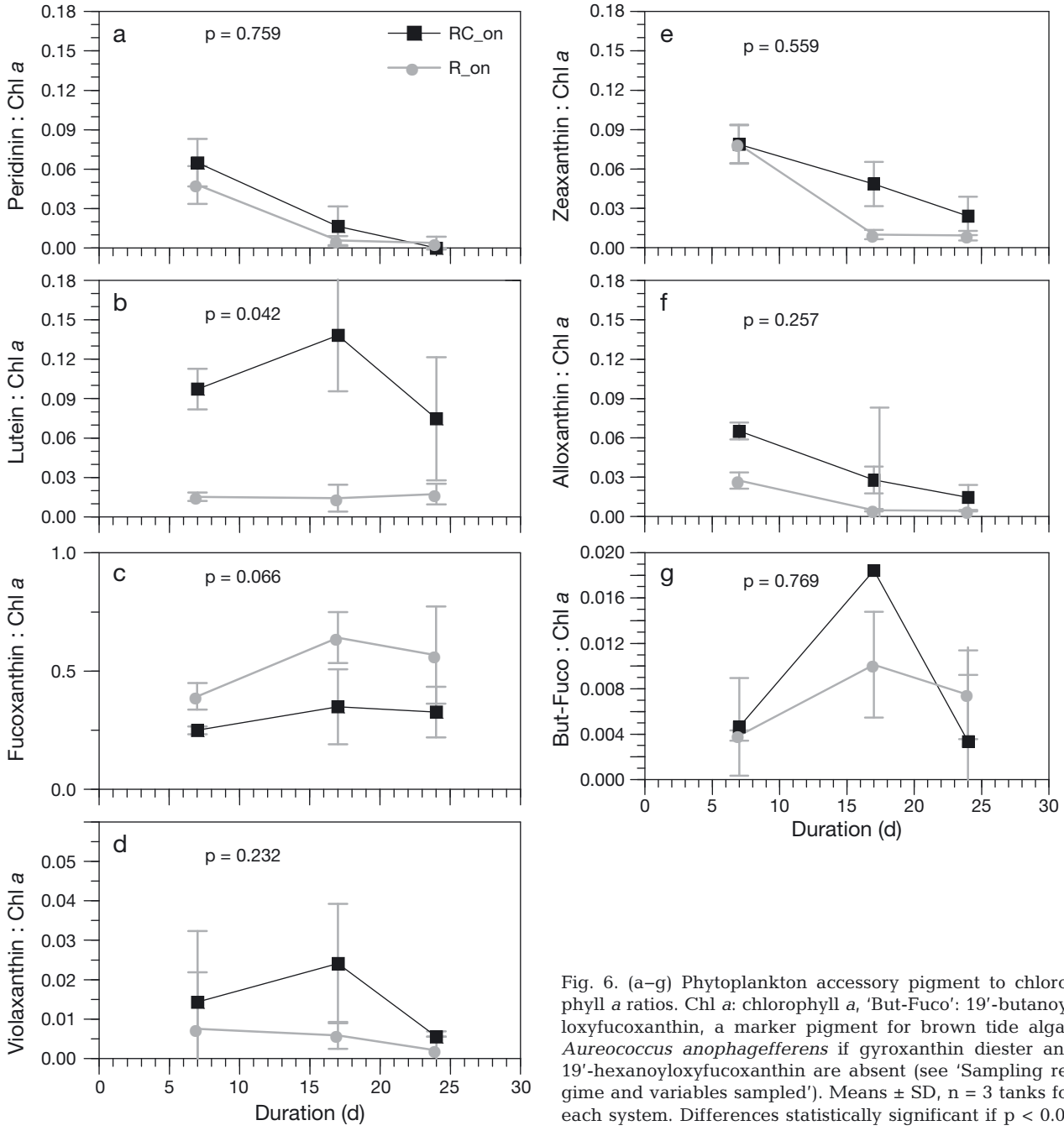


Fig. 6. (a–g) Phytoplankton accessory pigment to chlorophyll *a* ratios. Chl *a*: chlorophyll *a*, 'But-Fuco': 19′-butanoyloxyfucoxanthin, a marker pigment for brown tide algae *Aureococcus anophagefferens* if gyroxanthin diester and 19′-hexanoyloxyfucoxanthin are absent (see 'Sampling regime and variables sampled'). Means ± SD, n = 3 tanks for each system. Differences statistically significant if $p < 0.05$

ococcus anophagefferens, was found in all samples (Fig. 6g) with 19′-hexanoyloxyfucoxanthin and gyroxanthin diester being absent, except for Day 7 where 19′-hexanoyloxyfucoxanthin was present in the RC tanks indicating Prymnesiophyceae. But-fuco concentrations reached Category I bloom conditions of *Aureococcus anophagefferens* in 2 of the 3 RC tanks on Day 17 (8049 and 1375 cells ml^{-1}, respectively, based on cell count conversions by Trice et al. 2004) but did not reach bloom conditions in the R tanks. Levels of fucoxanthin, indicative of Chrysophyceae and Bacillariophyceae (Fig. 6c), peridinin indicating Dinophyceae (Fig. 6a), and zeaxanthin indicating Cyanobacteria (Fig. 6e) were similar in the RC and R tanks.

Nutrient concentrations were consistent with the differences noted above for algal biomass (Fig. 7b–e). Nitrate plus nitrite (Fig. 7a), dissolved PO_4^{3-} (Fig. 7c), DON (Fig. 7h), total dissolved nitrogen (Fig. 7e), and phosphorus concentrations (Fig. 7f) were significantly higher in the RC tanks than in the R tanks. As chl *a* increased in the R tanks (Fig. 5a), nitrate + nitrite (Fig. 7a), ammonium, and dissolved PO_4^{3-} levels decreased.

Defining nutrient limitation as dissolved silicate <5 µmol l^{-1}, DIN <2 µmol l^{-1}, and PO_4^{3-} <0.1 µmol l^{-1} (Fisher et al. 1992; T. Fisher, Horn Point Laboratory, University of Maryland Center for Environmental Science, pers. comm.), all nutrients became limiting in the R tanks after Day 17 (Fig. 7d,b,c). However, nutrient addition bioassays, such as those used by Fisher et al. (1999), would be needed to confirm which was the limiting nutrient. In contrast, the RC tanks generally did not meet the Fisher et al. (1992) criteria for nutrient limitation (Fig. 7b–d). Dissolved organic phosphorus concentrations were similar between the RC and the R tanks (Fig. 7g).

At the beginning of the experiment, total nitrogen (NH_4^+ + NO_2^- + NO_3^- + DON + PN) concentrations were about twice as high in the RC tanks as in the R tanks and decreased over time to become similar to the R tanks by the last week of the experiment (Fig. 7j). Total phosphorus concentrations (PO_4^{3-} + DOP + PP) were ~4-fold higher in the RC tanks than the R tanks at the beginning of the experiment (Fig. 7k) and decreased over time to become similar to the R tanks by the last week of the experiment. The pattern of TP and TN progressions (Fig. 7j,k,l) were similar to the patterns of the TSS concentrations over time, which suggests that resuspended sediment may have been a source of additional nutrients. Most of these increases in TP and TN were due to resuspended PP and TN, respectively, which was related linearly to TSS levels (PP mg l^{-1} = 0.00292 · TSS mg l^{-1} + 0.0083, R^2 = 0.99; PN mg l^{-1} = 0.00351 · TSS mg l^{-1} + 0.1414, R^2 = 0.87). The nitrogen budget (Table 1) showed the partitioning of nitrogen into zooplankton, phytoplankton, microphytobenthos, DON, DIN, hard clams, and porewater nitrogen. Seston quality (ratio of particulate organic matter to particulate inorganic matter, means ± SD) was significantly better in the R tanks (0.232 ± 0.006) than in the RC tanks (0.170 ± 0.005, $p < 0.001$).

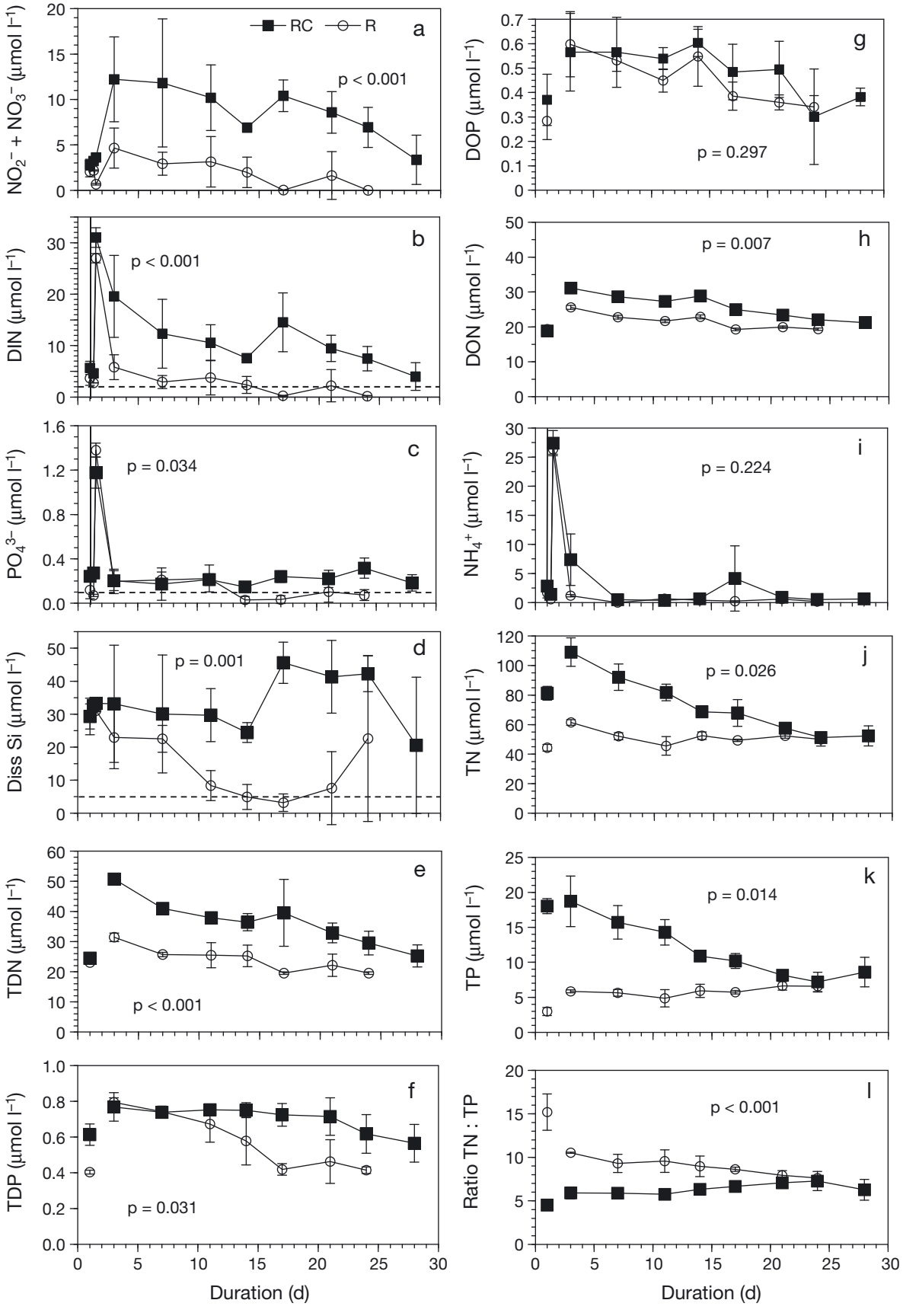
Fig. 7. Dissolved and particulate nutrient concentrations (mean ± SD, n = 3 tanks for each system). (a) Nitrite + nitrate (NO_2^- + NO_3^-), (b) dissolved inorganic nitrogen (DIN), (c) phosphate (PO_4^{3-}), (d) dissolved silicate, (e) total dissolved nitrogen (TDN), (f) total dissolved phosphorus (TDP), (g) dissolved organic phosphorus (DOP), (h) dissolved organic nitrogen (DON), (i) ammonium (NH_4^+), (j) total nitrogen (TN), (k) total phosphorus (TP), and (l) ratio of TN to TP. Vertical line in b and c: nutrient spike of nitrogen and phosphorus added to the water column. Horizontal dashed lines in b, c, and d: thresholds for nutrient limitation for the respective nutrients (see 'Water column processes'). Statistical results indicated with p value, statistical significance $p < 0.05$

Particle settling

Settling during the mixing 'off' phases showed similar patterns of settling and particle distributions in all of the systems (Fig. 8). Although TSS levels were ~3-fold higher in the RC tanks at the beginning of the settling period, both the rates of settling and the eventual steady state TSS concentrations were approximately the same in all tanks. Almost all of the material that

Table 1. Partitioning of nitrogen (N) within the resuspension (R) and resuspension with 50 clams (RC) tanks (see 'Discussion'). DIN: dissolved inorganic nitrogen (ammonium + nitrate + nitrite), DON: dissolved organic nitrogen. Porewater N estimates for R are taken from Porter et al. (2010)

Variable	R tanks		RC tanks	
	N (µmol $tank^{-1}$)	Distrib. (%)	N (µmol $tank^{-1}$)	Distrib. (%)
Zooplankton	337	0.6	392	0.7
Phytoplankton	14 813	24.9	4372	7.6
Microphytobenthos	19 318	32.5	9864	17.0
DON	21 507	36.2	25 906	44.7
DIN	2922	4.9	10 453	18.1
Mercenaria mercenaria	0	0.0	5927	10.2
Porewater N	490	0.8	989	1.7
Total N (µmol N per tank)	**59 387**	100.0	**57 903**	100.0


RC
R
a
p < 0.001
$NO_2^- + NO_3^-$ (µmol l^{-1})
b
p < 0.001
DIN (µmol l^{-1})
c
p = 0.034
PO_4^{3-} (µmol l^{-1})
d
p = 0.001
Diss Si (µmol l^{-1})
e
p < 0.001
TDN (µmol l^{-1})
f
p = 0.031
TDP (µmol l^{-1})
Duration (d)
g
p = 0.297
DOP (µmol l^{-1})
h
p = 0.007
DON (µmol l^{-1})
i
p = 0.224
NH_4^+ (µmol l^{-1})
j
p = 0.026
TN (µmol l^{-1})
k
p = 0.014
TP (µmol l^{-1})
l
p < 0.001
Ratio TN : TP
Duration (d)

settled did so within the first 30 min. Particles were dominated by particulate inorganic matter. Defining an estimate of the bulk settling velocity as the distance from the surface to the turbidity sensor (0.5 m depth) divided by the time to reduce the initial TSS concentration by 50% of the range to its steady state value, we estimated bulk settling velocities ranging between 0.9 and 1.7 mm s^{-1} with an average of 1.2 mm s^{-1}. There was no significant difference in settling velocity between the RC and R tanks. Particle size distributions (PSDs) from the LISST100C measurements showed, however, that settling was a dynamic process. During the first short period (~90 s) after settling began, the initial PSD simply decreased in magnitude without changing shape. Flocculation became apparent immediately afterward, with the sudden appearance of a peak in the largest sizes of the PSD. The average median diameter (D_{50}) during the 2 h settling period for all of the experiments was 86 µm. Floc diameters tended to be larger during the first 15 min (average of 117 µm). There was no significant difference in particle size between the RC and R tanks.

Light penetration and microphytobenthos levels

Measured bottom irradiance levels during the resuspension phase were low at ~1 and 2 µmol photons m^{-2} s^{-1} (Fig. 9a). While irradiance at the sediment surface was similar in the RC and R systems (Fig. 9a), geometric mean irradiance in the water column was higher in the R tanks (18.3 µmol photons) than in the RC tanks (8.9 µmol photons), which had higher TSS concentrations.

Light penetration, as measured by a home-made Secchi disk, penetrated ~40 cm into the R tanks during resuspension (Fig. 9b). During the 'off' phases, light in the R tanks occasionally reached to the bottom (Fig. 9b). In the RC tanks, light reached only 15 cm into the water column at the start of the experiment and reached ~40 cm by the end of the experiment during the 'on' phase, similar to the R tanks. At the end of the experiment, light reached the bottom during 'off' phases in the RC tanks, whereas in the R tanks, light reached ~85 cm into the water column (Fig. 9b), with implications for potential microphytobenthos growth.

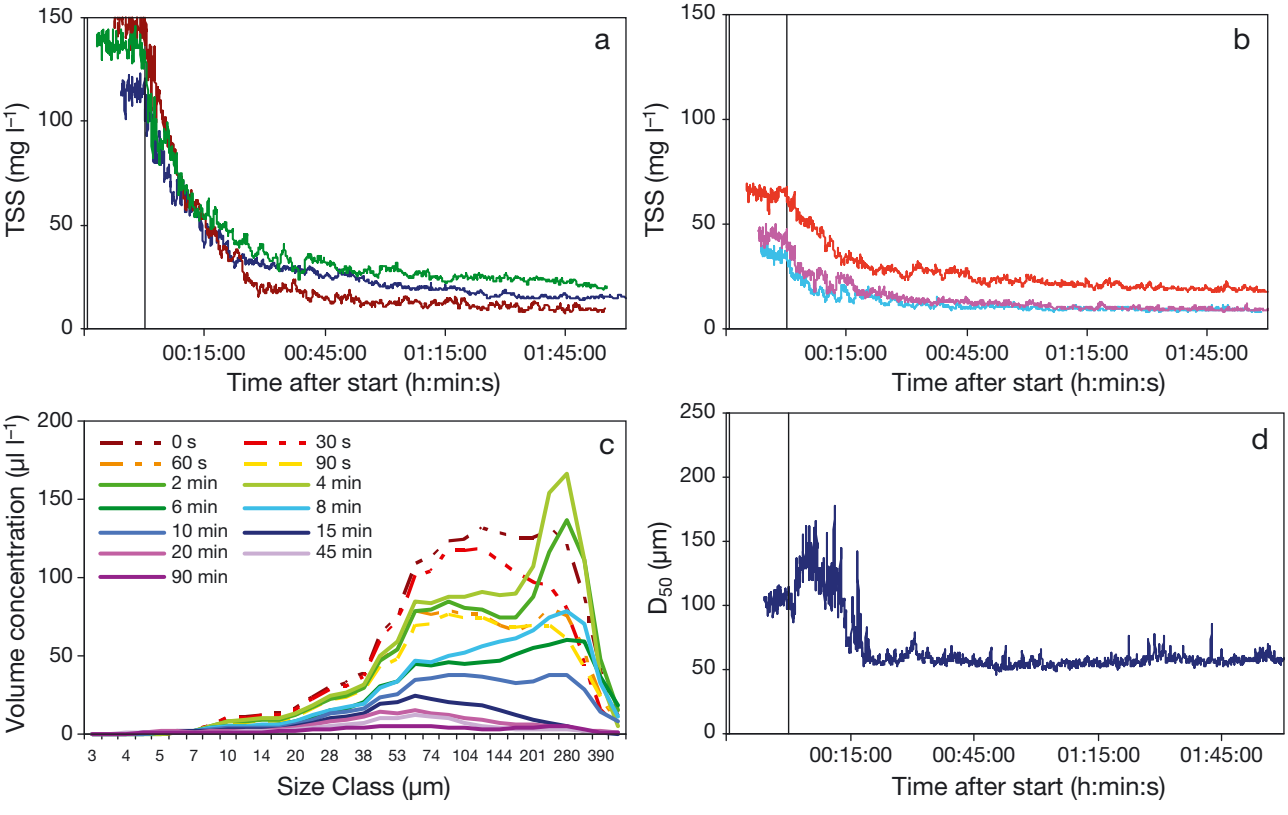


Fig. 8. Total suspended sediment (TSS) concentration time series during settling experiments in (a) 3 RC tanks and (b) 3 R tanks. Experiments were run in different tanks on Days 8, 10, 11, and 14. Vertical lines in panels a, b, and d mark the mixing 'off' time and the beginning of settling. (c) Sequence of particle size distributions measured with a LISST-100C in a RC tank during settling, typical of all of the tanks. Flocculation is apparent between ~2 and 15 min after mixing 'off'. (d) Median particle diameter (D_{50}) time series from the same RC tank, showing the formation and settling of large flocs during the first 15 min of settling

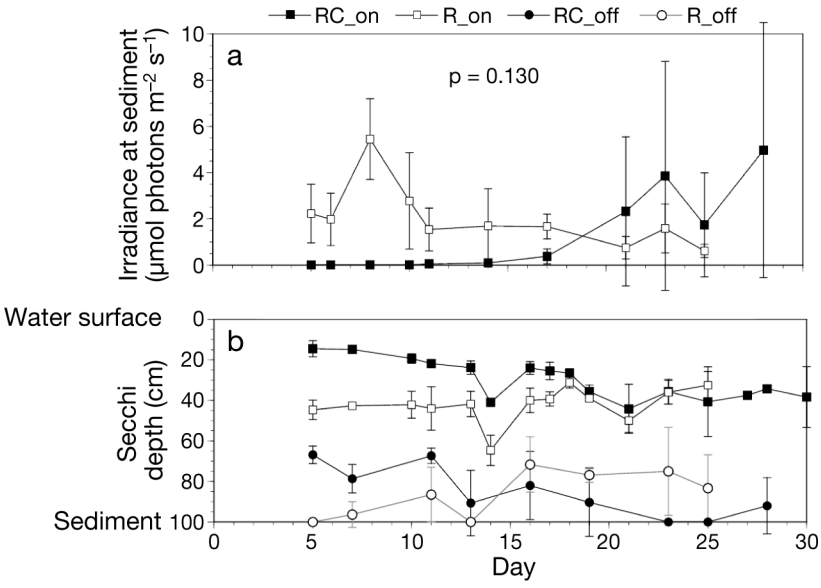


Fig. 9. (a) Irradiance calculated at the sediment surface during the mixing 'on' phases; n = 3 for each system, mean ± SD. (b) Secchi depth measured in the R and RC tanks during the mixing 'on' and 'off' phases over the experiment. Irradiance at the sediment was not significantly different, statistical significance $p < 0.05$

Sediment chl *a* concentrations were low overall but varied significantly between systems and over time. Sediment chl *a* levels were significantly higher in the R tanks (Fig. 10), which had a higher geometric mean irradiance and lower TSS levels but lacked clams. Sediment chl *a* levels increased from ~5 mg m^{-2} initially to ~13 and 18 mg m^{-2} midway through the experiment to ~14 mg m^{-2} and 26 mg m^{-2} in the RC and R tanks, respectively. We estimated how much sediment chl *a*: was eaten by the clams. Assuming that the ratios of chl *a*/phaeophytin would have been the same in the R and RC tanks in the absence of clams, we can use that assumption to calculate the expected concentration in the RC tanks: $RC_{SedChla} = RC_{SedPhae} \cdot (R_{SedChla}/R_{SedPhae})$, where $RC_{SedChla}$ removed is $RC_{SedChla}$ expected – $RC_{SedChla}$ actual. Thus, the amount of sediment chl *a* removed by the clams mid-experiment was 0.068 mg m^{-2} or 3.5 mg microphytobenthos carbon and by the end was 0.40 mg m^{-2} or 20.9 mg microphytobenthos carbon. Thus, 250 mg phytoplankton carbon and 8.1 microphytobenthos carbon combined were consumed per clam over the experiment. Other variables (particulate carbon and nitrogen concentrations and water content in the top 0.5 cm of sediment) were not significantly different between treatments.

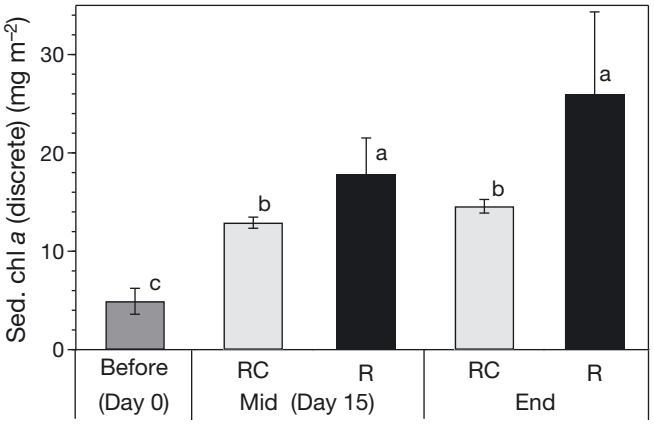


Fig. 10. Sediment (Sed.) chl *a* levels in the 0 to 1 cm depth section of sediment at the beginning (Day 0), on Day 15, and at the end of the experiment (Day 26 in the R tanks, Day 31 in the RC tanks), measured using HPLC. Different letters indicate statistically significant differences at $p < 0.05$. Means ± SD, n = 3 for each system

Macroinfauna and mesozooplankton

The sediment had been defaunated at the start of the experiment, and no macrofauna, besides the added *Mercenaria mercenaria,* developed in the RC or the R tanks over the course of the experiment. *M. mercenaria* live weight increased over the experiment in the 3 RC tanks by 0.135, 10.2, and 5.037 g (50 clams each) or by 103 ± 101 mg live weight per clam over the experiment. Converting live weight to carbon based on Frithsen et al. (1986) and length weight relationships, 6.2 mg carbon were gained per clam over the experiment. Assuming a trophic transfer efficiency of 10% (Reece et al. 2011), 62 mg carbon would be needed per clam over the experiment to achieve this weight. This estimate is within a factor of 4 of the value estimated above based on changes in phytoplankton biomass. Possible reasons for the differences in the 2 estimates is that we are assuming phytoplankton changes between the RC and R tanks are entirely due to clam feeding; however, differences in other factors, including differences in light, phytoplankton species composition, and the composition of the zooplankton between the RC and R tanks, could have all partially influenced the direct validity of the assumption. In addition, we used a POC:chl *a* ratio of 51.8 (Porter et al. 2010), and there is evidence that the ratios can vary depending on species composition (de Jonge 1980, Harding et al.

2002). Overall, given the basis of the calculations, the overall agreement between the estimates suggests that the differences between the RC and R tanks in phytoplankton abundance were most likely due to clam feeding.

Dominant mesozooplankton taxa found in the water column were adult *Acartia* sp. copepods, copepodites, copepod nauplii, and polychaete larvae (Fig. 11). Pumps likely destroyed the adult mesozooplankton stages (Adey & Loveland 1998) during the initial raw water fill of the tanks, and it took ~1 to 2 wk until adult copepods and polychaete larvae were found (Fig. 11). The R tanks contained ~7 adult *Acartia* sp. l^{-1} in the middle of the experiment, while the RC tanks contained 8 adult *Acartia* sp. l^{-1}. After 3 wk, *Acartia* sp. abundance decreased in all systems; however, polychaete larvae became abundant. About 25 to 40 polychaete larvae l^{-1} were found in the R tanks over the last 2 wk of the experiment (Fig. 11d), whereas after a phase of high abundance of polychaete larvae (75 ind. l^{-1}) in the RC tanks, the numbers decreased (Fig. 11d). *Acartia* sp., copepodite, copepod nauplii, and polychaete abundances were not significantly different between systems (Fig. 11). Linear regression analysis showed no significant relation between phytoplankton biomass and zooplankton biomass (both converted to carbon units), and thus, it was the hard clams that affected phytoplankton biomass.

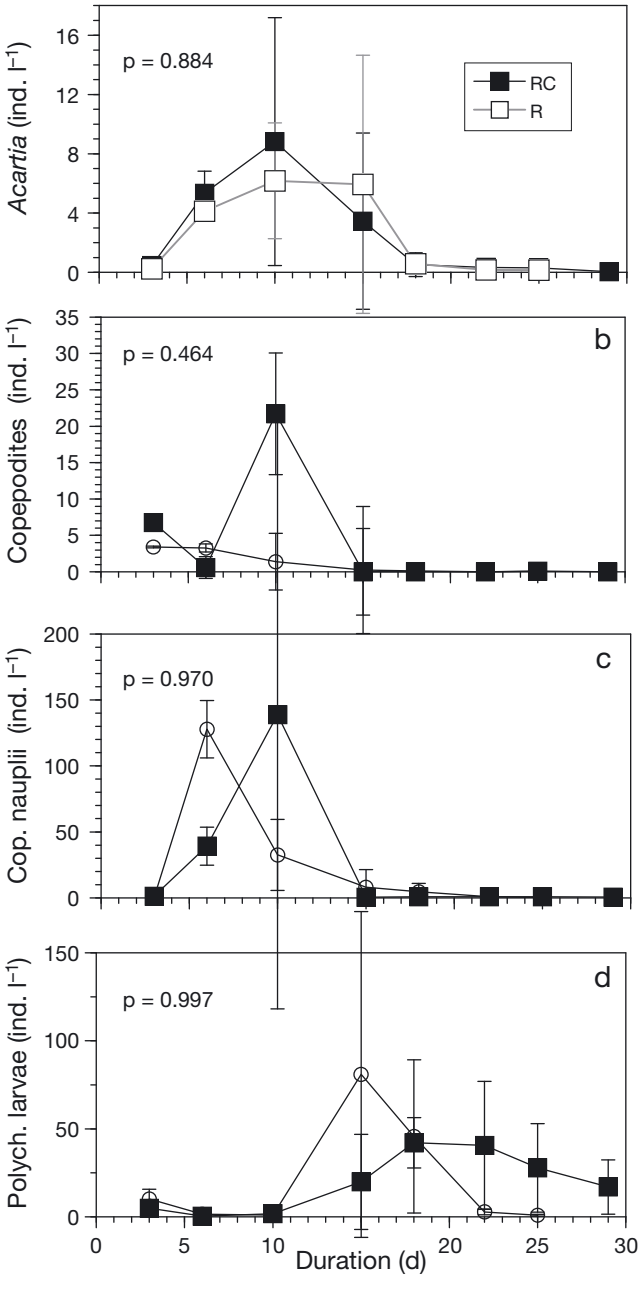


Fig. 11. Mesozooplankton abundance over the experiment: (a) adult *Acartia* sp., (b) copepodites, (c) copepod nauplii, and (d) polychaete larvae. Mean ± SD, n = 3. Statistical results indicated with p-value, statistical significance $p < 0.05$

DISCUSSION

Direct and indirect interactions between hard clams and high bottom shear stress affected phytoplankton biomass, TSS concentrations, light availability, microphytobenthos biomass, and nutrient dynamics (Fig. 12). In the following text, we discuss the findings in relation to the original hypotheses posed.

Hypothesis 1 that hard clams decrease phytoplankton abundance and affect phytoplankton diversity despite high total suspended solid concentrations has to be accepted. *Mercenaria mercenaria* feeding significantly decreased phytoplankton biomass. The R tanks developed a phytoplankton bloom, whereas the RC tanks did not, except for a small bloom of brown tide algae *Aureococcus anophagefferens* in 2 of the 3 tanks. A type I brown tide of *A. anophagefferens* bloom is not, however, expected to affect bivalve activity. Research examining the interaction of hard clams and *A. anophagefferens* generally finds that much higher cell densities are required to affect hard clam growth and activity (Bricelj & Lonsdale 1997, Wazniak & Glibert 2004). Cerrato et al. (2004) found that *M. mercenaria* prevented the formation of blooms of *A. anophagefferens*. Phytoplankton composition was shifted toward Chlorophyceae/Prasinophyceae in the RC tanks. *M. mercenaria* likely became food-limited during the experiments, as indicated by low phytoplankton biomass (Kemp et al. 2009) in the RC tanks. *M. mercenaria* selectively fed on phytoplankton and reduced phytoplankton concentrations in the RC tanks even though TSS lev-

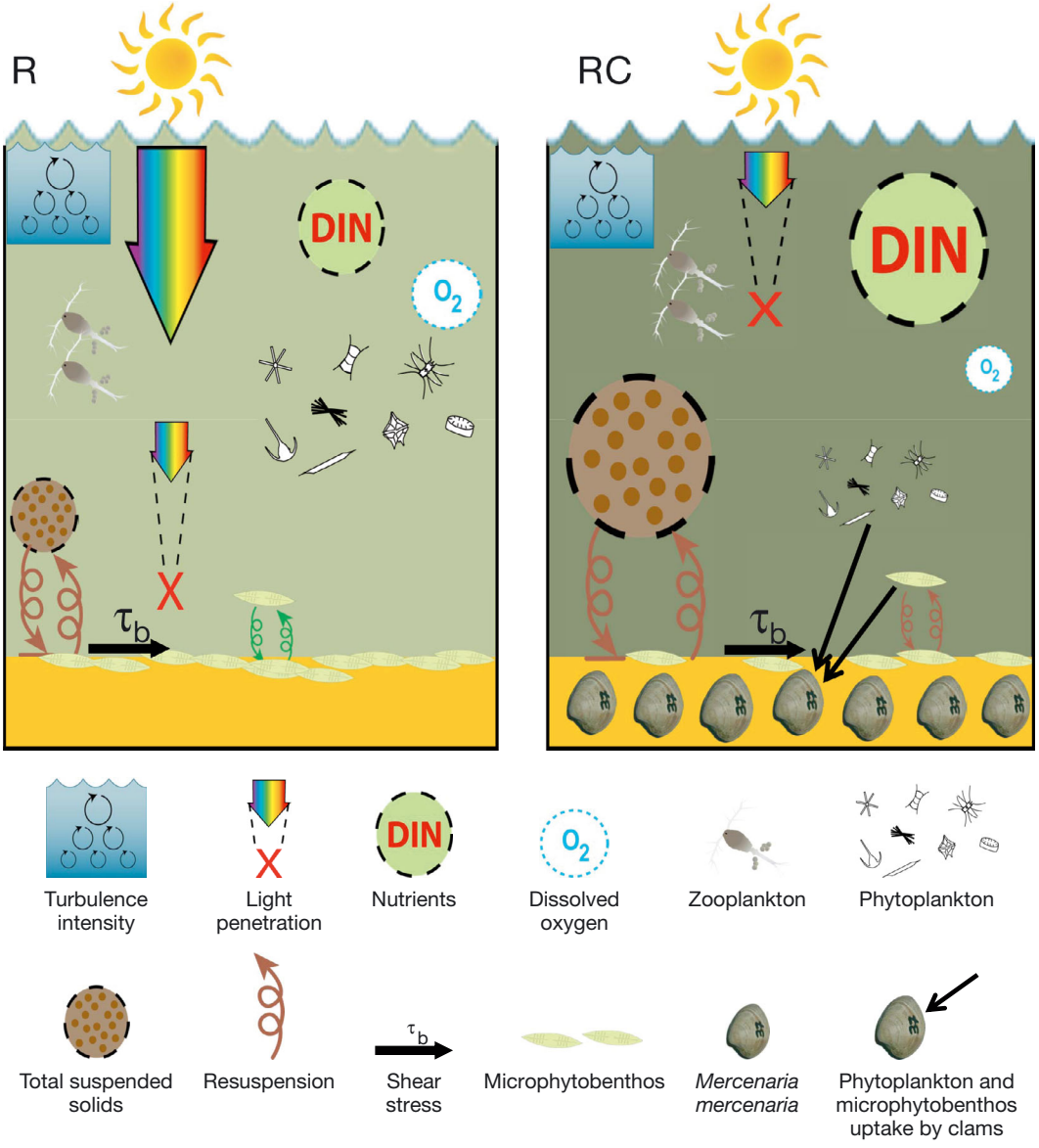


Fig. 12. Synthesis of experimental results comparing systems with tidal resuspension (R) and tidal resuspension with 50 hard clams (RC). Symbol size according to quantity or concentration. See 'Discussion'

els were as high as 200 mg l^{-1}. In previous feeding studies, *M. mercenaria* had been found to not feed at seston levels of ≥44 mg silt l^{-1} (Bricelj & Malouf 1984, Bricelj et al. 1984). In a mesocosm experiment with tidal resuspension and different densities of hard clams the following year, valve gape measurements on individual clams suspended in cages at mid-water column height showed that clams were gaping 59 ± 37% and 60 ± 37% of the time (mean ± SD, n = 15, unpubl. data) during mixing 'on' and 'off' phases, respectively. Hard clams controlled phytoplankton biomass, as shown for other bivalves (Cloern 1982, Officer et al. 1982, Porter et al. 2004b); however, *M. mercenaria* did not control pelagic biomass in non-resuspension experiments by Doering et al. (1986). Copepod abundance was not decreased by *M. mercenaria* unlike the pattern found by Lonsdale et al. (2007).

Hypothesis 2 that a high density of hard clams in combination with high shear stress does not affect the nitrogen dynamics has to be rejected. Resuspended sediments, resuspended biodeposits, and bivalve excretion contributed to the higher nutrient levels in the RC tanks. Hard clams and tidal resuspension significantly affected nitrogen partitioning within the system, and the nitrogen budget differed

between the R and the RC tanks. The R and RC systems accumulated similar amounts of N, with 59.4 vs. 57.9 mmol N, respectively. In the RC tanks, dissolved nutrients dominated the system with a TDN of 62.8% of the nitrogen in the tank due to resuspension and from bivalve excretion. In the R tanks, TDN was still dominant but accounted for only 41% of the N budget. In addition, 32.5% and 24.9% of the N was associated with the microphytobenthos and phytoplankton, respectively, in the R tanks. In contrast, only 17% and 7.6% of N was microphytobenthos and phytoplankton, respectively, in the RC tanks. The lower values likely reflect the feeding on phytoplankton and microphytobenthos by the clams.

An excretion experiment of hard clams after an identical setup of 50 hard clams m^{-2} and a month-long experiment with tidal resuspension in the following year showed that hard clams excreted ~3.31 ± 1.7 µmoles l^{-1} h^{-1} DIN per individual (data not shown). Scaled up to tank level, bivalve excretion added 165.5 µmoles DIN per tank per 50 hard clams l^{-1} h^{-1}or 3972 µmoles DIN $tank^{-1}$ d^{-1}. Sma & Baggaley (1976) also found a high rate of excretion in *Mercenaria mercenaria*. In the nitrogen budget, DIN in the RC tanks was 18.1% of the overall N budget, whereas it was only 4.9% in the R tanks. Decomposition of biodeposits may have taken place in the water column as biodeposits were resuspended, and this could be an additional nutrient source. Comparing these numbers to the average DIN content per RC tank (10320 ± 1080 µmoles), the contribution of excretion to nutrient concentrations is large, but release from resuspended sediment particles was likely another important source of nutrients.

A major N input was due to daily water exchanges with N in the fill water, which were identical between the R and the RC tanks. Another major input was expected from benthic DIN fluxes of ~2000 µmol $tank^{-1}$ d^{-1} in R systems, as measured in the R tanks the previous year (Porter et al. 2010). Effluxes are expected to be similar in the RC tanks as biodeposits were resuspended. However, some phytoplankton N was exported during the daily exchange in the R and RC tanks. We used estimates of porewater N release due to resuspension for the R tanks of 490 µmol m^{-2} d^{-1} from Porter et al. (2010) and scaled these up to the RC tanks, using the TSS concentrations. While porewater resuspension is slightly enhanced in the RC tanks, in both systems, it is at most a small additional source of N. In sandy sediments, stirring can induce additional porewater release due to advective flushing (Huettel & Gust 1992), but this is not the case with the muddy sediments used in this experiment.

Hypothesis 3 that hard clams and high bottom shear stress decrease microphytobenthos abundance due to both feeding and light limitation has to be accepted. *Mercenaria mercenaria* feeding significantly decreased microphytobenthos biomass, as has also been found for other bivalve species (Sauriau & Kang 2000, Rossi et al. 2004, Evrard et al. 2012). Microphytobenthos biomass was low in the RC tanks, which had reduced light penetration. During the last 9 d of the experiment, light levels were higher in the RC tanks with hard clams, yet overall microphytobenthos abundance was lower in the systems with hard clams, which suggests that bivalves were feeding on the microphytobenthos as it was resuspended (Fig. 12). Microphytobenthos is known to decrease nutrient regeneration (Sundbäck et al. 2000); thus, a reduction of microphytobenthos likely increased nutrient regeneration.

Fifty clams and high bottom shear stress initially destabilized the sediments in the RC tanks and reduced light penetration (Figs. 4, 9a,b, & 12). Willows et al. (1998) also observed sediment destabilization with high densities of *Macoma balthica* in an annular flume set to stepwise increasing levels of bottom shear stress. Ciutat et al. (2006, 2007) found that different densities of cockles destabilized the sediments, whereas Andersen et al. (2010) did not find destabilization by cockles. We directly observed bioresuspension by a hard clam 'puffing' materials up into the water, using a webcam in an underwater housing. In addition, the burying behavior of *Mercenaria mercenaria* could have led to the sediment destabilization. Adult hard clams burrow with a muscular foot. Adult hard clams moved laterally 5 cm in 38 d; however, juvenile hard clams 20 to 30 mm long moved 15 cm from the point of origin in an experiment by Chestnut (1951) as cited by Stanley (1985), which suggests a high potential for sediment destabilization. In our experiments, bivalves dug holes but seemed to stay within these holes with possible up and down movement. In a subsequent ecosystem experiment with different densities of *M. mercenaria* (unpubl. data), we observed similar effects of sediment destabilization at this hard clam density. Erosion studies in a 40 cm Gust microcosm (Gust & Müller 1997), with different densities of hard clams added to the sediment 6 d before stepwise increases in erosion, showed similar sediment destabilization at a hard clam density of 5 ind. per 100 cm^2 (Combs 2010). It has been shown that an enhanced water content can lead to sediment destabilization (de Deckere et al. 2001); however, the sediment water content in sediment cores from all

tanks was similar between treatments and over the experiment.

Sediment preparation techniques (Treatment HD-m in Porter et al. 2006) assured that sediments had stabilized geochemically at the start of the experiment, after 2 wk of consolidation time. Sediments in all tanks were prepared identically, and our defaunation technique did not lead to sediment destabilization.

The decrease in TSS concentrations in the RC tanks over the experiment suggests either that the burying behavior of the bivalves decreased during the experiment, that bivalves actively cleared the water column, or that the bed properties of the RC tanks changed, perhaps due to settled biodeposits by the clams. Microphytobenthos can stabilize sediments (Yallop et al. 1994), and significantly higher amounts of microphytobenthos biomass in the R tanks could have led to an enhanced sediment stabilization in the R tanks, thereby reducing resuspended TSS levels. Zimmerman & de Szalay (2007) found that streambeds were destabilized when mussels were burrowing, but sediments were stabilized when mussels were not burrowing. Similar to our experiments, they found that sediments destabilized the first week after adding bivalves and stabilized thereafter. While many experiments have found initial destabilization after experiments are started with infauna and sediments (e.g. Willows et al. 1998, Ciutat et al. 2006, 2007, Widdows et al. 2009), most experiments are short term. It is possible that hard clams respond in their burying behavior to the initial change in bottom shear stress that they are exposed to and subsequently become accustomed to the high shear stress levels. If this is an artifact, the short-term studies have to be revised. The precise reason(s) for the decreasing TSS concentrations in the RC tanks remain unknown.

Mercenaria mercenaria feeding, burrowing, and biodeposition activities did not significantly change the properties of suspended particles as measured by their settling behavior and particle size distributions during settling. The suspended particle population appeared to be separated into 2 sub-populations: one that settled rapidly after mixing was turned off, with a bulk settling velocity of ~1 mm s^{-1}, and one that stayed suspended for the remainder of the mixing 'off' phase. This settling behavior is very similar to that observed after tidal resuspension in Chesapeake Bay (e.g. Sanford & Halka 1993). Increases in particle size due to flocculation during initial settling accelerated the settling process. Overall, sediment destabilization due to the interaction of hard clams and high bottom shear stress greatly affected light penetration and the nutrient and oxygen dynamics (Fig. 12). High TSS concentrations increased the PN, PP, and PC concentrations. Dissolved inorganic nutrient concentrations were significantly higher in the RC tanks. In contrast, the dissolved inorganic nutrients were used by phytoplankton in the R tanks and lead to a phytoplankton bloom. DON concentrations were significantly higher in the RC tanks, and a combination of dissolved inorganic and organic nutrients available under low light conditions (Pustizzi et al. 2004) could have led to the small *Aureococcus anophagefferens* bloom in 2 of the RC tanks on Day 17.

Field studies have shown that dense populations of bivalve filter feeders can significantly affect benthic-pelagic coupling processes, including the transfer of materials to the bottom (Asmus et al. 1992, Norkko et al. 2001) and nutrient regeneration from the sediments (Dame et al. 1985, Doering et al. 1987, Porter et al. 2004b). *Mercenaria mercenaria* controlled phytoplankton biomass, as shown for other bivalve suspension-feeders (Cloern 1982, Officer et al. 1982, Porter et al. 2004b), while hard clams did not control pelagic biomass in a mesocosm experiment by Doering et al. (1986). Typical mesocosm studies have unrealistically low bottom shear stress (Porter et al. 2004a) so that resuspension cannot be mimicked. In the field, however, bottom shear stress is enhanced and can lead to TSS levels ranging from 40 mg l^{-1} (Sanford et al. 1991) to hundreds of mg l^{-1} (Schoellhamer 1995, 2002, Heyes et al. 2004).

Mercenaria mercenaria is now being farmed on a large scale in the high-salinity Virginia portion of the Chesapeake Bay ('aqua farming', M. Luckenbach, Virginia Institute of Marine Science, pers. comm.). Filtering by hard clams, even if there is resuspension, should decrease phytoplankton abundance at these farms. Densely planted hard clams in interaction with changing bottom shear stress during tidal cycles or resuspension during harvesting activity may, however, adversely affect TSS levels. Nutrient regeneration from resuspension and bivalve excretion may adversely affect the nitrogen dynamics. The balance of positive and negative ecosystem effects of hard clam aquaculture have to be weighed.

Overall, a high density of hard clams and high bottom shear stress affected phytoplankton abundance, microphytobenthos biomass, TSS concentrations, and the nitrogen dynamics in these experiments. The interplay of bottom shear stress and a dense assemblage of infaunal bivalve suspension feeders significantly affected benthic-pelagic coupling processes.

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Appendix 1

Analytical methods

Chlorophyll *a* (chl *a*) concentrations were measured using fluorometric techniques without acidification (Welschmeyer 1994) after extraction with 90% acetone to provide estimates of phytoplankton biomass. Phaeophytin was measured following Keefe et al. (2004). To determine total suspended seston (TSS) mass, seston filters were reweighed on a Mettler AE 240 microbalance after drying for 24 h to constant weight at 60°C. Then, the filters were ashed at 450°C for 4 h and re-weighed to determine particulate inorganic matter (PIM) concentrations. Particulate organic matter (POM) was determined by the difference between TSS and PIM (Berg & Newell 1986).

Water samples were analyzed for particulate phosphorus (PP) following techniques described by Keefe et al. (2004). Particulate carbon (PC) and nitrogen (PN) samples were analyzed on an Exeter Analytical (CHN) analyzer using Environmental Protection Agency method 440.0 (Zimmermann et al. 1997). Ammonium, nitrite plus nitrate, and phosphate were analyzed following the Technicon Industrial methods No. 804-86T, No. 158-71W, and No. 155-71W/Tentative, respectively, and total dissolved nitrogen (TDN) and phosphorus (TDP) were analyzed using methods described by Keefe et al. (2004). Dissolved organic phosphorus (DOP) was calculated by the difference between TDP and phosphate (PO_4^{3-}). We calculated dissolved organic nitrogen (DON) levels based on the difference between TDN and dissolved inorganic nitrogen (DIN). We analyzed dissolved silicate using a Technicon TrAAcs-800 nutrient analyzer with oxalic acid added to minimize interference from phosphates. Particulate silicate was analyzed as described by Keefe et al. (2004), as was DOC. For all variables, for quality control, every sixth sample was analyzed in duplicate. Nutrients and water-column chl *a* were analyzed by the Analytical Services Laboratory of the Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science (http://archive.chesapeakebay.net/pubs/quality_assurance/CBL_NASL_SOP_Feb_2004.pdf). These were the same techniques as used by Porter et al. (2010).

We extracted sediment chl *a* by adding 20 ml of 100% acetone to the sample tubes, sonicating each tube for 15 cycles at 0°C, and letting the sample extract further in a freezer overnight. After decanting, an additional 20 ml of 90% acetone was added, and the sample was again sonicated and left to extract for another 3 h. The supernatant was decanted after centrifugation and subsequently analyzed by high performance liquid chromatography (HPLC; Van Heukelem & Thomas 2001). HPLC was used to prevent interference from degradation products, such as chlorophyllides and pheophorbides, that interfere with fluorescence measurements (MacIntyre et al. 1996) and affect chl *a* readings. Chlorophyllides and pheophorbides can be abundant in areas with bivalves (e.g. Karakassis et al. 1998). In prior tests, we had found that this technique efficiently extracted ~97% of the sediment chl *a* from sediments containing a range of sediment chl *a* concentrations. We pooled extracts from samples taken in 3 quadrants of each tank and analyzed triplicate samples for each treatment for sediment chl *a* and phaeophytin.

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