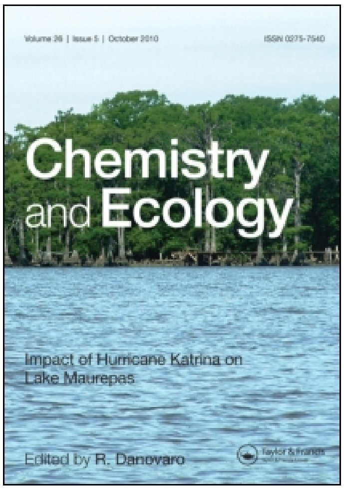

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Short Note

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Short Note

SEDIMENT ORGANIC MATTER AND ITS NUTRITIONAL QUALITY: A SHORT-TERM EXPERIMENT WITH TWO EXOTIC BIVALVE SPECIES

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This study was aimed to investigate the role of two exotic bivalves (*Ruditapes philippinarum* and *Musculista senhousia*) on the trophic enrichment and the nutritional quality of sediments from the Sacca di Goro (northern Adriatic Sea) through manipulative experiments. The concentrations of sedimentary total organic matter, biopolymeric carbon, carbohydrates, proteins, lipids, and chlorophyll-*a* were determined through a 64 days-long period. The experimental setup consisted in three replicated treatments (*R. philippinarum*, *M. senhousia* and simply sand), with treatments and time as fixed factors. All the biochemical classes showed wide fluctuation, and significant differences among treatments were detected during the experimental period. Both bivalve species played a role in sediment enrichment process. At the end of the experimental period, proteins represented the main biochemical class of organic compounds in the treatments with bivalves (48.5 and 44.5% of the biopolymeric fraction in the *M. senhousia* and *R. philippinarum* treatments, respectively), while in the control treatment they were only 32.7%. The accumulation of proteic nitrogen increased the nutritional quality of the sedimentary organic matter. Moreover, the presence of *M. senhousia* also enhanced the accumulation of microphytobenthic carbon.

Keywords: Biodeposition; Bivalve; Non-indigenous species

1 INTRODUCTION

Many non-indigenous species (NIS) are known to modify the invaded habitats in many ways. Estuarine and lagoonal ecosystems, being extremely vulnerable in terms of NIS introductions (Carlton, 1989), provide opportunities to investigate the effects of such introductions on the resident biota and on the habitat. In the Sacca di Goro, a shallow water lagoon in the Po River deltaic area (Northern Italy), two NIS, the Manila clam, *Ruditapes philippinarum* Adams and Reeve, 1850, and the Asian date mussel, *Musculista senhousia* Benson in Cantor, 1842, are nowadays the most abundant bivalve species (Mistri *et al.*, 2001). The infaunal Manila clam was deliberately introduced in the Eighties, and is actively farmed. In farming areas, clam densities are maintained at about 10^3 ind m^{-2}. The mytilid *M. senhousia* was accidentally introduced in the lagoon at the beginning of the Nineties, and reaches densities often exceeding 10^4 ind m^{-2}. The species

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is an ecosystem engineer (Crooks, 2001), building extensive mats in the upper layer of bottom sediments. In the Sacca, the presence of large mussel populations create economic impacts on clam farming, notwithstanding mussel abundance may be greatly reduced by harsh summer conditions (Mistri, 2002a), and predation (Mistri, 2002b).

Whether cultivated or accidentally introduced, dense populations of suspension feeding organisms contribute significantly to physico-chemical and biological changes in the bottom sediments, mostly due to deposition of faeces and pseudofaeces (Kroncke, 1996; Mirto *et al.*, 2000; Chamberlain *et al.*, 2001). Such biodeposits may have an important trophic significance, depending on their nutritional composition. The effects of dense NIS populations on sedimentary properties has, so far, received little attention. Aims of this study were (1) to investigate the role of *R. philippinarum* and *M. senhousia* on the trophic enrichment of sediments from the Sacca di Goro through manipulative experiments, and (2) to assess the quality of biodeposits which are eventually usable as food by benthic consumers.

2 MATERIALS AND METHODS

2.1 Experimental Setup

Ruditapes philippinarum individuals (shell length, mm: 19.0 ± 0.5 SD) were supplied by the local shellfish farmer syndicate (COPEGO), white *Musculista senhousia* (shell length, mm: 21.1 ± 0.8 SD) were collected from natural banks in the Sacca at the beginning of August 2001. Bivalves were separately kept for two weeks in suspended cages in a 5000 l tank in running, unfiltered brackish water, to acclimatize. Only undamaged bivalves in apparently good condition (fast closure of the valves or vigorous siphon withdrawal responses upon handling) were used in the experiment. In August 2001, a sufficient amount of well-sorted fine sand was gathered from a licensed area, and made free from any kind of organic matter through prolonged combustion in a muffle furnace (500 °C, 12 h). Eighteen aluminium containers were then filled with this sand (volume of sand in each container: 300 cm^3), and carefully submerged in an flow-through system tank (250 l) with running, unfiltered brackish water. The tank was built with the inflow on a corner, and the overflow on the opposite one to ensure efficient water turnover. Containers were placed in the center of the tank to avoid edge effects. Each container was then randomly assigned to one of the following conditions: (a) with five *R. philippinarum* (RP treatment), (b) with ten *M. senhousia* (MS treatment), and (c) with simply sand (SS treatment). The number of bivalves was chosen in order to have grossly the same biomass in the RP and MS treatments. Manila clams took 20 to 30 min to bury, while Asian date mussels were partially buried by hand, leaving a few mm of shell exposed, to mimic the sediment-water interface position of mussel mats in the field. The experiment started on August 13, immediately after placing bivalves into containers. Subsequent sampling was scheduled on a $\times 2$ geometric scale, *i.e.* at 2–4–8–16–32–64 days from the beginning of the experiment. On each sampling occasion, three randomly chosen containers (RP, MS, SS) were removed from the tank, and sediments were sampled by means of plastic cores (i.d. 3 cm). Following retrieval, the top 0–1 cm of each core was immediately deep frozen (-20 °C) and stored until subsequent analysis.

2.2 Analytical Procedures

For each analysis about 1 g of sediment was used. Protein (PRT) analyses were carried out according to Hartree (1972) modified by Rice (1982), and presented as albumin equivalents. Carbohydrates (CHO) were analyzed according to Gerchacov and Hatcher (1972) and expressed as glucose equivalents; this method is specifically adapted for carbohydrate determination in sediments. Lipids (LIP) were extracted from sediment samples by direct elution with chloroform and methanol according to Bligh and Dyer (1959) and Marsh and Weinstein (1966). Protein, carbohydrate, and lipid concentrations were converted to carbon equivalent assuming a conversion factor of 0.49, 0.40 and 0.75 respectively (Fichez, 1991). The sum of protein, carbohydrate and lipid carbon was referred as biopolymeric fraction (BPC: BioPolymeric Carbon; Fabiano and Danovaro, 1994; Danovaro and Fabiano, 1997). For each analysis, blanks were made using the same previously calcinated sediments. Total organic matter (TOM) was determined as the weight loss of the dried samples (80 °C, 48 h) after combustion for 4 h at 450 °C. Before drying, samples were acidified with 0.1 N HCl to remove carbonates. Finally, microphytobenthic chlorophyll-*a* was analyzed according to Lorenzen and Jeffrey (1980), using 90% cold acetone to extract pigments, and converted into microphytobenthic carbon (C-Chl*a*) using a conversion factor of 40 (De Jonge, 1980). All analyses were carried out in three replicates. Sediment trophic concentrations were normalized to dry weight after desiccation at 80 °C until constant weight (48 h).

2.3 Analysis of Data

Experimental design followed a two-way ANOVA layout with treatments (RP, MS, SS) and days (2–4–8–16–32–64) as fixed factors. Data were log-transformed to meet homoscedasticity. Every time a significant difference resulted for the main effect, a post-hoc Tukey HSD test set at the 5% significance level was performed.

3 RESULTS AND DISCUSSION

Before running the experiment, aliquots of the combusted sediment were analyzed for the various classes of biochemical compounds under investigation: all concentrations resulted $<0.01\,\mu g\,g^{-1}$. The temporal trends of CHO, PRT, LIP, BPC, TOM and C-Chl*a* in the MS, RP and SS treatments are shown in Figure 1. In Table I, results of two-way ANOVAs are reported. Significant differences were always detected among the two main factors except for CHO across time, and TOM, among treatments. All the biochemical classes showed wide fluctuation during the experimental period. CHO ranged between 17.8 and $48.6\,\mu g\,g^{-1}$, and significant differences were detected between MS and SS treatments (Tukey HSD test: $p < 0.05$). PRT ranged between 63.8 and $369.4\,\mu g\,g^{-1}$, and, on average, differed among the three treatments (SS *vs*. MS: $p < 0.001$; SS *vs*. RP: $p < 0.01$; MS *vs*. RP: $p < 0.05$). LIP ranged between 46.9 and $222.7\,\mu g\,g^{-1}$, with SS different from the other treatments (SS *vs*. MS: $p < 0.001$; SS *vs*. RP: $p < 0.01$; MS *vs*. RP: $p = 0.105$). BPC ranged from 82.4 and $308.0\,\mu g\,g^{-1}$ (SS *vs*. MS: $p < 0.001$; SS *vs*. RP: $p < 0.001$; MS *vs*. RP: $p < 0.05$). TOM ranged between 0.36% and 2.15%, and differences were detected only between days (from day 2 onwards: $p < 0.05$). Finally, C-Chl*a* ranged between 0.8 and $12.0\,\mu g\,g^{-1}$ (SS *vs*. MS: $p < 0.001$; SS *vs*. RP: $p = 0.528$; MS *vs*. RP: $p < 0.01$).

In Figure 2, the contribution of CHO, LIP and PRT as percentage to BPC is shown. At the end of the experimental period (64 days), proteins represented the main biochemical class of organic compounds in the treatments with bivalve NIS (48.5 and 44.5% of BPC pool for the MS and RP treatments, respectively), while in the control treatment (SS) they were only 32.7% of BPC pool. Conversely, lipids constituted the main biochemical class in SS (56.3% of BPC pool), and were lower in the MS (38.6%) and RP (42.5) treatments. The carbohydrate contribution to BPC was low, ranging between 11.0% (SS) and 12.9–13.0% (MS and RP, respectively). A temporal increase in lipid content and a concomitant decrease in protein content resulted in the SS treatment during the experimental period. TOM slightly

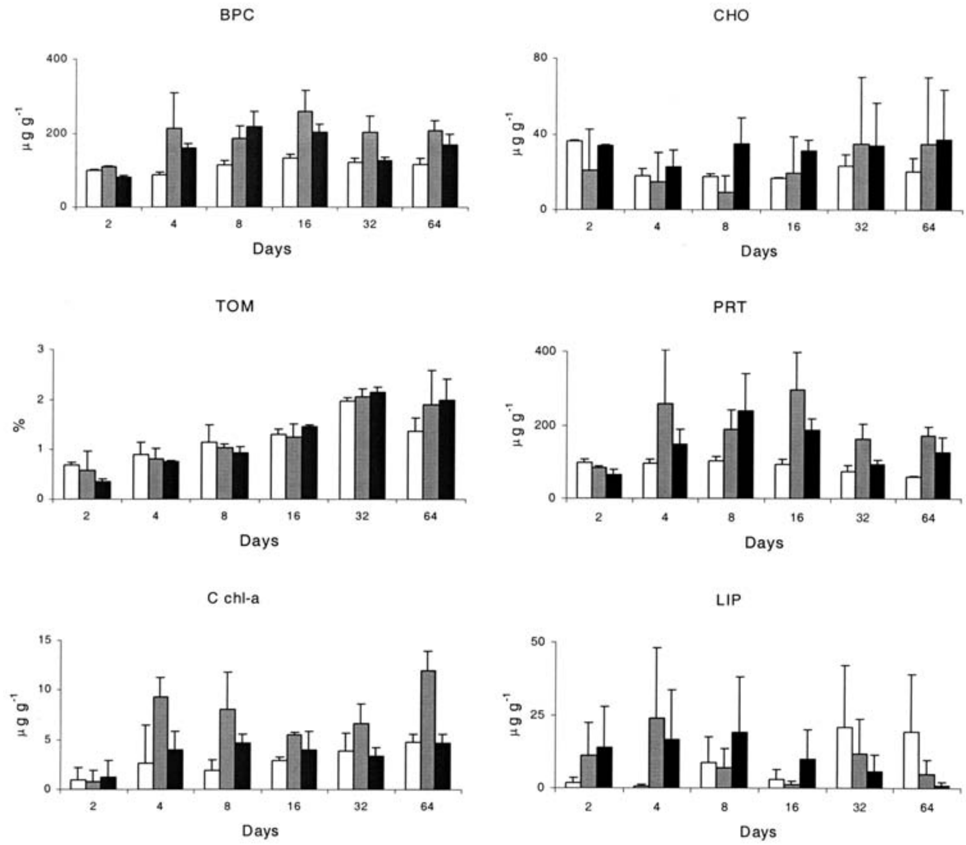

FIGURE 1 Temporal changes in the concentration of the biochemical classes investigated; BPC: biopolymeric carbon; TOM: total organic matter; C-Chl*a*: microphytobenthic carbon; CHO: carbohydrates; PRT: proteins; LIP: lipids (white: control; grey: *M. senhousia*; black: *R. philippinarum*; bars are 1 standard deviation).

differed among treatments (1.9, 2.0 and 1.4% in the MS, RP and SS treatments, respectively), while microphytobenthic carbon was highest in MS ($12.0\,\mu g\,g^{-1}$), followed by SS ($6.8\,\mu g\,g^{-1}$), and RP ($4.7\,\mu g\,g^{-1}$).

Sediments are complex systems affected, among the others, by biological parameters. There are relatively few studies on the impact that infaunal, non-indigenous species exert on sediments of newly-invaded habitats. This paper investigates the role of two exotic bivalves, the mussel *Musculista senhousia* and the clam *Ruditapes philippinarum*, on sedimentary trophic enrichment

TABLE I *F* Values from ANOVAs. Main Factors: Treatments (MS, RP, SS) and Time (from Day 2 to 64 on $a \times 2$ Geometric Scale).

Factors	*CHO*	*PRT*	*LIP*	*BPC*	*C-Chla*	*TOM*
Treatment	377*	22.70***	21.08***	28.47***	16.81***	0.03
Time	0.99	7.82***	31.59***	11.16***	7.46**	30.17***
Treatment × Time	0.26	2.52*	3.48**	2.37	1.58	1.02

Notes: CHO = carbohydrates; PRT = proteins; LIP = lipids; BPC = biopolymeric carbon; C-Chl*a* = microphytobenthic carbon; TOM = total organic matter.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

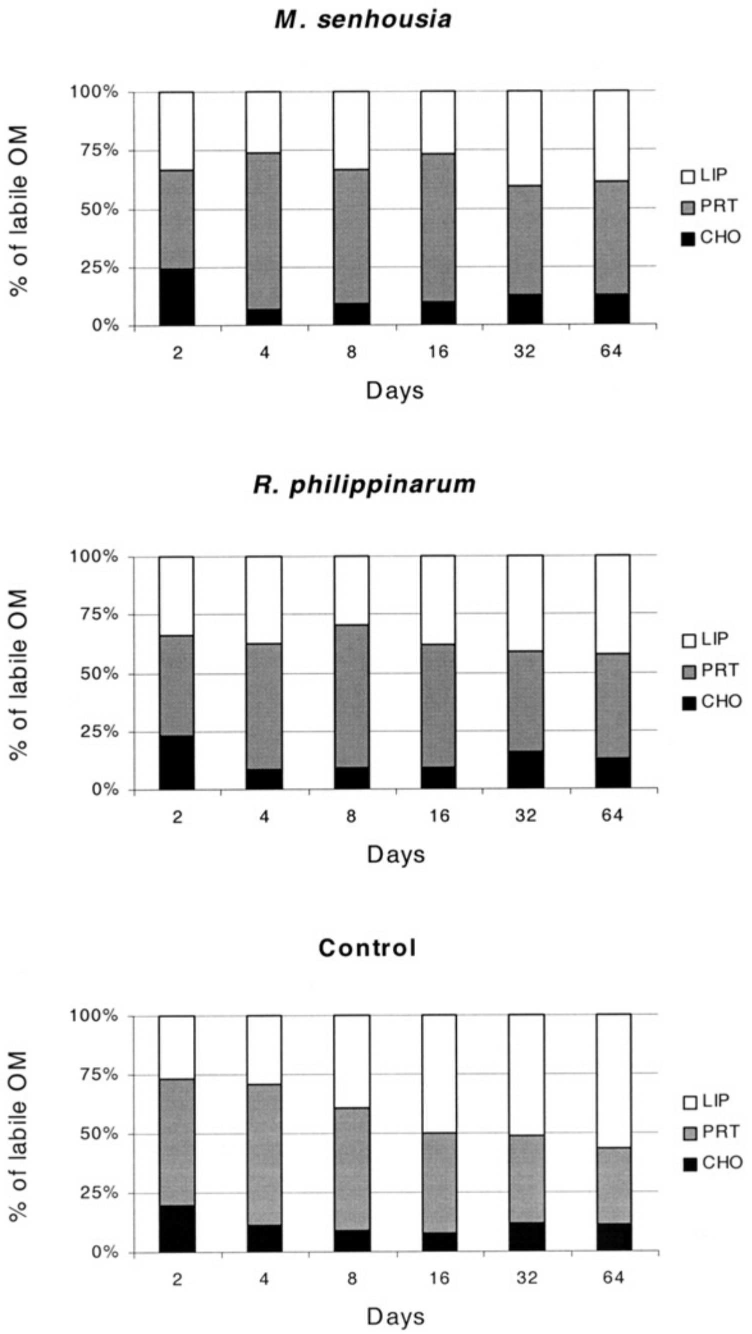

FIGURE 2 Percent contribution of carbohydrates (CHO), proteins (PRT) and lipids (LIP) to the labile organic matter in the three treatments (*M. senhousia*, *R. philippinarum*, control) as a function of time.

and the nutritional quality of the biodeposits. This study showed that both NIS play a role in sedimentary organic enrichment process. Over our short-term study period, however, the presence of *M. senhousia* exerted different effects on sediments with respect to *R. philippinarum*. We found, for example, that microphytobenthic carbon (C-Chl*a*) was at least twice higher in the MS than in the RP and SS treatments. It has been suggested that high values of chlorophyll-*a* are due to deposition of pseudofaeces (Navarro and Thompson, 1997), and *M. senhousia* (at least in Japan) seems characterized by high ingestion rates (Inoue and Yamamuro, 2000). However, it should also be stressed that *M. senhousia* is typically raised several millimeters relative to ambient sediments, and this biogenic structure may potentially alter hydrodynamic features on flat bottoms. Consequently, it may trap a range of living materials (*e.g.* microalgae), leading to an increase in their rate of accumulation and, thus, to very high values of microphytobenthic carbon. After only 8 weeks of biodeposition and accumulation, C-Chl*a* concentrations in the sediments with *M. senhousia* were comparable to those reported in highly productive coastal systems (*e.g.* Danovaro *et al.*, 1999a; Koster and Meyer-Reil, 2001; Dell'Anno *et al.*, 2002).

Sedimentary organic enrichment has been reported in most studies dealing with shellfish farming impacts (Kroncke, 1996; Chamberlain *et al.*, 2001). However, it has been hypothesized (Mirto *et al.*, 2000) that TOM is probably a poor descriptor of shellfish biodeposition impact. Results from this study seem to confirm such finding, since slight differences in TOM accumulation between experimental and control treatments were evidenced. It should be stressed, however, that the determination of TOM through combustion is a technique not free from bias, since many inorganic compounds may be oxidized at temperatures above 400 °C. For this reason, Danovaro *et al.* (1999b) stressed that changes in the trophic state of marine sediments were better evidenced in terms of organic matter composition than in terms of TOM. Notwithstanding the short-term study period, we observed the existence of temporal changes in the quality of sediment organic matter, as already shown in other Mediterranean aquatic environments (Grémare *et al.*, 1997; Pusceddu *et al.*, 1999). The analysis of the different biochemical classes of organic compounds contributing to the BPC pool revealed that an higher accumulation of proteins was present at both MS and RP treatments. The temporal trend of protein concentration was decoupled from that of carbohydrates, so we can exclude that protein accumulation was consequent to microphytobenthic accumulation. Such finding indicates that the quality and bio-availability of organic matter in sediments where *M. senhousia* and *R. philippinarum* are present is high. The organic matter available to benthic organisms inhabiting lagoonal systems is highly heterogeneous, and such an heterogeneity refers both to the origin of organic matter and to its degradation state. Organic nitrogen is considered the major limiting factor for the detritus food-chain and, thus, for deposit-feeding organisms (Carey and Mayer, 1990), but it is also recognized that, in the aquatic environment, a large proportion of sediment nitrogen is bound into refractory materials, and cannot be readily absorbed by primary consumers (Rice, 1982). So, living bacteria represent the main protein resource for sediment-ingesting organisms (Danovaro *et al.*, 1994), and their activity parameters seem controlled primarily by the nutritional quality of organic matter (Fabiano and Danovaro, 1998). The accumulation of proteic nitrogen increases the nutritional quality of the sedimentary organic matter where bivalve NIS are abundant. As a matter of fact, Mistri (2002a) found higher abundance of deposit-feeder organisms within *M. senhousia* beds than on neighboring bare sediments in the Sacca di Goro. Due to the preliminary nature of this study, microbial biomass in the various treatments was not quantified. Future studies will be aimed to deepen the knowledge of the interaction between sediment properties, nutritional quality of organic matter and microbial biomass to better understand the effects of dense non-indigenous, filter-feeder assemblages in coastal lagoons.

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