Behavioral responses of juvenile golden grey mullet *Liza aurata* to changes in coastal temperatures and consequences for benthic food resources

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Abstract

Temperature is an important factor for fish. Yet, little is known about its effects on the feeding behavior of fish and the subsequent consequences of these behavioral changes on the spatial distribution of resources. We analyzed the differences in the feeding behavior of two sizes of juvenile *Liza aurata* at two water temperatures (i.e. 10°C and 20°C), using laboratory mesocosms. We also examined whether temperature-induced changes in feeding behavior of the smaller size of *L. aurata* would affect the spatial distribution of the microphytobenthos (MPB) biomass, an important resource in coastal systems.

Both the number of feeding events and the swimming velocity during feeding in juvenile *L. aurata* were higher at 20°C than at 10°C, independently of the fish size. The time spent feeding did not vary between 10°C and 20°C, while the distance covered during feeding was significantly smaller at 20°C than at 10°C. Grazing did not affect the mean MPB biomass, but did increase its spatial variance at the smaller scale (i.e. a few centimeters) at 20°C.

A high number of feeding events, a high swimming velocity during feeding and a small distance covered during feeding in 20°C-acclimated *L. aurata* most likely represented an adaptation to an increase in metabolism, as well as to the need to reduce the energy costs of feeding at 20°C. Results also indicated that changes in feeding behavior of the 20°C-acclimated *L. aurata* were responsible for the increase in small-scale spatial variability in the MPB biomass. We suggested that the enhanced spatial patchiness due to grazing by fish at 20°C might yield a local increase in the mean MPB biomass, probably increasing photosynthetic efficiency of cells and algal growth that counterbalance the negative effect of algal removal by fish, and resulting in the lack of an overall significant effect on the mean.

Keywords: Grey mullets; *Liza aurata*; feeding behavior; temperature; grazing; microphytobenthos; spatial variance.

1 Introduction

Temperature is one of the most important environmental factors for the physiology and metabolism of fish (McKenzie and Claireaux, 2010). Consequently, it can affect all the behavioral traits of fish, including feeding behavior, as well as influence the outcomes of the consumer-resource interactions. If few studies have explicitly investigated the effect of temperature on the feeding behavior of fish (e.g., Nowicki et al., 2012; Theodorou et al., 2012), even less has been done to understand how these behavioral changes influence the spatial or temporal distribution of resources in coastal areas (Polunin and Klumpp, 1992; Smith 2008). Furthermore, most of these studies have been focused on tropical herbivorous fish in coral reef systems. Very little is known about the effects of temperature on the feeding behavior of other fish inhabiting coastal systems. An example is the grey mullet (Mugilidae), one one of the most abundant fish in coastal areas worldwide (Laffaille et al., 2002; Cardona, 2006; Lebreton et al., 2011; Whitfield et al., 2012).

Rises in temperature (within naturally occurring ranges) are usually accompanied by an increase in metabolism and energy demands (Ferreira et al., 1998; Biro et al., 2007,2010) and, consequently, in food consumption and feeding activity (Polunin and Klumpp 1992; Smith 2008). In particular, an increase in the number of feeding events (Polunin and Klumpp, 1989; Ferreira et al., 1998; Floeter et al., 2005; Smith, 2008; Nowicki et al., 2012), the amount of time spent feeding (Biro et al., 2007) and the distances covered during feeding (Biro et al., 2010; Theodorou et al., 2012) have been observed with temperature increases.

In general, it has been shown through experiments that the addition of grazers or predators results in the reduction of the mean biomass (or density) of their resources (Berlow, 1999; Armitage et al., 2009). 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). In coral reef systems, Polunin and Klumpp (1992) showed that the increase in the number of feeding events at increasing temperatures can lead to increases in algal consumption. It is therefore possible that the amount of algae removed and the amount of variance lost in an area both increase as

water temperatures increase, even if the density of grazers and the availability of their resources do not vary in that area (Polunin and Klumpp, 1989; Beukers-Stewart al., 2011).

However, whether or not grazing has negative effects on the mean and the spatial variance of the algal biomass depends on the time-scale of measurement and the interplay between the rates of algal removal and algal growth. Grazing is effective in reducing the mean and the spatial variance of algal biomass only if the rates of algal removal exceed the rates of algal growth. Furthermore, whether or not grazing decreases the overall spatial variance of a resource biomass depends not only on the mean effect of the trophic interaction, the overall biomass of the resource and the mathematical relationship between the mean and the variance, but also on the variance of the trophic interaction as well as the residual variability of the resource (i.e. the component of variation that is not due to grazing) (Benedetti-Cecchi, 2000,2003; Benedetti-Cecchi et al., 2005). The overall spatial variance of the resource is expected to decrease if the loss of variance caused by the reduction in the mean abundance of the resource is larger than the residual variability in the resource abundance, and if the negative effect caused by the reduction in the mean abundance of the resource is larger than the positive effect due to increased spatial variance of grazing.

In this study, we tested the effects of changes in water temperature on the feeding behavior of juvenile golden grey mullet *Liza aurata*, one of the most abundant European grey mullets inhabiting the Northwestern Atlantic coasts and the Mediterranean Sea (Cardona, 2006; Lebreton et al., 2011). The feeding behavior was analyzed at 10°C and 20°C, two temperatures representing low and high seasonal values (which occur naturally during winter and summer, respectively) in the geographic distribution of *L. aurata* (Cardona, 2006; Lebreton et al., 2011). We were also interested in understanding whether temperature-induced changes in the feeding behavior of *L. aurata* would affect the mean and the spatial variance of the microphytobenthos (MPB) biomass, an important benthic food resource for *L. aurata* (Laffaille et al., 2002; Pasquaud et al., 2010; Lebreton et al., 2011). We used a series of laboratory experiments in mesocosm with natural sediment, where we measured the feeding behavior of juvenile *L. aurata* and the mean and the spatial variance of MPB at each temperature (i.e. 10°C and 20°C). As juveniles must meet higher metabolic rates and energy demands at increasing temperatures, we expected

(i) the number of feeding events, (ii) the total time spent feeding and/or (iii) the distance covered during feeding to be higher at increasing temperatures; as temperature can affect swimming performance in fish (Claireaux et al., 2006; Deslauriers and Kieffer, 2012), we also expected (iv) the velocity of swimming during feeding to be higher at higher temperatures. Whether or not the grazing of grey mullets operates with large spatial or temporal variance able to enhance the patchiness of MPB is not known. Grey mullets, however, feed in schools (Whitfield et al., 2012) and their gregarious behavior should increase the likelihood of homogeneous grazing in an area (Adler et al., 2001). At each experimental temperature, it is possible that the amount of variance in the MPB biomass caused by a heterogeneous grazing would be lower than the amount of MPB variance lost due to the reduction in the mean MPB biomass. Therefore, we expected grazing to have a negative effect (v) on the mean of the MPB biomass and (vi) on the amount of spatial variability of MPB but we also expected that (vii) the amount of microalgae removed and the amount of variance lost due to grazing would be higher at 20°C than at 10°C. Because feeding activity in juveniles often varies with fish size (Choat and Clements, 1993; Buckle and Booth, 2009), tests on the feeding behavior were repeated with two sizes of juveniles (i.e. J0 and J1). However, only J0 was taken into account to test for the grazing effects on MPB, as this size dominates the natural populations in shallow coastal areas (Hotos and Katselis, 2011; Lebreton et al., 2011).

2 Material and Methods

2.1 Experimental fish

Thirty-six J0 juveniles and 36 J1 juveniles (mean total length (TL) ± SE: 9.71± 0.23 cm and 16.10 ± 0.12 cm, respectively) were used in this experiment. The fish were collected along the Sardinian coast of Italy and then transported to the Marine Station of the University of La Rochelle (France), where the experiment was carried out. Fish were maintained for one month in 1000 liter holding tanks (1 × 1 × 1 m) filled with re-circulated filtered sea water. Temperatures and salinity ranged between 14 and 19°C (mean ± S.E: 17 °C ± 0.2, n=30) and 29 and 35 psu (mean ± S.E: 32 psu ± 0.3, n=30), respectively. After the mullets were transferred into experimental tanks, water temperatures were

adjusted by 0.5 °C per day, until experimental temperatures were reached (10°C and 20°C). The fish were then allowed to acclimate to the experimental temperature for at least three months. During this period fish were under natural photoperiod of the location (46°10 N) and fed with natural periphyton daily and with dry pellets (BioMar®) twice a week as an integration of proteins and vitamins. The use of natural periphyton allowed us to maintain the fish in laboratory conditions without compromising their natural foraging behavior (Richard et al., 2010). Natural periphyton was developed on artificial substrates in an adjacent pond, following the methods described in Richard et al. (2010).

2.2 Protocol

Grey mullets are a gregarious fish and exhibit schooling behavior (Whitfield et al., 2012). Therefore, we used 3 fish for each run of the experiment. Each fish was used only once. Before being transferred into the mesocosm (4 × 1 × 1 m; Fig. 1), fish were marked on the opercula with plastic tags of different colors using Krazy glue®, following the procedure described in Lefrancois et al. (2005). For marking, fish were lightly sedated with MS222 (0.08 g l^{-1}). 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.). Each fish group was placed in the acclimatization chamber of the mesocosm under natural photoperiod and was left undisturbed for 12 hours before the experiment in order to allow them to recover from any stress caused by this handling. Fish were not fed during this period.

An hour and a half before the beginning of the experiment, the water level of the working chamber of the mesocosm was raised by about 50 cm and the fish were encouraged to move from the acclimatization to the working chamber (where the sediment was positioned; see below). This was done by opening the sliding door and lifting up the floor of acclimatization chamber (Fig. 1). In general, the fish entered rapidly (less than 3 min for transfer). The experiment began when the fish started feeding on the sediment placed on the floor and lasted for 3 hours. The cameras were turned on as the fish entered the working chamber and recorded their foraging for the entire duration of the experiment. At the end, the fish were encouraged to move back to the acclimatization chamber and the water level was progressively reduced in the working chamber All the experimental runs were performed in the same

period of the day (in the morning) in order to avoid a bias in the results done by daily variations in the fish feeding activity (Almeida, 2003). All precautions were taken to limit fish stress during the experiments. Each fish manipulation was done with careful handling. None of the fish showed abnormal behaviour or died during the experiment.

The sediment used in this study was collected on an intertidal mudflat in Aiguillon Bay (France; 46°10'N, 1°15'W) close to the Marine Station, by scraping off the upper 2 mm. The sediment was sieved through 5 mm screens to eliminate plant debris and then kept on plastic plates (0.60 × 0.40 × 0.05 m), laid on the bottom of a 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. The water in the tanks was renewed daily to avoid excessive increases in water temperature and salinity due to water evaporation. Sediment was kept in the greenhouse for a period of three days before being used in the mesocosm. This was a sufficient period of time to recover from disturbance for both microalgal biomass and community structure (Peterson and Stevenson, 1992). For each run of the experiment, a different series of sediment plates was used. The mesocosm was set up in a room with natural sources of light in order to allow for photosynthesis to be carried out by microalgae during each run of the experiment.

2.3 Experimental designs

The experiment on the effect of changes in temperature on the feeding behavior of juvenile *L. aurata* consisted in the manipulation of two fixed, crossed factors: Temperature (T; T_{10} and T_{20}) and Size (S; J0 and J1). For each combination, there were 6 replicates (i.e. replicate runs) with 3 juvenile fish. In order to compare the feeding behavior of fish of different sizes, the same ratio between fish biomass and foraging area (i.e. the area of the working chamber) was used for both fish sizes. A constant biomass of fish per unit area was thus used, ≈ 55 g m^{-2}. To attain this biomass, the surface area of the working chamber was set to 0.48 m² (i.e. 1.20 × 0.40 m) and to 1.44 m^2 (1.80 × 0.80 m) for J0

and J1, respectively. It corresponded to two and six adjacent plastic plates respectively (with the reconstructed sediment), which perfectly fitted on the bottom of the working chamber.

The influence of temperature on the effects of grazing on MPB biomass was assessed through the manipulation of two fixed, crossed factors: Temperature (T; T_{10} and T_{20}) and Grazing. At each temperature, two out of the six replicate runs belonging to the J0 level of the factor Size were used in this experiment. Grazing effects were assessed, at both 10°C and 20°C, by comparing these two replicate runs of the grazed mesocosm (hereafter called grazed treatment, G) with two replicate runs of the mesocosm without *L. aurata* (hereafter called Un-grazed treatment, U). For U, the same experimental procedure was followed as for G except that fish were not introduced into the mesocosm (i.e. the surface area of the working chamber was set to 0.48 m² and two plastic plates with sediment were positioned on the bottom). U enabled us to estimate both the mean biomass of MPB in absence of grazers and the spatial components of variance in the distribution of MPB biomass that was not due to the grazing by *L. aurata* (i.e. residual variability). The fish density used in this experiment, which corresponded to 6.25 individual m^{-2}, was ecologically realistic, as it is within the natural range of densities of grey mullets observed in marine coastal and transitional systems (Pais et al., 2007; Mwandya et al., 2010; Nicolas et al., 2010).

Since only one mesocosm was available, only one replicate of the experimental treatments was run each day and the study lasted 28 days (between the end of March and the end of June 2009). Each day, the replicate should have been randomly assigned to one of the six experimental combinations ($T_{10}J0=T_{10}G$, $T_{20}J0=T_{20}G$, $T_{10}J1$, $T_{20}J1$, $T_{10}U$, $T_{20}U$). However, changing the grazing area, the arrangement of cameras or the water temperature in the water reserve tank of the mesocosm every day was practically impossible. Therefore, the replicates for each combination were grouped together and done over a period of 6-8 consecutive days. Such a temporal stratification could have introduced a bias in the results, if the MPB availability had varied naturally in the sediment used during the study period. Changes in availability or nutritional values of food are known to affect the feeding behavior of fish (Polunin and Klumpp, 1989; Beukers-Stewart al., 2011). To check for this potential bias, 6 sediment samples were collected from the experimental replicates of each combination of T × S before each run,

in order to estimate the MPB biomass (as a concentration of Chlorophyll-*a*; µg chl-*a* cm^{-2}; see paragraph below for the description of the methodology). Data were analyzed through a one-way ANOVA with Time as a fixed factor (4 levels, which corresponded to the combinations of T × S). The analysis did not reveal any difference among the four times (see Appendix A).

Temporal segregation could have also introduced a bias in the results of the experiment done to test for the effects of Grazing and T on MPB biomass. To check for this potential bias, sediment samples were collected in two additional tanks located in the greenhouse where the MPB was maintained during both periods when the replicates of G and U for the two levels of T (i.e. T_{10} and T_{20}) were run. Sediment samples were collected following the same sampling design used in G and U (see paragraph below for the description of the sampling collection) and the mean and the components of variance were estimated accordingly. Data were then analyzed through a one-way ANOVA with Time as a fixed factor (2 levels, which corresponded to the two times when T_{10} and T_{20} replicates were run, respectively). Both the mean and the components of variance of MPB biomass did not vary between times (see Appendix B). These results indicated that the availability of MPB biomass and its spatial distribution did not vary in the sediment used during the study period; we can therefore reasonably exclude any bias due to the temporal segregation of the replicate runs of both experiments.

2.4 Data collection and analysis

2.4.1 Feeding behavior of *L. aurata*

We assessed the grazing behavior throughout the total time spent feeding, the number of feeding events, swimming velocity during feeding and the distance covered during feeding. The total time spent feeding (in min) was calculated as the sum of the time lengths of all grazing events for each fish whereas the number of feeding events was estimated as the number of times a fish fed on the sediment. These variables were estimated for each individual and analyzed with the open source ODRec (Observational Data Recorder). The tags positioned on the opercula of the fish allowed us to monitor each single individual for the entire duration of the experiment. These variables were estimated during the 3 hours of the video tracking, minus the first 20 minutes. A pre-analysis of the videos showed that during this initial phase fish exhibited an abnormal feeding activity. This represents a compensatory

response to the 12-hours of food deprivation they underwent before the experiment (Ali et al., 2003). For these two variables, the mean values of the test group were then calculated. Differently, the distance covered during feeding was calculated as the distance (in cm) covered on the sediment surface at each grazing event. This was assessed during 20 grazing events of one individual from each group test (i.e. from each run of the experiment) and analyzed with WINanalyze software. Since the position of the video cameras provided a lateral view along the longest side of the working chamber, the position of the fish could only be followed on a X-axis. Therefore, in order to increase the accuracy of the assessment of the distance travelled by the fish, the 20 grazing events were randomly selected from those that occurred parallel to the longest axis of the working chamber. The swimming velocity during feeding was then calculated by summing the horizontal vectors of distance travelled by the fish (cm), divided by the total duration of the 20 grazing events considered and is expressed as centimeters per second ($cm \cdot sec^{-1}$).

As additional data, droppings left on the sediment surface were collected at the end of each run of the experiment (i.e. for each combination of Temperature and Size factors) for determination of the content of Chlorophyll-*a* (i.e. µg chl-*a* g^{-1}) in fish droppings.

2.4.2 Mean and variance of MPB biomass

At the end of the two replicate runs of both G, and U treatments, at both temperatures, the top 5 millimeters of the sediment layer were collected using a hand corer (12 mm inner diameter) for the determination of MPB biomass. 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. A total of thirty-six sediment cores were therefore collected at the end of each replicate run. Samples were immediately frozen, freeze-dried and then kept in the dark at -80°C until further processing. The chlorophyll-*a* (chl-*a*) concentration (i.e. µg chl-*a* cm^{-2}) in the sediment was analysed following the method described in Guarini et al. (1998) and used as proxy of MPB biomass.

2.4.3 Statistical analyses

To test for the effects of temperature and fish size on the feeding behavior of *L. aurata*, a two-way analysis of variance (ANOVA, Underwood, 1997) was used, with Temperature (T; T_{10} and T_{20})

and Size (S; J0 and J1) as crossed, fixed factors, and 6 replicates (i.e. replicate runs). The same model of ANOVA was used to analyze mean values of chl-*a* content (µg chl-*a* g^{-1}) in the droppings found within each mesocom.

The mean biomass of MPB was estimated for each replicate run (n=36 cores) and data were analyzed using a two-way ANOVA with Temperature (T; T_{10} and T_{20}) and Grazing (G and U) as fixed crossed factors and two replicates (i.e. replicate runs). The components of 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), according to a fully nested sampling design. They were calculated 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). 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 analyzed separately, using the same model of ANOVA as for mean biomass of MPB. Homogeneity of variances was checked using Cochran's C-test (Winer et al, 1991). All analyses were done using Statistica (StatSoft 6.1, 1994).

3 Results

3.1 Effect of Temperature and Size on feeding behavior of *L. aurata*

The analysis of variance showed a significant effect of Size on the total time spent feeding, independently of the level of Temperature (Table 1). The total time spent feeding by J0 individuals was higher than that spent by J1 ones during the three hours of the experiment (i.e. about 14 and 5 minutes, respectively) (*a posteriori* multiple comparison, Student–Newman–Keuls, SNK, p = 0.05; test, Fig. 2).

Results also highlighted significant main effects of both Temperature and Size on the number of feeding events, the swimming velocity during feeding and the cumulative distance covered during feeding (Table 1). SNK tests revealed that the number of feeding events and swimming velocity attained during feeding were consistently higher in 20°C-acclimated *L. aurata* than in 10°C-acclimated ones. In

contrast, the cumulative distance covered during feeding events was consistently lower in 20°C-acclimated *L. aurata* than in 10°C-acclimated *L. aurata*, by an average factor of three (i.e. 287 and 102 cm, respectively) (Fig. 2). In addition, the swimming velocity and distance covered during feeding was higher in J1 than in J0 individuals, while the opposite trend was observed in the number of feeding events (Fig. 2).

Finally, a significant main effect of Temperature ($F_{1,12}$ = 40.47, p<0.00) on the mean chl-*a* content in the droppings was found. In particular, values were higher at 10°C than at 20°C (mean chl-a content [mg chl-*a* g sed^{-1}] ± SE: 10°C = 1815.05 ± 158.84; 20°C = 524.85 ± 124.16).

3.2 Effect of Grazing and Temperature on mean and spatial variance of MPB biomass

The analysis of variance did not detect any effect of Grazing, Temperature or Grazing × Temperature interaction on the mean values of MPB biomass or on the spatial variance of MPB biomass at the largest spatial scales (i.e. Sites and Plots) (Table 2; Fig. 3a,c-d). On the other hand, a significant Grazing × Temperature effect was observed on the spatial variance of MPB biomass at the smallest spatial scale (i.e. Cores) (Table 2; Fig. 3b). SNK tests showed that the spatial variance of MPB biomass on the scale of cores was three-times higher in the presence of J0 *L. aurata* (G) than in controls (U) at 20°C (G>U), while no differences between G and U were found at 10°C. In addition, in the presence of grazing (G), variance was higher at 20°C than at 10°C, while no differences were found in U between the two temperatures.

4 Discussion

Results showed that both the number of feeding events and the swimming velocity during feeding were higher in 20°C-acclimated *L. aurata* than in 10°C-acclimated ones, independent of their size. In addition, results showed the swimming velocity and the distance covered during feeding to be higher in J1 than J0 individuals, while the opposite was observed for the total time spent feeding and the number of feeding events.

Contrary to our expectations, grazing did not have any negative effect on the mean biomass of MPB at either acclimation temperature. However, it increased the spatial variance in the MPB biomass

at the smaller spatial scale (i.e. a few centimeters) at 20°C, indicating a relationship with the observed changes in feeding behavior at this temperature.

4.1 Effect of Temperature and Size on feeding behavior of *L. aurata*

Rising temperatures increase metabolism, thus influencing feeding activity of fish (Biro et al., 2007,2010). The higher number of feeding events in juvenile *L. aurata* at 20°C than at 10°C was in accordance with studies on other species (both in the laboratory and in the field), showing a positive relationship between the number of feeding events and water temperature (Polunin and Klumpp, 1989; Ferreira et al., 1998; Floeter et al., 2005; Smith, 2008; Theodorou et al., 2012). It is worth noting that an interpretation of this result in the laboratory, however, needs to be made with caution. The number of feeding events that an individual displays in a mesocosm may, in fact, be influenced by behavioral constraints due to the experimental set up (i.e. the tank size). In particular, if in nature an individual prefers to feed by doing one single trace instead of many distinct ones, this behavior could be modified within a mesocom, if the swimming velocity increases (as observed in our study at 20°C), and if it is due to the increased distance covered during feeding. In this case, in fact, fish should be forced to interrupt their feeding within the mecosom, resulting in an increase in the number of feeding events. However, the cumulative distance covered during feeding was found to be three times lower at 20°C than at 10°C in our study. Therefore, the increase in swimming velocity at 20°C was not due to the increased distance covered at each feeding event, but to an even larger decrease in the time spent feeding per event at that temperature. This suggests that the higher number of feeding events in 20°C-acclimated *L. aurata* than in 10°C-acclimated ones represents a voluntary modification of the individual behavior of *L. aurata* at 20°C, as a consequence of the need to maximize food consumption at this temperature. The observed reduction in the distance covered during feeding at 20°C is in contrast with other studies on different species (Biro et al., 2010; Theodorou et al., 2012). In particular, Theodorou et al. (2012) showed a concomitant increase in the number of feeding events and in the distance covered during feeding as temperature increased in the Atlantic cod *Gadus morhua*. Reducing distance and time spent feeding while increasing the number of feeding events in *L. aurata* may represent a foraging strategy, which contributes to decreasing the energy costs involved in feeding activity as metabolism

increases (Rice and Hale, 2009). The suction feeding strategy typical of grey mullets involves the integrated mechanical act of swimming, jaw movement and related muscle activity and it is energetically costly (Rice and Hale, 2009). At 20°C, feeding by making long feeding traces might not pay off in terms of energy expenditures with respect to energy gains. In fact, ingesting a large amount of sediment at one time might not provide any energetic advantages due to the rapid gastric evacuation and intestinal translocation which reduce the time of interaction between ingested food and enzymatic activities involved in the digestion and assimilation processes. Indeed, the gut transit time in fish decreases with increasing temperatures (Halver and Hardy, 2002; Evans and Clairborne, 2006), so that long feeding traces can even represent a disadvantage. It is worth noting, however, that the enzymatic activities involved in the extraction of nutrients from food (i.e. digestion) and nutrients absorption through the intestinal walls (i.e. assimilation) are known to increase with temperature (Halver and Hardy, 2002; Evans and Clairborne, 2006), so that this higher efficiency in digestion and assimilation can counterbalance the short gut transit time. The efficiency of such a process is highlighted by the lower amount of MPB found in droppings at 20°C than in those at 10°C, suggesting a more complete algal digestion in 20°C-acclimated *L. aurata*. Grey mullets are generally active swimming foragers. Maintaining the capacity for locomotion is more important in active fishes than in sit-and-wait foragers because the former are more likely to encounter predators and need to be able to avoid capture, and they continue to hunt or graze while still digesting (Fu et al., 2009). Therefore, grey mullets should be able to modulate their feeding behavior in order to save energy for other activities such as locomotion.

In accordance with other fish species (Choat and Clements, 1993; Videler, 1993; Buckle and Booth, 2009), size influenced the feeding behavior of juvenile *L. aurata* in this study. In general, the swimming velocity was observed to increase (Videler, 1993) and the number of feeding events to decrease with size in herbivorous fish (Choat and Clements, 1993; Buckle and Booth, 2009). This latter result has been related to the fact that the amount of food ingested during each feeding event increases with size so that a fish fulfils its energetic requirement after a lower number of feeding events at increasing size (Buckle and Booth, 2009).

4.2 Effect of grazing and temperature on mean and spatial variance of MPB biomass

Our results provide the first evidence that the temperature-induced changes in the feeding behavior of *L. aurata* can influence the spatial distribution of their main food resource, MPB. However, the mechanisms linking the behavioral changes of *L. aurata* to the spatial distribution of the MPB biomass are more complex than previously thought (Polunin and Klumpp, 1992; Smith, 2008). We had predicted that the direct negative effects of grazing on the mean and spatial variance of the MPB biomass would be higher at 20°C than at 10°C because of an increase both in the amount of algae removed and in the amount of variance lost due to grazing by fish at increasing water temperatures, as was found to be the case for herbivorous tropical fish (Polunin and Klumpp, 1992). Our results showed that grazing at 20°C did not induce any change in the mean MPB biomass but it significantly increased the variability in the spatial distribution of the MPB biomass at the smallest spatial scale. This latter result was in accordance with studies showing that grazing can increase the spatial patchiness of resources (Hillebrand, 2008). In our study, it is likely that the enhanced spatial patchiness of MPB due to grazing at 20°C counterbalanced the direct negative effect of algal removal by fish through an enhanced growth rate of algae. Spatial variability at a scale of a few cm is known to favor primary productivity of microalgae (Cardinale et al., 2002) by affecting the flow and uptake of nutrients (Riber and Wetzel, 1987), as well as the ability of small benthic grazers to recognize and select food patches (Kawata and Agawa, 1999). For instance, the small-scale spatial variability of MPB biomass generated by grazing at 20°C may have increased the photosynthetic efficiency of cells and algal growth by preventing nutrient depletion or CO_2 limitation at the base of the biofilm (Riber and Wetzel, 1987). In our mesocosms, it is likely that all these effects yielded a local increase in the mean MPB biomass that counterbalanced the direct negative effects of grazing, resulting in the lack of an overall significant effect (Berlow, 1999).

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; Hillebrand 2008) and if the amount of variance generated by grazing activity is larger than the residual variability in the resource abundance (Benedetti-Cecchi, 2000). Most of these studies

have focused on the grazing pattern of benthic invertebrates while little is known about the ability of epibenthic fish to cause variability in the spatial distribution of the resources (Hillebrand, 2008). Flecker and Taylor (2004) showed that the heterogeneous grazing of the epibenthic fish *Parodon apolinari* significantly increased the spatial variability in biofilm abundance in stream. As with *P. apolinari*, our results provided indications of a heterogeneous grazing of *L. aurata* at 20°C, able to enhance the patchiness of MPB within the mesocosm.

Although previous studies have investigated the effects of grazing on the spatial patchiness of resources (Hillebrand 2008), little attention has been given to the effects of behavioral plasticity of grazers. The effects of temperature-induced behavioral changes of grazing fish on the spatial patchiness of their resources, like those observed in our study, should be taken into account in future ecological studies in natural systems. Indeed, grazing fish such as grey mullets are one of the most abundant fish in coastal areas (Laffaille et al., 2002; Cardona, 2006; Lebreton et al., 2011; Whitfield et al., 2012). Maintaining high level of spatial patchiness of MPB by affecting the behavior of grazing fish may be a way through which high seasonal temperatures favor primary productivity in areas characterized by high biomass or during spring blooms, when nutrient depletion or CO_2 limitation inhibits the photosynthetic efficiency of cells and algal growth (Guarini et al., 1998; Jesus et al., 2005).

5 Conclusion

In summary, this study provides the first evidence of clear-cut changes in the feeding behavior of juvenile *L. aurata* at high seasonal temperatures. These changes most likely reflect not only the need to maximize food consumption due to the temperature-induced increases in metabolism, but also the ability of *L. aurata* to modulate individual feeding behavior, in order to save energy for other activities, like locomotion, which are energetically costly.

Our results also provide the first evidence that the temperature-induced changes in the feeding behavior of *L. aurata* influence the spatial distribution of MPB, their main food resource. However, the mechanisms linking the behavioral changes of *L. aurata* to the response of the MPB biomass to grazing are more complex than those expected from a mere increase of algal consumption at higher water

temperatures. We suggest that the changes in the feeding behavior of *L. aurata* at high seasonal temperatures enhance the spatial patchiness of the MPB biomass at the smallest spatial scale, thus increasing photosynthetic efficiency of cells and algal growth and favoring primary productivity.

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Tables

Table 1 Results of ANOVAs to test for the effect of Temperature and Size on the total time spent feeding (min), the number of feeding events (n. traces), the swimming velocity during feeding (cm sec^{-1}) and the distance covered during feeding (cm). Significant P values (<0.05) are indicated with an asterisk.

		Time (min)			Events (n. traces)		
Source	DF	MS	F	P	MS	F	P
Temperature = T	1	49.65	4.03	0.06	514.54	21.94	0.00*
Size = S	1	539.41	43.76	0.00*	819.22	34.93	0.00*
T × S	1	14.08	1.14	0.30	3.18	0.14	0.72
residual [A]	20	12.33			23.45		
Total	23						
Cochran's C		0.54 (P>0.05)			0.47(P>0.05)		
Transformation		None			None		

		Velocity (cm sec^{-1})			Distance (cm)		
Source	DF	MS	F	P	MS	F	P
Temperature = T	1	325.98	102.85	0.00*	205873.88	103.94	0.00*
Size = S	1	84.71	26.73	0.00*	15898.57	8.03	0.01*
T × S	1	3.83	1.21	0.28	2039.28	1.03	0.32
residual [A]	20	3.17			1980.66		
Total	23						
Cochran's C		0.49 (P>0.05)			0.58(P>0.05)		
Transformation		Sqrt(X+1)			none		

[A] denominator for T, S and T × S

Table 2 Results of ANOVAs to test for the effect of Grazing and Temperature on mean MPB biomass (estimated as chlorophyll-*a* concentration; µg cm^{-2}) and components of variance (CV) in MPB biomass at the scales of Cores, Plots and Sites. Significant P values (<0.05) are indicated with an asterisk.

		Mean MPB biomass			CV_{cores}			CV_{plots}			CV_{sites}		
Source	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Grazing	1	8.64	0.69	0.45	22.64	15.69	0.02	306.03	0.32	0.60	347.56	1.44	0.30
Temperature = T	1	14.90	1.20	0.34	12.24	8.48	0.04	290.16	0.30	0.61	62.11	0.26	0.64
Grazing × T	1	55.39	4.45	0.10	33.81	23.43	0.01*	1617.10	1.68	0.26	0.22	0.00	0.98
residual [A]	4	12.46			1.44			962.02			241.29		
Total	7												
Cochran's C		0.62 (P>0.05)			0.89 (P>0.05)			0.87 (P>0.05)			0.74 (P>0.05)		
Transformation		none			Sqrt(X+1)			none			none		

[A] denominator for Grazing, T and Grazing × T

Captions:

Figure 1 Schematic illustration of the mesocosm (viewed from the side) and the video-tracking system, which consisted of four polychrome video cameras (25 image sec^{-1}), a multichannel video interface and a DVD recorder. The cameras were placed along the longest side of the working chamber in order to provide a lateral view. Not shown: a series of lamps connected to a timer which provided a supplementary light source in addition to the natural illumination, and a buffer tank connected to a thermostat which controlled temperature, circulation and the water level inside the mesocosm.

Figure 2 Mean values (n=6 ± SE) of the total time spent feeding (min), the number of feeding events (n. traces), the swimming velocity during feeding (cm sec^{-1}) and the distance covered during feeding (cm) for each acclimation temperature (i.e. 10°C and 20°C) and fish size (i.e. J0 and J1). Asterisk (*) indicates where significant differences ($P<0.05$) between the two acclimation temperatures and the two fish sizes occur.

Figure 3 Mean values (±1 SE, n=2) of MPB biomass (estimated as chlorophyll-a concentration; µg chl-*a* cm^{-2}) and components of variation (CV) at the scales of Cores, Plots and Sites in each grazing treatment (i.e. G and U) at each temperature (i.e. 10°C and 20°C). G, Grazed treatment (black bars); U, Un-grazed treatment (grey bars). Experiment done with J0 individuals, only. Asterisk (*) indicates where significant differences ($P<0.05$) occur.

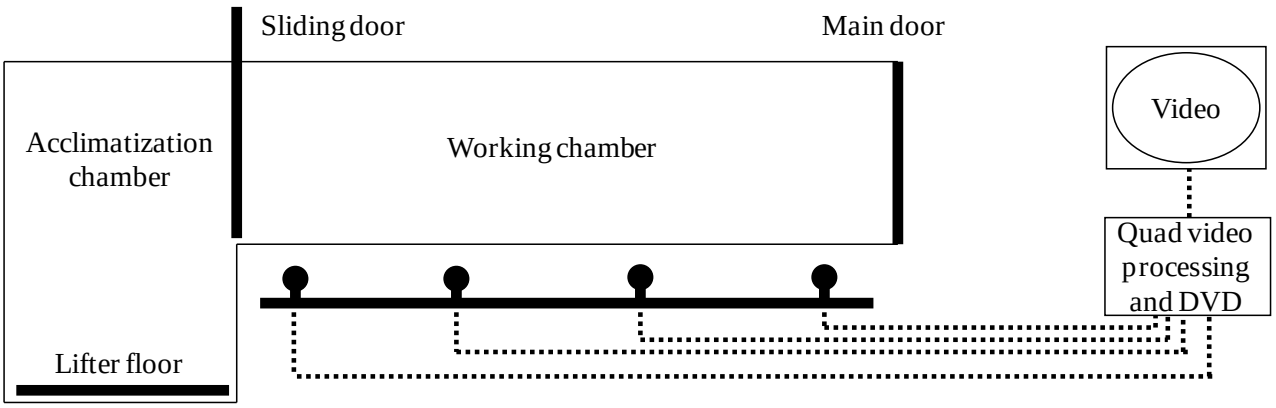

Fig. 1 Como et al.

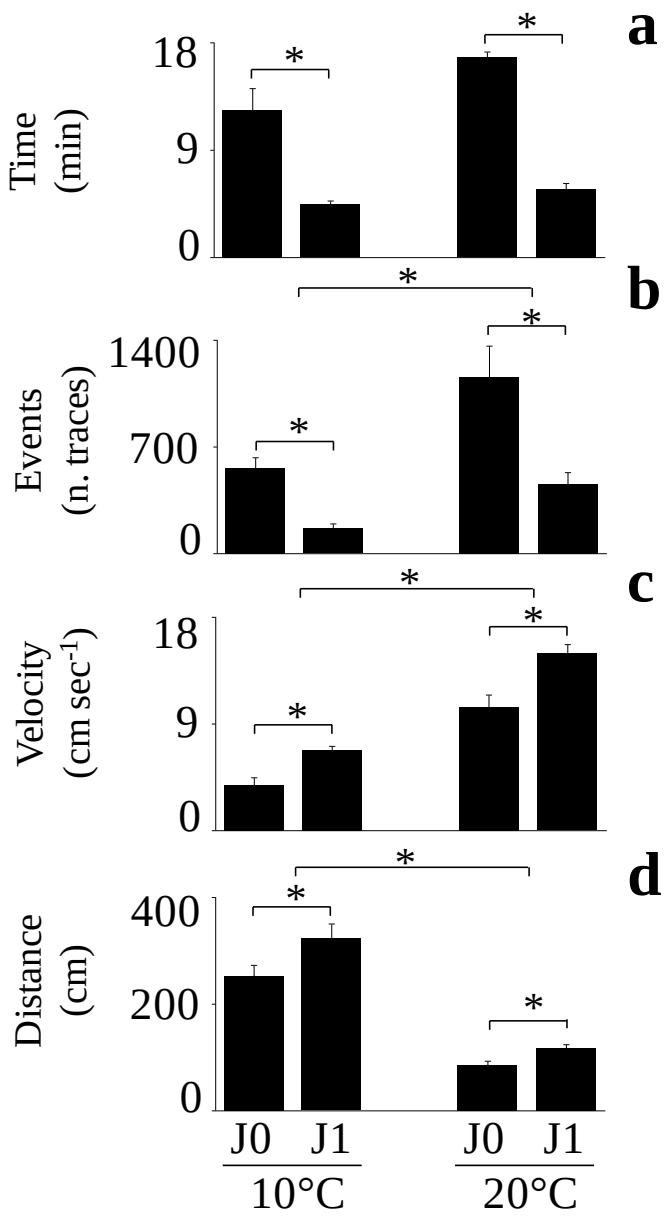

Fig. 2 Como et al.

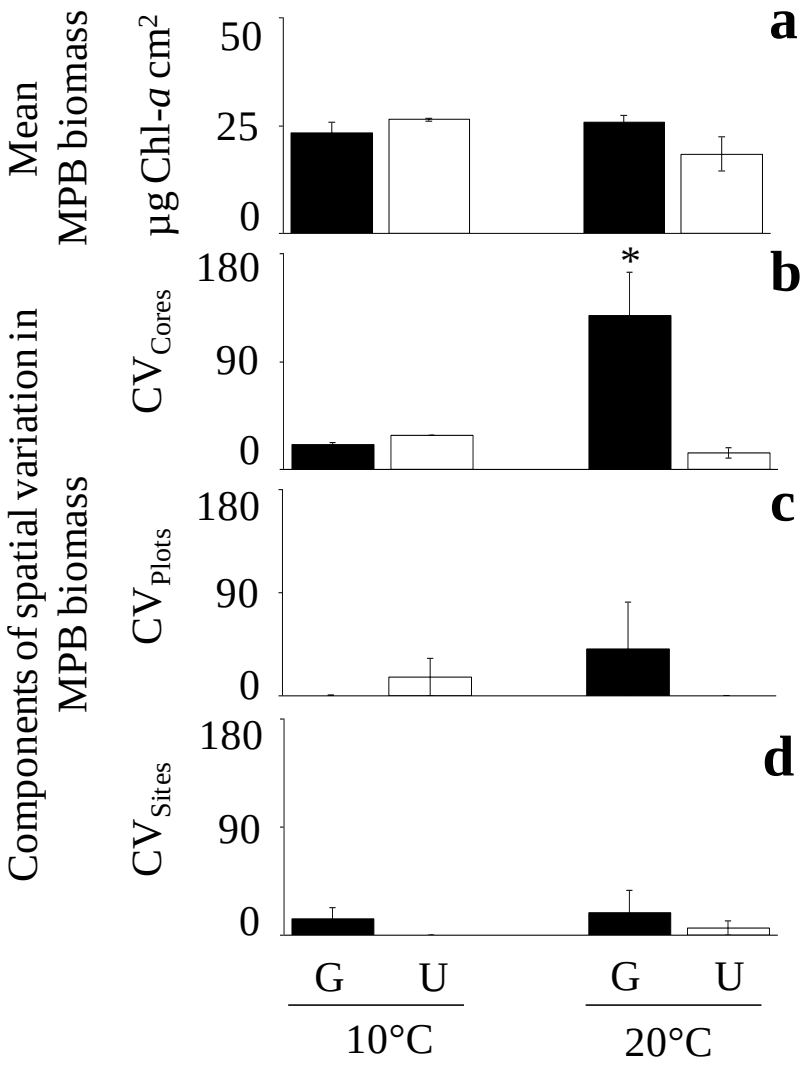

Fig. 3 Como et al.

Appendices

Appendix A Results of the 1-way ANOVA on the effect of Time (4 levels, which corresponded to the combinations of the two levels of Temperature = T and Size = S) on mean MPB biomass (estimated as chlorophyll-*a* concentration; µg cm^{-2}). The number of experimental replicates for each time (i.e. each combination of T × S) was six. Samples were collected before each run.

		Mean MPB biomass		
Source	DF	MS	F	P
Time	3	25.66	0.10	0.96
Residual [A]	20	245.37		
Total	23			
Cochran's C		0.48 (P>0.05)		
Transformation		none		

[A] denominator for Time

Appendix B Results of the 1-way ANOVA on the effect of Time (2 levels, which corresponded to the two periods when the replicates of G and U for the two temperatures, T_{10} and T_{20}, were run) on mean MPB biomass (estimated as chlorophyll-*a* concentration; µg cm^{-2}) and on components of variation (CV) in MPB biomass at the scales of Cores, Plots and Sites. Samples were collected from two additional tanks located in the greenhouse where the MPB was maintained.

		Mean MPB biomass			CV_{cores}			CV_{plots}			CV_{sites}		
Source	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Time	1	54.08	5.47	0.14	142.92	2.95	0.23	842.54	0.48	0.56	143.64	0.45	0.57
Residual [A]	2	9.89			48.41			1748.64			321.67		
Total	3												
Cochran's C		0.89 (P>0.05)			0.92 (P>0.05)			0.93 (P>0.05)			0.99 (P>0.05)		
Transformation		none			none			none			none		

[A] denominator for Time