The effects of grazing by the golden grey mullet Liza aurata on microphytobenthos in intertidal mudflats: evidences from mesocosm experiments

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experiments

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Abstract

Grey mullets are one of the most abundant fish in coastal areas that periodically inspect the intertidal flats at high tide. Although they are one of the few fish to exploit directly microphytobenthos (MPB), very little is known about their effects on MPB assemblages. In this study, we experimentally tested hypotheses on the effects of grazing by juveniles of *Liza aurata* on either the mean and the variance of MPB biomass in an intertidal mudflat in the Northeast Atlantic, using laboratory mesocosms filled with natural sediments. The experiment was repeated twice (on April and on June 2009) to test for consistency on different dates of the year.

Grazing by *L. aurata* did not influence the mean MPB biomass at any of the two dates . In contrast, it increased the spatial variance in MPB biomass in June, at the scale of few centimeters. Video analyses showed that the grazing velocity was higher in June in comparison to April suggesting a relationship between the intensity of grazing and its ability to generate spatial variability in MPB biomass. We hypothesize that grazing by juveniles of grey mullets might generate variability in the spatial distribution of MPB biomass in natural systems, in a way consistent with that observed under our mesocosm conditions, with important implications for the ecology of mudflats.

Keywords: Grey mullets; microphytobenthos; intertidal mudflats; grazing; *Liza aurata*;; spatial variance.

Introduction

Microphytobenthos (MPB) are a key component of marine intertidal soft sediments, as they are important primary producers (Yallop et al., 1994; MacIntyre & Cullen, 1996; Underwood et al., 2005), contribute to carbon budgets (Sullivan and Moncrieff, 1988; Underwood & Kromkamp, 1999), can stabilize sediments (Tolhurst & Chapman, 2007) and provide food for herbivores (Decho 2000). Grey mullets are one of the most abundant fish in coastal areas worldwide, where they inspect periodically the intertidal flats at high tide (Thomson 1966; Odum 1973; Brusle 1981; Collins 1981; Lafaille et al. 2002; Almeida 2003; Richard et al. 2006). Studies based on diet composition and stable isotope analyses have provided evidence that grey mullets are one of the few fish able to directly exploit benthic microalgae (Ferrari and Chieregato1981; Cardona 2001; Lafaille et al. 2002; Pasquaud et al. 2010; Lebreton et al. 2011), thus potentially affecting MPB assemblages.

The effect of grey mullets on MPB inhabiting the sediment can be very complex, rather than merely a consumer–resource relationship. Grey mullets can create pits and release droppings on the surface of the sediment (Lafaille et al. 2002; Almeida 2003; Richard et al. 2006). As a consequence, they can alter the heterogeneity of the substratum available for benthic microalgae. Many species of grey mullets can also feed on small benthic grazers such as foraminifers, nematodes and polychaetes (Ferrari and Chieregato1981; Cardona and Castelló 1990; Cardona 2001; Lafaille et al. 1998; Lebreton et al. 2011), thus indirectly influencing MPB by modifying trophic relationships of local infauna.

Although grey mullets are generally present at high abundances in coastal areas, their benthic feeding habit, and in particular their exploitation of benthic microalgae, is poorly known. This lack of knowledge stems in part from the practical difficulties due to the experimental manipulation of epibenthic fish abundances in the field, especially in areas completely exposed to air at low tide such as intertidal flats (Reise 1985). Benthic mesocosms, containing natural sediments, are types of facility designed to conduct manipulative experiments in laboratory

conditions, and may represent an alternative method to test specific hypotheses about the effects of grey mullets on MPB at small spatial scales (i.e., $< 1m^2$) (Benton et al. 2007).

In general, the experimental addition of a grazer or predator (both in laboratory and field conditions) results in the reduction in the mean biomass (or density) of their resources (Smith et al. 1996; Berlow 1999; Uthicke 2001, Armitage and Fong 2004; Armitage et al 2009). However, a consumer may also alter the spatial variability in the distribution of a resource, with important consequences, among others, for the stability of the ecosystem (May 1973). A grazer might prefer foraging on certain patches of resources, thus increasing the spatial variability of its activity (Benedetti-Cecchi 2000; Sommer 2000; Adler et al. 2001; Flecker and Taylor 2004; Alvarez and Peckarsky 2005; Hillebrand 2008), or create pits and release droppings on the surface of the sediment (Lafaille et al. 2002; Almeida 2003; Richard et al. 2006), thus altering the heterogeneity of the substratum. Whether these activities increases, decreases or has no effect on the overall spatial variance of a resource abundance, however, is difficult to anticipate. This is because this effect depends on many factors, such as the overall abundance of the resource, the variance and the mean effect of the trophic interaction, the residual variability of the resource (i.e., the component of variation that is not due to grazing), and the mathematical relationship between the mean and the variance (Benedetti-Cecchi, 2000,2003; Benedetti-Cecchi et al. 2005). For instance, if a consumer reduces the mean biomass of a resource, this should also cause a reduction in spatial variance of the biomass itself, due to the relationship between the mean and the variance (Taylor 1961). The overall spatial variance of the resource, however, is expected to increase if the amount of variance generated by the grazing activity is larger than the background spatial variability in the resource abundance, and if the positive effect due to increased spatial variance of grazing is larger than the negative effect caused by the reduction in the mean abundance of the resource.

In this study, we experimentally investigated the effects of grazing by juveniles of the grey mullet *Liza aurata* on either the mean and the spatial variance in MPB biomass, using laboratory mesocosms, filled with sediments from an intertidal mudflat in the Northeast Atlantic. Specifically,

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we predicted a negative effect of grazing on the mean abundance of MPB (Ferrari and Chieregato1981; Cardona 2001; Lafaille et al. 2002; Pasquaud et al. 2010; Lebreton et al. 2011). Differently, a singular hypothesis on the effect on the spatial variability in MPB biomass could not be stated *a priori*, due to the lack of knowledge on the characteristics of the trophic interaction. In fact, we could expect a null (or negative) effect of grazing on the spatial variance of MPB biomass if the negative effect due to the removal of a large amount of algae from the sediment (i.e., strong effects) was equal (or larger) than the residual variability of MPB, if the grazing pressure distributed homogenously in space. On the contrary, a positive effect would be detected if the amount of variance generated by the grazing activity was larger not only than the residual variability of MPB biomass, but also than the loss of variation that might follow the reduction in its mean. The effect of grazing on the mean abundance and the variance in MPB biomass was tested at three different spatial scales. Because intertidal mudflats are variable systems (Blanchard et al. 1997; Guarini et al. 1998) and because the grazing activity of grey mullets may be temporally variable (Almeida et al. 1993; Cardona 1999; Lafaille et al. 2002; Venkatesha et al. 2002), the experiment was repeated twice, to examine if these effects were consistent on different dates of the year.

2. Material and Methods

2.1 Study area

The sediment used in this study was collected on an intertidal mudflat in Aiguillon Bay, located in the Vendèe and Charentes Maritime districts of France (Atlantic coast) (46°10'N, 1°15'W). The golden grey mullet *Liza aurata* is one of the most abundant grey mullets inhabiting the European Atlantic coasts and it is a permanent species in Aiguillon Bay (Lafaille et al. 1998; Parlier et al. 2006; Lebreton et al. 2011). In particular, we focused on 1 year-old juveniles, because they dominate the local populations of *L. aurata* in Auguillon Bay (Parlier et al. 2006; Lebreton et

al. 2011) and their benthic feeding habit is well known (Albertini-Berhaut 1974; Ferrari and Chieregato 1981; Lebreton et al. 2011).

2.2 Experimental set-up

For each replicate mesocom, sediment was collected in the study area at low tide, by scraping off the upper 2 mm. The sediment was then brought back to the laboratory and sieved through 500 µm screens to eliminate large macrograzers. The reconstructed sediment was then maintained on plastic plates (0.60 × 0.40 × 0.05 m), laid on the bottom of a tank (two adjacent plates within each tank), which had the same shape and area of the working chamber of the mesocosm. Tanks were positioned inside an external greenhouse, so that the sediment experienced the same natural light conditions as in the field. Sediment was exposed to the natural tidal cycles of the area of collection, through a series of pumps which controlled the flow of water in and out of the tanks. Sediment was kept for three days before being used. The water in the tanks was renewed daily to avoid an excessive increase in water temperature and salinity due to water evaporation.

Due to practical difficulties, it was not possible to collect *L. aurata* in Aiguillon Bay. Therefore, we used individuals of a natural populations of *L. aurata* appropriately collected from a Mediterranean coastal lagoon in June 2008. Once in the laboratory (3 October 2008), fish (standard length, mean ± S.E.: 9.68 ± 0.54 cm) were maintained in 1000 liter rearing tanks (1 × 1 × 1 m) filled with filtered re-circulated natural sea water using a natural photoperiod (46°10 N). Temperature in the tanks ranged between 14 and 19°C (mean ± S.E: 17 °C ± 0.2, n=30), while salinity ranged between 29 and 35 psu (mean ± S.E: 32 psu ± 0.3, n=30). Fish were fed with dry pellets (BioMar®) and natural periphyton daily. Natural periphyton is usually used as supplementary food in aquatic production systems (Richard et al., 2009).

The benthic mesocosm (4 × 1 × 1 m; Fig. 1) was located in a quiet, closed room, with controlled temperature, at the marine station of the University of La Rochelle (France). The photoperiod (12:12; dark:light) and the light intensity were controlled through a series of lamps

connected to a timer. The mesocosm was divided into two chambers, separated by a sliding door (Fig. 1): (i) a working chamber and (ii) an acclimatization chamber, a deeper compartment where the fish were acclimatized before the experiment. For the purpose of this study, the surface area of the working chamber was set to 0.48 m² (i.e. 1.20 × 0.40 m), so that two adjacent plastic plates (with the reconstructed sediment) could perfectly fit on the bottom. The acclimatization chamber was equipped with a vertically-mobile floor, which was lifted up in order to help the fish moving to the working chamber while the sliding door was open. The mesocosm was connected to a buffer tank and a thermostat to control the water temperature. This system also enabled us to control the inner circulation and level of water inside the mesocosm. In addition, the mesocosm was equipped with a video-tracking system, which allowed us to estimate the grazing activity by fish (Fig. 1). In particular, four polychrome video cameras were placed along the longest side of the working chamber for a lateral vision and were connected to a quad video processing and a DVD recorder (Fig. 1).

The day before the experiment, the fish were randomly chosen from the holding tanks and transferred to the acclimatization chamber of the mesocosm. Before being transferred into the mesocosm, fish were marked using plastic tags of different colors. Krazy glue® was used to adhere the tags on the opercula of the fish. For marking, fish were lightly sedated (McFarland and Klontz 1969). During the procedure, the gills were continuously perfused with oxygenated water containing an anesthetic (0.08 g l^{-1} of MS222). Each tagging procedure took <2 min and fish ventilated continuously with no noticeable decrease in ventilation rate. Fish never lost balance and resumed swimming as soon as they were transferred to the acclimatization chamber (Como pers. obs.). Fish were then left undisturbed for 12 hours to allow them to recover from the stress induced by this handling (Lefrancois et al. 2005). One hour and a half before the beginning of the experiment, the two plastic plates of reconstructed sediment were transferred into the working chamber of the mesocosm through the main door. The water level was then raised (about 50 cm water height) and the fish were encouraged to move from the acclimatization to the working

chamber. This was done by gently opening the sliding door and lifting up the floor (Fig. 1). In general, the school of fish moved rapidly (less than 3 min for transfer) from the acclimatization chamber to the working chamber. The experiment began when the fish started feeding on the sediment and lasted 3 hours. The cameras were turned on as the school of fish entered the working chamber and recorded the feeding activity of the fish continuously for the entire duration of the experiment. At the end, the water level was progressively reduced, the main door opened and the sediment sampled. It is worth noting that both at the beginning and at the end of the experiment, the water level in the mesocosm was altered very slowly, in order to avoid resuspension of sediment, and thus of MPB. Each of these phases lasted approximately one hour and a half. The experiment was done in the morning.

2.3 Experimental design

The effect of grazing by *L. aurata* on the mean and the spatial variance in MPB biomass was assessed by comparing mesocosms with *L. aurata* (hereafter called Grazed treatment, G) and without *L. aurata* (hereafter called Un-grazed treatment, U). For G, three randomly chosen juvenile mullets were introduced in each replicate mesocoms. Since no data on the actual densities of mullet populations on mudflats were available (Degrè et al. 2006), fish density (i.e., 6.25 individuals $\cdot$ m^{-2} corresponding to a concentration of grazing fish of 14.48 ± 0.89 g fresh weight $\cdot$ m^{-2}) was chosen taking into account the constraints related to the feeding behavior of grey mullets (they are gregarious fish, with individual behavior during swimming and feeding depending on the behavior of the school) and of the size of fish with respect to those of the mesocom. For U, the same experimental procedure used for G was followed except that fish were not introduced into the mesocosms. In particular, U enabled us to estimate the component of variance in the spatial distribution of MPB biomass that was not due to the grazing by *L. aurata* (i.e. residual variability). Benthic mesocosms are a type of facility designed for conducting manipulative experiments using laboratory-based systems (Benton et al. 2007). In our case, however, the mesocosm might cause

changes in the mean or the variance in MPB biomass. This is because the environmental conditions inside the mesocosm (mainly light) could differ from those on the field. If the mesocosm *per se* alters the mean or the variance in MPB biomass, this would affect the components of spatial variation in MPB biomass. In this case, the effect of grazing would be tested on a residual variance that may be very different from that of MPB in natural conditions, and it would be difficult to evaluate the relevance of the results for natural systems. The procedural effect of the mesocosm was thus assessed at each date by comparing U with the tanks in the greenhouse where the MPB was maintained, which experienced light conditions more similar to those naturally present in the field, during the run of the experiment. In particular, the procedural control (PC) was represented by two adjacent plastic plates maintained within the tanks in the greenhouse.

There were 2 experimental replicates (i.e. mesocosms or tanks, hereafter called Mesocom) for each treatment (i.e., G, U and PC). Since only one mesocosm was available, only one experimental replicate was run each day, and the entire experiment lasted a period of 6 consecutive days, at each date. Each day, the experimental replicate was randomly assigned to one of the treatments and a new set of two plastic plates was used. In order to avoid using sediment kept in the greenhouse for a different period of time (and possibly differing in both biotic or abiotic characteristics), the sediment used for each mesocom was collected in different days, so that it was always kept for three days before being used. At the end of each daily run of the experiment (i.e. within each replicate mesocosm), the mean and variance in MPB biomass were estimated over a range of three spatial scales: Cores (3 cm apart), Plots (10 cm apart) and Sites (20 cm apart). We used a corer of 12 mm diameter (area of 1.13 cm^2) and collected samples at the distances specified by the scales in a fully nested design (Underwood, 1997). Thus, moving up in the hierarchy, three sediment cores were collected 3 cm apart in each of three plots chosen approximately 10 cm apart, at each of four sites spaced about 20 cm apart, for a total of thirty-six sediment cores collected within each replicate mesocosm.

The study was repeated in two different periods: April (from the 1st to the 7th) and June (from the 7th to the 14th) 2009. Fish were allowed to acclimate for at least 3 months before the set up of each run of the experiment, at the mean water temperature recorded in Aiguillon Bay during the same period of the year before the study (C. Lefrancois, pers. comm.): 10 and 20 °C in June and April, respectively.

2.4 Collection and analysis of data

The sediment at the top 0.5 cm layer of the cores was freeze-dried and then kept in the dark at -80°C until further processing. The chlorophyll-*a* (chl-*a*) content of the dried sediment was extracted by adding 90% acetone for 18 h in the dark with agitation; chl-*a* was then measured using the fluorometric method (Lorenzen 1967). The chl-*a* content of the sediment was then divided by the weight of the dried top 0.5 cm layer of the sediment core and expressed as concentration (i.e. µg chl-*a* cm^{-2}). Chl-*a* concentration was used as proxy of MPB biomass (Guarini et al. 1998).

The components of variance in MPB biomass at each scale (i.e., Sites, Plots and Cores), for each treatment (i.e., G, U and PC) was then calculated for each period (i.e., April and June), following the method described in Underwood (1997), in order to provide independent measures of the amount of variability of MPB biomass at each spatial scale. Occasionally, negative estimates were obtained. In these cases, variances were set to zero and the other components were recalculated following the "pool-the-minimum-violator" procedure, as recommended by Fletcher and Underwood (2002).

The values of mean and variance were analyzed separately for each period (i.e., April and June), using different models of analyses of variance. Data on the mean MPB biomass were analyzed with a 4-way ANOVA which included the following factors: Treatment (3 levels; fixed), Mesocosm (2 levels; random and nested within Treatment), Site (4 levels; random and nested within Mesocosm (Treatment) and Plot (3 levels; random, nested within Site (Mesocosm (Treatment)), and the 3 Cores as replicates. As the components of variance in MPB biomass at each

scale (i.e., Cores, Plots and Sites) were not independent from each other, they were separately analyzed with a 1-way ANOVA which included the factor Treatment (3 levels; fixed) and the two Mesocosms as replicates.

Cochran's C-test was used to check the assumption of homogeneity of variances (Winer et al. 1991) and, when necessary, data were transformed to remove heterogeneity. Transformation was appropriately indicated in Table 1. When homogeneity of variances could not be achieved by transformation (see "Results"), data were analysed nonetheless, since analysis of variance is robust relative to departures from this assumption when there are many independent estimates of residual variability (Underwood, 1997). However, results were interpreted with caution by judging significance more conservatively ($\alpha = 0.01$). When significant differences were found in the factors of interest, SNK (Student-Newman-Keuls) tests were used for *a-posteriori* comparisons (at $\alpha = 0.05$). All analyses were done using Statistica (StatSoft 6.1, 1994).

As complementary data, the grazing activity of *L. aurata* was estimated by video analysis. The following variables were taken into account: (i) time, (ii) frequency and (iii) velocity of grazing. Grazing time is the total actual time that a fish spent feeding on the sediment surface. This was calculated as the sum of the time lengths of all grazing events for each fish expressed in seconds. The grazing frequency is the number of grazing events per hour for each fish (n. events · hour $^{-1}$). The grazing velocity was estimated each time a fish fed on the sediment surface and was expressed as centimeters per second (cm · sec $^{-1}$). The grazing time and frequency were estimated for each fish in each G mesocosm during the three hours of the video tracking, and analyzed with the open source ODRec (Observational Data Recorder). The tags positioned on the opercula of the fish allowed us to monitor every single fish for the entire duration of the experiment. The grazing velocity of each fish was quantified during two grazing events, which were randomly chosen within each video. The mean value of the two grazing events for each fish were used. The grazing velocity was estimated using WINanalyze 2.1 software. Differences in grazing time, frequency and velocity of *L. aurata* between the two periods were tested using a 2-way nested ANOVA with Period (2

levels: April and June; fixed) and Mesocosms (2 levels; random and nested within Period) as factors and 3 individual juveniles as replicates.

3. Results

The addition of *L. aurata* did not affect the mean MPB biomass both in April and June. The analyses of variance did not reveal any procedural effect due to the mesocosm. (Table 1a; Fig. 2a,b). A significant variability in MPB biomass was detected at the scale of the Site and Plot in April (Table 1a).

In contrast, the addition of *L. aurata* significantly affected the spatial variance in MPB biomass into the mesocosms. This effect, however, was not consistent across the levels of spatial scales and the two periods. In particular, in June the variance at the smaller spatial scale (i.e. Cores) was significantly higher in G than in U and PC, which did not differed from each other (SNK test: $p<0.05$) (Table 1b, Fig. 2d); no significant effects were detected at the higher spatial scales (i.e., Plots and Sites) or in April (Table 1b; Fig. 2f-h).

Finally, the video analyses showed that the velocity of grazing of *L. aurata* was significantly smaller in April than in June (MS=48.705 $F_{1,2}$=48.37, P<0.05), by an average factor of 3 (Fig. 3a). In contrast, no significant differences between Periods were detected on the grazing times (MS=462 $F_{1,2}$=0.01, P>0.05; Fig. 3b) or frequencies (MS=56.25 $F_{1,2}$=0.01, P>0.05; Fig. 3c).

Discussion

Grazing by juveniles of *L. aurata* did not induce any detectable change in the mean MPB biomass, both in April and June, contrary to our expectations. In contrast, grazing increased the spatial variance in MPB biomass at the scale of few centimeters, but only in June. In April, a significant variability in MPB biomass at the scale of Plots and Sites (i.e. at 10s of centimeters) was detected, and a similar trend (although not significant) was present in June; however spatial

variance at the smaller scale of Cores (i.e. a few centimeters) was always larger than those at the scales of Plots and Sites (Fig. 2c-h).

The lack of an effect of grazing by juvenile mullets on mean MPB biomass could be evidence of a weak direct effect of this fish species on MPB. However, alternative scenarios could explain our results. For example, *L. aurata* feeds by consuming the top sediment surface, thus increasing sediment disturbance. This likely adds micro-ripples at the surface, as do many other benthic animals (Fries et al. 1999; Guillén et al. 2008; Peine et al. 2009), thus enhancing MPB biomass at the top of these structures (Blanchard et al. 2000; Lanuru et al. 2007). In addition, droppings released by consumers are known to positively affect MPB (Cooper 1973; Steinman et al. 1992; Kuwae et al. 2008). In a companion study, the amount of microalgae in the droppings of juveniles of *L. aurata* was found to be significantly higher than in the surrounding sediment (unplublished data). Our fish released droppings during the experiments. Thus, the partial algal digestion potentially contributed to enrich the sediment. Finally, *L. aurata* is known to feed also on small benthic consumers such as foraminifers, nematodes and polychaetes (Ferrari and Chieregato1981; Cardona and Castelló 1990; Lafaille et al. 1998; Cardona 2001; Lebreton et al. 2011). Hence, grazing by mullets could have yielded a local increase in mean MPB biomass via trophic cascades (Rzeznik-Orignac et al. 2003), by releasing benthic microalgae from infaunal grazing. It is therefore possible that, although juvenile mullets exerted a strong negative direct effect on mean MPB biomass, this effect was counterbalanced by indirect positive effects related to their grazing activity, resulting in the lack of an overall significant effect (Berlow 1999). Finally, an alternative explanation could be that the intensity of grazing was too low to lead to a statistical change in the mean biomass of MPB assemblages in our study. That is, experimental fish density was not large enough to detect realist effects of grazing by juveniles of *L. aurata*. Further experiments must be carried out to determine the extent to which increases in densities of these fish could affect the mean biomass of MPB. It is worth noting that, in contrast, the concentrations of chl-*a* we found in the sediment, reflected those of the area where the sediments were collected (Guarini

et al. 1998; Degrè et al. 2006). These concentrations also felt within the ranges of values found in other intertidal mudflats (Brotas at al. 1995; Brito et al. 2009).

Interestingly, our results showed that even in the absence of strong effects on the mean biomass, grazing by juveniles of *L. aurata* can inject variability in the spatial distribution of MPB. So far, the role of grazers (mostly benthic invertebrates) on the spatial variability of MPB in intertidal mudflats has been addressed only through correlation analyses (Decho and Fleger 1988; Blanchard 1990; Pinckney and Sandulli 1990). Although these studies established quantitative, objective observations about possible similarities between patterns of spatial variability in MPB abundance and those of grazers, only experimental manipulations of grazers can really test for a direct (or indirect) effect on spatial variance in MPB biomass. To our knowledge, our study represents the first attempt to experimentally test this hypothesis.

Theoretical and empirical studies have shown that trophic interactions that have weak effects on a resource may enhance the patchiness of that resource, if foraging operates with large spatial or temporal variance (Berlow 1999; Benedetti-Cecchi 2000; Sommer 2000; Adler et al. 2001; Flecker and Taylor 2004; Alvarez and Peckarsky 2005). However, the overall spatial variance of the resource is expected to increase if the amount of variance generated by grazing activity is also larger than the background spatial variability in the resource abundance (Benedetti-Cecchi 2000). Sommer (2000) has shown that the heterogeneous grazing of the gastropod *Littorina littorea* (which moves over the biofilm producing macroscopically visible feeding tracks) significantly increased the variability in biofilm abundance, in laboratory experiments. Similar to other species of grey mullets, individuals of *L. aurata* feed by sucking up the top layer of the sediment (Thomson 1966; Odum 1973; Brusle 198; Collins 1981). In doing so, they leave complex mosaics of traces and pits on the sediment surface, which are visible at emersion of the mudflats in the field (Lafaille et al. 2002; Almeida 2003; Richard et al. 2006) and could affect the spatial variability of MPB. However, the ability of juveniles of *L. aurata* to generate such a spatial variability was observed only in June. Video analyses showed that, although the grazing time and frequency were similar in April and

June (indicating a similar feeding behaviour of fish), average velocities during grazing were very different. In fact, it was lower in April than in June, by a factor of three, suggesting that in April the fish were less prone to impinge on MPB assemblages. This result is in accordance with studies based on stomach content of natural populations, which showed that the grazing activity of different species of grey mullets was the highest in Summer and the lowest during the colder period of the year (Almeida et al. 1993; Cardona 1999; Lafaille et al. 2002; Venkatesha et al. 2002). Water temperature is known to be one of the most important environmental factors affecting the energetic requirements of fish and, as a consequence, their feeding activities (Jobling et al. 1977; Persson 1986; Shekk et al. 1990; Clements and Raubenheimer 2006). We hypothesize that, in April, the reduced grazing velocity of *L. aurata* (likely due to lower water temperature) affected the intensity of this activity, which became too feeble to generate variability in the spatial distribution of MPB biomass. Although our results suggest a relationship between the intensity of grazing and spatial variance in benthic microalgae, this study was not tailed to test how changes in intensity of grazing influence the ability of grey mullets to generate variability in the spatial distribution of MPB. Only an appropriate experimental design in which levels of mean intensity and variability of grazing are treated as orthogonal and fixed factors (Benedetti-Cecchi 2003), could test these hypotheses.

As complementary analyses, we tested for differences in the mean and the components of variance in MPB biomass in the control treatments (i.e., U and PC), between April and June. Mean MPB biomass was higher in April (28.30 ± 0.99 µg chl-*a* cm^{-2}) than in June (20.63 ± 0.76 µg chl-*a* cm^{-2}) ($F_{1,6}=8.34$, P<0.05). The larger mean biomass in April, however, did not correspond to larger values in spatial variances. In fact, one-way ANOVAs did not reveal any difference between April and June in the components of variance at the scale of Sites ($F_{1,6}=0.11$, P>0.7), Plots ($F_{1,6}=1.29$, P>0.3) or Cores ($F_{1,6}=4.49$, P>0.05). These results suggests that the lack of significant effects on spatial variability in MPB biomass in April were not due to a larger background variability in biomass at the scale of few centimeters (i.e of Cores), in comparison to that present in June.

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To summarize, this study provides evidences that the grazing of grey mullets can enhance spatial patchiness of MPB in intertidal mudflats at the scale of few centimeters. Spatial variability is an important feature of MPB biomass in these systems (Blanchard 1990; Saburova et al. 1995; Guarini et al. 1998; Seuront and Spilmont 2002; Jesus et al. 2005; Murphy et al. 2008; Brito et al. 2009). By being one of the most abundant group of fish in coastal areas, grey mullets can therefore have important effects on the coastal ecology of microphytobenthos. For example, the spatial patchiness generated by *L. aurata* may increase the photosynthetic efficiency of the MPB, by preventing nutrient depletion or CO_2 limitation in areas characterized by large algal biomass (Jesus et al. 2005). In addition, its grazing activity might affect the species composition in MPB assemblages. For instance, disturbance of the sediment surface (e.g., due to the creation of pits and ripples) might alter patterns of algal colonization, by favoring motile algae against non motile ones (de Jong and Admiral 1984; Barranguet et al. 1998). Whilst we would not attempt to directly extrapolate the results of our mesocosm studies to the natural field, our results suggest that, in natural systems, grazing by grey mullets might generate variability in the spatial distribution of MPB biomass in a manner consistent with that observed under mesocosm conditions. We conclude that a thorough evaluation of the effects of grey mullets on MPB should be taken into account in assessing the spatial and temporal changes in distribution of MPB in intertidal mudflats.

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Table 1. Results of ANOVAs on (a) mean MPB biomass (estimated as chlorophyll-*a* concentration; µg cm^{-2}) and (b) components of variation (CV) in MPB biomass at the scales of Cores, Plots and Sites, in April and June. Data were not transformed, except the CV at the scale of Cores in June (Sqrt (x+1)). Significance was judged at a more conservative level (α = 0.01) in the analysis on mean MPB biomass in June, since transformations failed to remove heterogeneous variances. Significant P values are in bold.

(a) Mean MPB biomass		April			June		
Sources of variation	*df*	MS	*F*	P	MS	*F*	P
Treatments = T	2	874.17	2.35	0.24	983.36	2.12	0.27
Mesocosm (T) = M (T)	3	371.50	2.18	0.13	463.80	2.57	0.09
Sites (M (T))	18	170.23	2.08	**0.02**	180.37	1.94	0.03
Plots (Sites (M (T)))	48	81.83	2.77	**0.00**	92.97	1.58	0.02
Residual	144	29.53			59.00		
Total	215						

(b) Components of spatial variation in MPB biomass	April			June		
	Treatments			Treatments		
	MS	$F_{2,6}$	P	MS	$F_{2,6}$	P
CV$_{Cores}$	178.83	5.74	0.09	32.89	14.97	**0.03**
CV$_{Plots}$	808.19	0.64	0.59	874.59	0.73	0.55
CV$_{Sites}$	171.66	0.63	0.59	111.43	0.42	0.69

Captions:

Figure 1 Schematic illustration of the mesocosm and video-tracking system.

Figure 2 Mean (±1 SE, *n*=72) values of MPB biomass (estimated as chlorophyll-*a* concentration; µg chl-*a* cm^{-2}) and mean (±1 SE, *n*=2) values of components of variation at the scale of Cores, Plots and Sites in each experimental treatment (Grazed, G (*black bars*); Un-grazed, U (*grey bars*); Procedural Control, PC (*white bars*)), in April and June.

Figure 3 Mean (±1 SE, *n*=6) values of (a) grazing velocity (cm sec^{-1}), (b) grazing time (sec), and (c) grazing frequency (n. traces hour^{-1}) of *L. aurata* within Grazed treatments, in April (*grey bars*) and June (*black bars*).
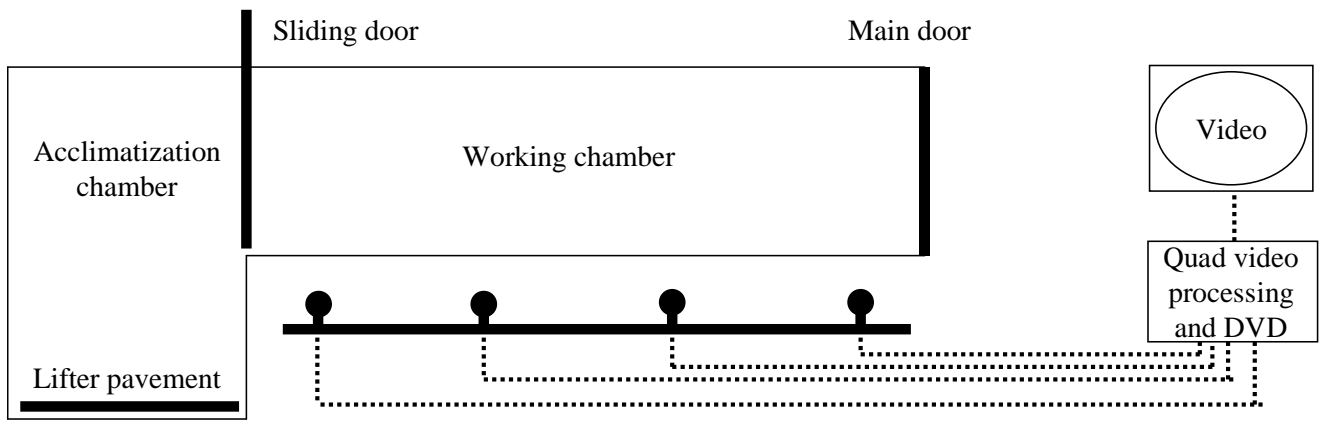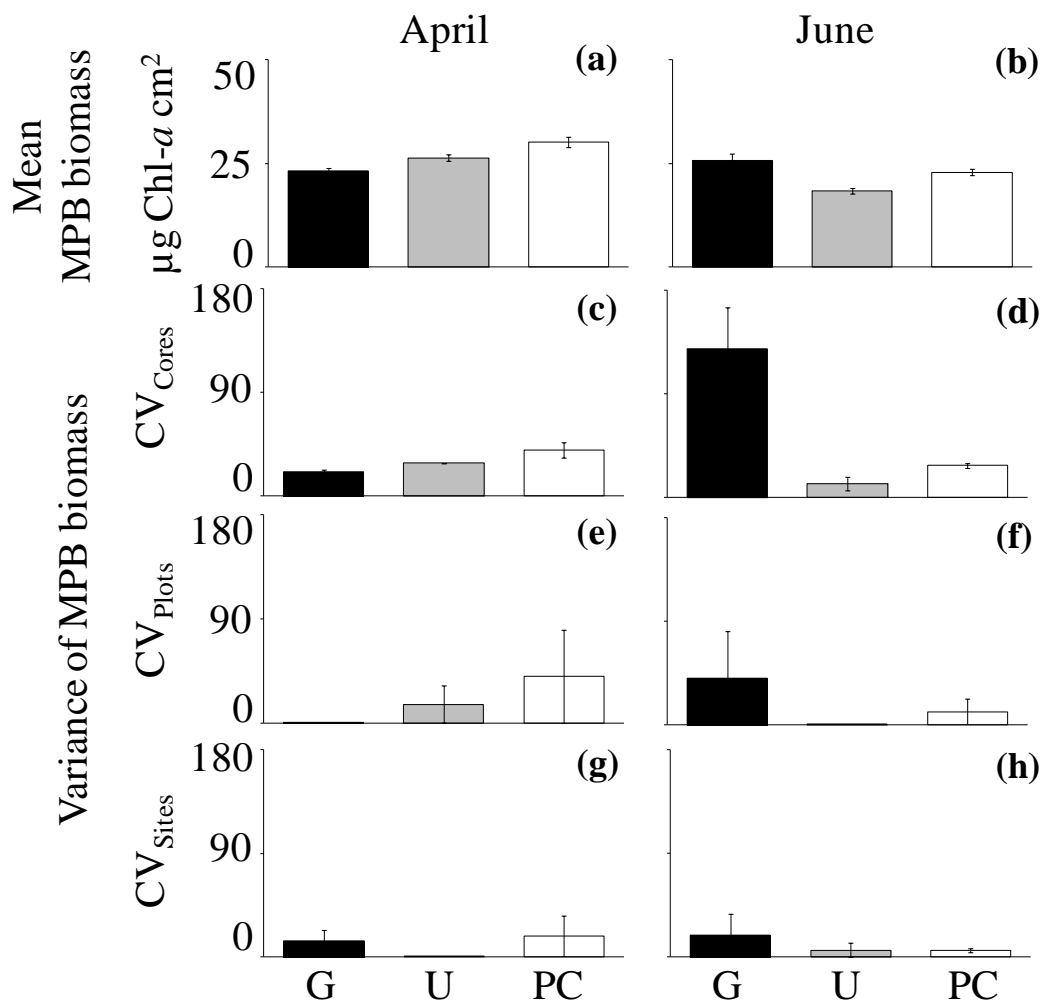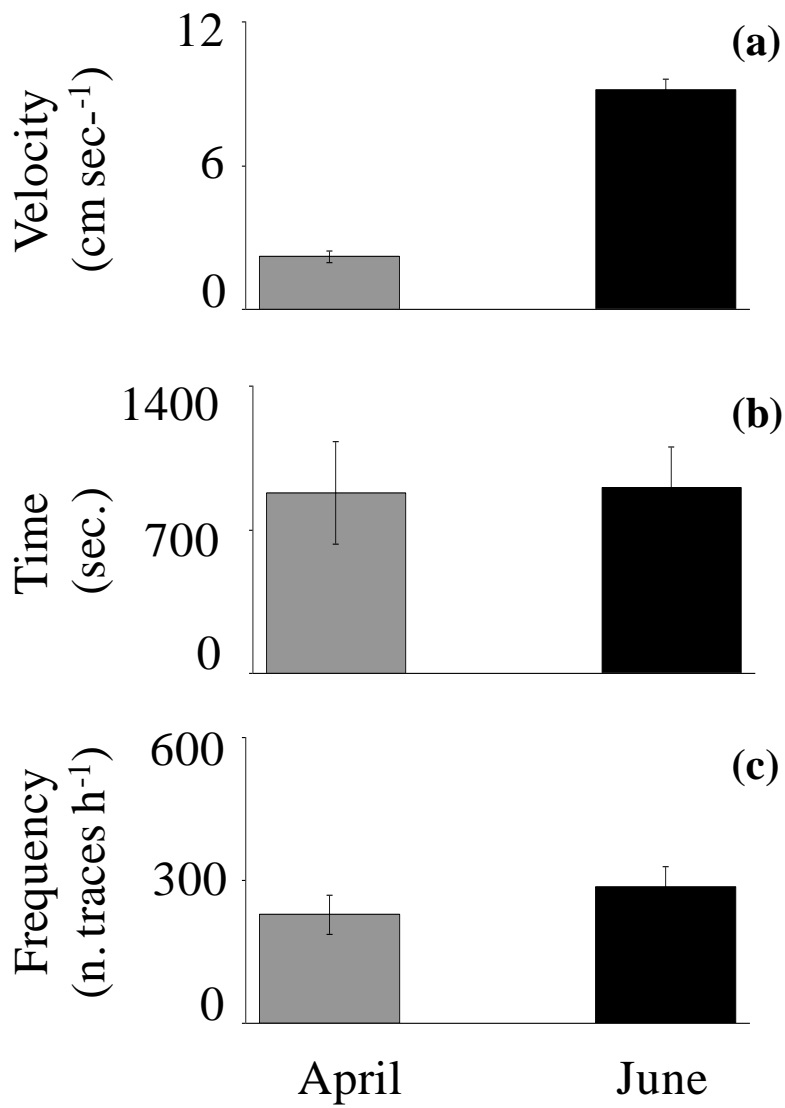
Fig. 1 Como et al.

Fig. 2 Como et al.

Fig. 3 Como et al.