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10 Behavioral responses of juvenile golden grey mullet *Liza aurata* to
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Abstract

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2 Temperature is an important factor for fish. Yet, little is known about its effects on the feeding
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4 behavior of fish and the subsequent consequences of these behavioral changes on the spatial distribution
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6 of resources. We analyzed the differences in the feeding behavior of two sizes of juvenile *Liza aurata* at
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8 two water temperatures (i.e. 10°C and 20°C), using laboratory mesocosms. We also examined whether
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10 temperature-induced changes in feeding behavior of the smaller size of *L. aurata* would affect the
11
12 spatial distribution of the microphytobenthos (MPB) biomass, an important resource in coastal systems.
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16 Both the number of feeding events and the swimming velocity during feeding in juvenile *L.*
17
18 *aurata* were higher at 20°C than at 10°C, independently of the fish size. The time spent feeding did not
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20 vary between 10°C and 20°C, while the distance covered during feeding was significantly smaller at
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22 20°C than at 10°C. Grazing did not affect the mean MPB biomass, but did increase its spatial variance at
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24 the smaller scale (i.e. a few centimeters) at 20°C.
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28 A high number of feeding events, a high swimming velocity during feeding and a small distance
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30 covered during feeding in 20°C-acclimated *L. aurata* most likely represented an adaptation to an
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32 increase in metabolism, as well as to the need to reduce the energy costs of feeding at 20°C. Results also
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34 indicated that changes in feeding behavior of the 20°C-acclimated *L. aurata* were responsible for the
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36 increase in small-scale spatial variability in the MPB biomass. We suggested that the enhanced spatial
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38 patchiness due to grazing by fish at 20°C might yield a local increase in the mean MPB biomass,
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40 probably increasing photosynthetic efficiency of cells and algal growth that counterbalance the negative
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42 effect of algal removal by fish, and resulting in the lack of an overall significant effect on the mean.
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49 *Keywords:* Grey mullets; *Liza aurata*; feeding behavior; temperature; grazing;
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51 microphytobenthos; spatial variance.
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1 Introduction

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

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27 Rises in temperature (within naturally occurring ranges) are usually accompanied by an increase
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29 in metabolism and energy demands (Ferreira et al., 1998; Biro et al., 2007,2010) and, consequently, in
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31 food consumption and feeding activity (Polunin and Klumpp 1992; Smith 2008). In particular, an
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33 increase in the number of feeding events (Polunin and Klumpp, 1989; Ferreira et al., 1998; Floeter et al.,
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35 2005; Smith, 2008; Nowicki et al., 2012), the amount of time spent feeding (Biro et al., 2007) and the
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37 distances covered during feeding (Biro et al., 2010; Theodorou et al., 2012) have been observed with
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39 temperature increases.
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44 In general, it has been shown through experiments that the addition of grazers or predators
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46 results in the reduction of the mean biomass (or density) of their resources (Berlow, 1999; Armitage et
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48 al., 2009). If a consumer reduces the mean biomass of a resource, this should also cause a reduction in
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50 spatial variance of the biomass itself, due to the relationship between the mean and the variance (Taylor,
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52 1961). In coral reef systems, Polunin and Klumpp (1992) showed that the increase in the number of
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54 feeding events at increasing temperatures can lead to increases in algal consumption. It is therefore
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56 possible that the amount of algae removed and the amount of variance lost in an area both increase as
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1 water temperatures increase, even if the density of grazers and the availability of their resources do not
2 vary in that area (Polunin and Klumpp, 1989; Beukers-Stewart al., 2011).
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4 However, whether or not grazing has negative effects on the mean and the spatial variance of the
5 algal biomass depends on the time-scale of measurement and the interplay between the rates of algal
6 removal and algal growth. Grazing is effective in reducing the mean and the spatial variance of algal
7 biomass only if the rates of algal removal exceed the rates of algal growth. Furthermore, whether or not
8 grazing decreases the overall spatial variance of a resource biomass depends not only on the mean effect
9 of the trophic interaction, the overall biomass of the resource and the mathematical relationship between
10 the mean and the variance, but also on the variance of the trophic interaction as well as the residual
11 variability of the resource (i.e. the component of variation that is not due to grazing) (Benedetti-Cecchi,
12 2000,2003; Benedetti-Cecchi et al., 2005). The overall spatial variance of the resource is expected to
13 decrease if the loss of variance caused by the reduction in the mean abundance of the resource is larger
14 than the residual variability in the resource abundance, and if the negative effect caused by the reduction
15 in the mean abundance of the resource is larger than the positive effect due to increased spatial variance
16 of grazing.
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34 In this study, we tested the effects of changes in water temperature on the feeding behavior of
35 juvenile golden grey mullet *Liza aurata*, one of the most abundant European grey mullets inhabiting the
36 Northwestern Atlantic coasts and the Mediterranean Sea (Cardona, 2006; Lebreton et al., 2011). The
37 feeding behavior was analyzed at 10°C and 20°C, two temperatures representing low and high seasonal
38 values (which occur naturally during winter and summer, respectively) in the geographic distribution of
39 *L. aurata* (Cardona, 2006; Lebreton et al., 2011). We were also interested in understanding whether
40 temperature-induced changes in the feeding behavior of *L. aurata* would affect the mean and the spatial
41 variance of the microphytobenthos (MPB) biomass, an important benthic food resource for *L. aurata*
42 (Laffaille et al., 2002; Pasquaud et al., 2010; Lebreton et al., 2011). We used a series of laboratory
43 experiments in mesocosm with natural sediment, where we measured the feeding behavior of juvenile *L.*
44 *aurata* and the mean and the spatial variance of MPB at each temperature (i.e. 10°C and 20°C). As
45 juveniles must meet higher metabolic rates and energy demands at increasing temperatures, we expected
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1 (i) the number of feeding events, (ii) the total time spent feeding and/or (iii) the distance covered during
2 feeding to be higher at increasing temperatures; as temperature can affect swimming performance in fish
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4 (Claireaux et al., 2006; Deslauriers and Kieffer, 2012), we also expected (iv) the velocity of swimming
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6 during feeding to be higher at higher temperatures. Whether or not the grazing of grey mullets operates
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8 with large spatial or temporal variance able to enhance the patchiness of MPB is not known. Grey
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10 mullets, however, feed in schools (Whitfield et al., 2012) and their gregarious behavior should increase
11
12 the likelihood of homogeneous grazing in an area (Adler et al., 2001). At each experimental
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14 temperature, it is possible that the amount of variance in the MPB biomass caused by a heterogeneous
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16 grazing would be lower than the amount of MPB variance lost due to the reduction in the mean MPB
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18 biomass. Therefore, we expected grazing to have a negative effect (v) on the mean of the MPB biomass
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20 and (vi) on the amount of spatial variability of MPB but we also expected that (vii) the amount of
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22 microalgae removed and the amount of variance lost due to grazing would be higher at 20°C than at
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24 10°C. Because feeding activity in juveniles often varies with fish size (Choat and Clements, 1993;
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26 Buckle and Booth, 2009), tests on the feeding behavior were repeated with two sizes of juveniles (i.e. J0
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28 and J1). However, only J0 was taken into account to test for the grazing effects on MPB, as this size
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30 dominates the natural populations in shallow coastal areas (Hotos and Katselis, 2011; Lebreton et al.,
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32 2011).
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42 **2 Material and Methods**

43 **2.1 Experimental fish**

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46 Thirty-six J0 juveniles and 36 J1 juveniles (mean total length (TL) \pm SE: 9.71 ± 0.23 cm and
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48 16.10 ± 0.12 cm, respectively) were used in this experiment. The fish were collected along the Sardinian
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50 coast of Italy and then transported to the Marine Station of the University of La Rochelle (France),
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52 where the experiment was carried out. Fish were maintained for one month in 1000 liter holding tanks
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54 ($1 \times 1 \times 1$ m) filled with re-circulated filtered sea water. Temperatures and salinity ranged between 14
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56 and 19°C (mean \pm S.E: $17 \text{ }^\circ\text{C} \pm 0.2$, n=30) and 29 and 35 psu (mean \pm S.E: $32 \text{ psu} \pm 0.3$, n=30),
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58 respectively. After the mullets were transferred into experimental tanks, water temperatures were
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1 adjusted by 0.5 °C per day, until experimental temperatures were reached (10°C and 20°C). The fish
2 were then allowed to acclimate to the experimental temperature for at least three months. During this
3 period fish were under natural photoperiod of the location (46°10 N) and fed with natural periphyton
4 daily and with dry pellets (BioMar®) twice a week as an integration of proteins and vitamins. The use
5 of natural periphyton allowed us to maintain the fish in laboratory conditions without compromising
6 their natural foraging behavior (Richard et al., 2010). Natural periphyton was developed on artificial
7 substrates in an adjacent pond, following the methods described in Richard et al. (2010).
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16 **2.2 Protocol**

18 Grey mullets are a gregarious fish and exhibit schooling behavior (Whitfield et al., 2012).
19 Therefore, we used 3 fish for each run of the experiment. Each fish was used only once. Before being
20 transferred into the mesocosm (4 × 1 × 1 m; Fig. 1), fish were marked on the opercula with plastic tags
21 of different colors using Krazy glue®, following the procedure described in Lefrancois et al. (2005). For
22 marking, fish were lightly sedated with MS222 (0.08 g l⁻¹). Each tagging procedure took <2 min and
23 fish ventilated continuously with no noticeable decrease in ventilation rate. Fish never lost balance and
24 resumed swimming as soon as they were transferred to the acclimatization chamber (Como pers. obs.).
25 Each fish group was placed in the acclimatization chamber of the mesocosm under natural photoperiod
26 and was left undisturbed for 12 hours before the experiment in order to allow them to recover from any
27 stress caused by this handling. Fish were not fed during this period.
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42 An hour and a half before the beginning of the experiment, the water level of the working
43 chamber of the mesocosm was raised by about 50 cm and the fish were encouraged to move from the
44 acclimatization to the working chamber (where the sediment was positioned; see below). This was done
45 by opening the sliding door and lifting up the floor of acclimatization chamber (Fig. 1). In general, the
46 fish entered rapidly (less than 3 min for transfer). The experiment began when the fish started feeding
47 on the sediment placed on the floor and lasted for 3 hours. The cameras were turned on as the fish
48 entered the working chamber and recorded their foraging for the entire duration of the experiment. At
49 the end, the fish were encouraged to move back to the acclimatization chamber and the water level was
50 progressively reduced in the working chamber. All the experimental runs were performed in the same
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1 period of the day (in the morning) in order to avoid a bias in the results done by daily variations in the
2 fish feeding activity (Almeida, 2003). All precautions were taken to limit fish stress during the
3 experiments. Each fish manipulation was done with careful handling. None of the fish showed abnormal
4 behaviour or died during the experiment.
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9 The sediment used in this study was collected on an intertidal mudflat in Aiguillon Bay (France;
10 46°10'N, 1°15'W) close to the Marine Station, by scraping off the upper 2 mm. The sediment was sieved
11 through 5 mm screens to eliminate plant debris and then kept on plastic plates (0.60 × 0.40 × 0.05 m),
12 laid on the bottom of a tank, which had the same shape and area of the working chamber of the
13 mesocosm. Tanks were positioned inside an external greenhouse, so that the sediment experienced the
14 same natural light conditions as in the field. Sediment was exposed to the natural tidal cycles of the area
15 of collection, through a series of pumps, which controlled the flow of water in and out of the tanks. The
16 water in the tanks was renewed daily to avoid excessive increases in water temperature and salinity due
17 to water evaporation. Sediment was kept in the greenhouse for a period of three days before being used
18 in the mesocosm. This was a sufficient period of time to recover from disturbance for both microalgal
19 biomass and community structure (Peterson and Stevenson, 1992). For each run of the experiment, a
20 different series of sediment plates was used. The mesocosm was set up in a room with natural sources of
21 light in order to allow for photosynthesis to be carried out by microalgae during each run of the
22 experiment.
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41 **2.3 Experimental designs**

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43 The experiment on the effect of changes in temperature on the feeding behavior of juvenile *L.*
44 *aurata* consisted in the manipulation of two fixed, crossed factors: Temperature (T; T₁₀ and T₂₀) and
45 Size (S; J0 and J1). For each combination, there were 6 replicates (i.e. replicate runs) with 3 juvenile
46 fish. In order to compare the feeding behavior of fish of different sizes, the same ratio between fish
47 biomass and foraging area (i.e. the area of the working chamber) was used for both fish sizes. A
48 constant biomass of fish per unit area was thus used, $\approx 55 \text{ g m}^{-2}$. To attain this biomass, the surface area
49 of the working chamber was set to 0.48 m² (i.e. 1.20 × 0.40 m) and to 1.44 m² (1.80 × 0.80 m) for J0
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1 and J1, respectively. It corresponded to two and six adjacent plastic plates respectively (with the
2 reconstructed sediment), which perfectly fitted on the bottom of the working chamber.
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4 The influence of temperature on the effects of grazing on MPB biomass was assessed through
5 the manipulation of two fixed, crossed factors: Temperature (T; T₁₀ and T₂₀) and Grazing. At each
6 temperature, two out of the six replicate runs belonging to the J0 level of the factor Size were used in
7 this experiment. Grazing effects were assessed, at both 10°C and 20°C, by comparing these two
8 replicate runs of the grazed mesocosm (hereafter called grazed treatment, G) with two replicate runs of
9 the mesocosm without *L. aurata* (hereafter called Un-grazed treatment, U). For U, the same
10 experimental procedure was followed as for G except that fish were not introduced into the mesocosm
11 (i.e. the surface area of the working chamber was set to 0.48 m² and two plastic plates with sediment
12 were positioned on the bottom). U enabled us to estimate both the mean biomass of MPB in absence of
13 grazers and the spatial components of variance in the distribution of MPB biomass that was not due to
14 the grazing by *L. aurata* (i.e. residual variability). The fish density used in this experiment, which
15 corresponded to 6.25 individual m⁻², was ecologically realistic, as it is within the natural range of
16 densities of grey mullets observed in marine coastal and transitional systems (Pais et al., 2007;
17 Mwandya et al., 2010; Nicolas et al., 2010).
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37 Since only one mesocosm was available, only one replicate of the experimental treatments was
38 run each day and the study lasted 28 days (between the end of March and the end of June 2009). Each
39 day, the replicate should have been randomly assigned to one of the six experimental combinations
40 (T₁₀J0=T₁₀G, T₂₀J0=T₂₀G, T₁₀J1, T₂₀J1, T₁₀U, T₂₀U). However, changing the grazing area, the
41 arrangement of cameras or the water temperature in the water reserve tank of the mesocosm every day
42 was practically impossible. Therefore, the replicates for each combination were grouped together and
43 done over a period of 6-8 consecutive days. Such a temporal stratification could have introduced a bias
44 in the results, if the MPB availability had varied naturally in the sediment used during the study period.
45 Changes in availability or nutritional values of food are known to affect the feeding behavior of fish
46 (Polunin and Klumpp, 1989; Beukers-Stewart al., 2011). To check for this potential bias, 6 sediment
47 samples were collected from the experimental replicates of each combination of T × S before each run,
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1 in order to estimate the MPB biomass (as a concentration of Chlorophyll-*a*; $\mu\text{g chl-}a\text{ cm}^{-2}$; see paragraph
2 below for the description of the methodology). Data were analyzed through a one-way ANOVA with
3
4 Time as a fixed factor (4 levels, which corresponded to the combinations of $T \times S$). The analysis did not
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6 reveal any difference among the four times (see Appendix A).
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9 Temporal segregation could have also introduced a bias in the results of the experiment done to
10
11 test for the effects of Grazing and T on MPB biomass. To check for this potential bias, sediment
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13 samples were collected in two additional tanks located in the greenhouse where the MPB was
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15 maintained during both periods when the replicates of G and U for the two levels of T (i.e. T_{10} and T_{20})
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17 were run. Sediment samples were collected following the same sampling design used in G and U (see
18
19 paragraph below for the description of the sampling collection) and the mean and the components of
20
21 variance were estimated accordingly. Data were then analyzed through a one-way ANOVA with Time
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23 as a fixed factor (2 levels, which corresponded to the two times when T_{10} and T_{20} replicates were run,
24
25 respectively). Both the mean and the components of variance of MPB biomass did not vary between
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27 times (see Appendix B). These results indicated that the availability of MPB biomass and its spatial
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29 distribution did not vary in the sediment used during the study period; we can therefore reasonably
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31 exclude any bias due to the temporal segregation of the replicate runs of both experiments.
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37 **2.4 Data collection and analysis**

38 **2.4.1 Feeding behavior of *L. aurata***

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41 We assessed the grazing behavior throughout the total time spent feeding, the number of feeding
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43 events, swimming velocity during feeding and the distance covered during feeding. The total time spent
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45 feeding (in min) was calculated as the sum of the time lengths of all grazing events for each fish
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47 whereas the number of feeding events was estimated as the number of times a fish fed on the sediment.
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49 These variables were estimated for each individual and analyzed with the open source ODRec
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51 (Observational Data Recorder). The tags positioned on the opercula of the fish allowed us to monitor
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53 each single individual for the entire duration of the experiment. These variables were estimated during
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55 the 3 hours of the video tracking, minus the first 20 minutes. A pre-analysis of the videos showed that
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57 during this initial phase fish exhibited an abnormal feeding activity. This represents a compensatory
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1 response to the 12-hours of food deprivation they underwent before the experiment (Ali et al., 2003).
2 For these two variables, the mean values of the test group were then calculated. Differently, the distance
3 covered during feeding was calculated as the distance (in cm) covered on the sediment surface at each
4 grazing event. This was assessed during 20 grazing events of one individual from each group test (i.e.
5 from each run of the experiment) and analyzed with WINalyze software. Since the position of the
6 video cameras provided a lateral view along the longest side of the working chamber, the position of the
7 fish could only be followed on a X-axis. Therefore, in order to increase the accuracy of the assessment
8 of the distance travelled by the fish, the 20 grazing events were randomly selected from those that
9 occurred parallel to the longest axis of the working chamber. The swimming velocity during feeding
10 was then calculated by summing the horizontal vectors of distance travelled by the fish (cm), divided by
11 the total duration of the 20 grazing events considered and is expressed as centimeters per second (cm ·
12 sec⁻¹).

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

16 **2.4.2 Mean and variance of MPB biomass**

17 At the end of the two replicate runs of both G, and U treatments, at both temperatures, the top 5
18 millimeters of the sediment layer were collected using a hand corer (12 mm inner diameter) for the
19 determination of MPB biomass. Three sediment cores were collected 3 cm apart in each of three plots
20 chosen approximately 10 cm apart, at each of four sites spaced about 20 cm apart. A total of thirty-six
21 sediment cores were therefore collected at the end of each replicate run. Samples were immediately
22 frozen, freeze-dried and then kept in the dark at -80°C until further processing. The chlorophyll-*a* (chl-
23 *a*) concentration (i.e. µg chl-*a* cm⁻²) in the sediment was analysed following the method described in
24 Guarini et al. (1998) and used as proxy of MPB biomass.

25 **2.4.3 Statistical analyses**

26 To test for the effects of temperature and fish size on the feeding behavior of *L. aurata*, a two-
27 way analysis of variance (ANOVA, Underwood, 1997) was used, with Temperature (T; T₁₀ and T₂₀)

1
2 and Size (S; J0 and J1) as crossed, fixed factors, and 6 replicates (i.e. replicate runs). The same model of
3 ANOVA was used to analyze mean values of chl-*a* content ($\mu\text{g chl-}a\text{ g}^{-1}$) in the droppings found within
4 each mesocom.
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7 The mean biomass of MPB was estimated for each replicate run (n=36 cores) and data were
8 analyzed using a two-way ANOVA with Temperature (T; T₁₀ and T₂₀) and Grazing (G and U) as fixed
9 crossed factors and two replicates (i.e. replicate runs). The components of variance in MPB biomass
10 were estimated over a range of three spatial scales: Cores (3 cm apart), Plots (10 cm apart) and Sites (20
11 cm apart), according to a fully nested sampling design. They were calculated following the method
12 described in Underwood (1997), in order to provide independent measures of the amount of variability
13 of MPB biomass at each spatial scale. Occasionally, negative estimates were obtained. In these cases,
14 variances were set to zero and the other components were recalculated following the “pool-the-
15 minimum-violator” procedure, as recommended by Fletcher and Underwood (2002). As the components
16 of variance in MPB biomass at each scale (i.e. Cores, Plots and Sites) were not independent from each
17 other, they were analyzed separately, using the same model of ANOVA as for mean biomass of MPB.
18 Homogeneity of variances was checked using Cochran’s C-test (Winer et al, 1991). All analyses were
19 done using Statistica (StatSoft 6.1, 1994).
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40 **3 Results**

41 **3.1 Effect of Temperature and Size on feeding behavior of *L. aurata***

42 The analysis of variance showed a significant effect of Size on the total time spent feeding,
43 independently of the level of Temperature (Table 1). The total time spent feeding by J0 individuals was
44 higher than that spent by J1 ones during the three hours of the experiment (i.e. about 14 and 5 minutes,
45 respectively) (*a posteriori* multiple comparison, Student–Newman–Keuls, SNK, $p = 0.05$; test, Fig. 2).
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53 Results also highlighted significant main effects of both Temperature and Size on the number of
54 feeding events, the swimming velocity during feeding and the cumulative distance covered during
55 feeding (Table 1). SNK tests revealed that the number of feeding events and swimming velocity attained
56 during feeding were consistently higher in 20°C-acclimated *L. aurata* than in 10°C-acclimated ones. In
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1 contrast, the cumulative distance covered during feeding events was consistently lower in 20°C-
2 acclimated *L. aurata* than in 10°C-acclimated *L. aurata*, by an average factor of three (i.e. 287 and 102
3 cm, respectively) (Fig. 2). In addition, the swimming velocity and distance covered during feeding was
4 higher in J1 than in J0 individuals, while the opposite trend was observed in the number of feeding
5 events (Fig. 2).
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10 Finally, a significant main effect of Temperature ($F_{1,12} = 40.47$, $p < 0.00$) on the mean chl-*a*
11 content in the droppings was found. In particular, values were higher at 10°C than at 20°C (mean chl-*a*
12 content [mg chl-*a* g sed⁻¹] ± SE: 10°C = 1815.05 ± 158.84; 20°C = 524.85 ± 124.16).
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18 **3.2 Effect of Grazing and Temperature on mean and spatial variance of MPB biomass**

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20 The analysis of variance did not detect any effect of Grazing, Temperature or Grazing ×
21 Temperature interaction on the mean values of MPB biomass or on the spatial variance of MPB biomass
22 at the largest spatial scales (i.e. Sites and Plots) (Table 2; Fig. 3a,c-d). On the other hand, a significant
23 Grazing × Temperature effect was observed on the spatial variance of MPB biomass at the smallest
24 spatial scale (i.e. Cores) (Table 2; Fig. 3b). SNK tests showed that the spatial variance of MPB biomass
25 on the scale of cores was three-times higher in the presence of J0 *L. aurata* (G) than in controls (U) at
26 20°C (G>U), while no differences between G and U were found at 10°C. In addition, in the presence of
27 grazing (G), variance was higher at 20°C than at 10°C, while no differences were found in U between
28 the two temperatures.
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44 **4 Discussion**

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46 Results showed that both the number of feeding events and the swimming velocity during
47 feeding were higher in 20°C-acclimated *L. aurata* than in 10°C-acclimated ones, independent of their
48 size. In addition, results showed the swimming velocity and the distance covered during feeding to be
49 higher in J1 than J0 individuals, while the opposite was observed for the total time spent feeding and the
50 number of feeding events.
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58 Contrary to our expectations, grazing did not have any negative effect on the mean biomass of
59 MPB at either acclimation temperature. However, it increased the spatial variance in the MPB biomass
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2 at the smaller spatial scale (i.e. a few centimeters) at 20°C, indicating a relationship with the observed
3 changes in feeding behavior at this temperature.

4 **4.1 Effect of Temperature and Size on feeding behavior of *L. aurata***

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6 Rising temperatures increase metabolism, thus influencing feeding activity of fish (Biro et al.,
7 2007,2010). The higher number of feeding events in juvenile *L. aurata* at 20°C than at 10°C was in
8 accordance with studies on other species (both in the laboratory and in the field), showing a positive
9 relationship between the number of feeding events and water temperature (Polunin and Klumpp, 1989;
10 Ferreira et al., 1998; Floeter et al., 2005; Smith, 2008; Theodorou et al., 2012). It is worth noting that an
11 interpretation of this result in the laboratory, however, needs to be made with caution. The number of
12 feeding events that an individual displays in a mesocosm may, in fact, be influenced by behavioral
13 constraints due to the experimental set up (i.e. the tank size). In particular, if in nature an individual
14 prefers to feed by doing one single trace instead of many distinct ones, this behavior could be modified
15 within a mesocom, if the swimming velocity increases (as observed in our study at 20°C), and if it is
16 due to the increased distance covered during feeding. In this case, in fact, fish should be forced to
17 interrupt their feeding within the mecosom, resulting in an increase in the number of feeding events.
18 However, the cumulative distance covered during feeding was found to be three times lower at 20°C
19 than at 10°C in our study. Therefore, the increase in swimming velocity at 20°C was not due to the
20 increased distance covered at each feeding event, but to an even larger decrease in the time spent
21 feeding per event at that temperature. This suggests that the higher number of feeding events in 20°C-
22 acclimated *L. aurata* than in 10°C-acclimated ones represents a voluntary modification of the individual
23 behavior of *L. aurata* at 20°C, as a consequence of the need to maximize food consumption at this
24 temperature. The observed reduction in the distance covered during feeding at 20°C is in contrast with
25 other studies on different species (Biro et al., 2010; Theodorou et al., 2012). In particular, Theodorou et
26 al. (2012) showed a concomitant increase in the number of feeding events and in the distance covered
27 during feeding as temperature increased in the Atlantic cod *Gadus morhua*. Reducing distance and time
28 spent feeding while increasing the number of feeding events in *L. aurata* may represent a foraging
29 strategy, which contributes to decreasing the energy costs involved in feeding activity as metabolism
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1 increases (Rice and Hale, 2009). The suction feeding strategy typical of grey mullets involves the
2 integrated mechanical act of swimming, jaw movement and related muscle activity and it is
3 energetically costly (Rice and Hale, 2009). At 20°C, feeding by making long feeding traces might not
4 pay off in terms of energy expenditures with respect to energy gains. In fact, ingesting a large amount of
5 sediment at one time might not provide any energetic advantages due to the rapid gastric evacuation and
6 intestinal translocation which reduce the time of interaction between ingested food and enzymatic
7 activities involved in the digestion and assimilation processes. Indeed, the gut transit time in fish
8 decreases with increasing temperatures (Halver and Hardy, 2002; Evans and Clairborne, 2006), so that
9 long feeding traces can even represent a disadvantage. It is worth noting, however, that the enzymatic
10 activities involved in the extraction of nutrients from food (i.e. digestion) and nutrients absorption
11 through the intestinal walls (i.e. assimilation) are known to increase with temperature (Halver and
12 Hardy, 2002; Evans and Clairborne, 2006), so that this higher efficiency in digestion and assimilation
13 can counterbalance the short gut transit time. The efficiency of such a process is highlighted by the
14 lower amount of MPB found in droppings at 20°C than in those at 10°C, suggesting a more complete
15 algal digestion in 20°C-acclimated *L. aurata*. Grey mullets are generally active swimming foragers.
16 Maintaining the capacity for locomotion is more important in active fishes than in sit-and-wait foragers
17 because the former are more likely to encounter predators and need to be able to avoid capture, and they
18 continue to hunt or graze while still digesting (Fu et al., 2009). Therefore, grey mullets should be able to
19 modulate their feeding behavior in order to save energy for other activities such as locomotion.

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

28 **4.2 Effect of grazing and temperature on mean and spatial variance of MPB biomass**

1 Our results provide the first evidence that the temperature-induced changes in the feeding
2 behavior of *L. aurata* can influence the spatial distribution of their main food resource, MPB. However,
3 the mechanisms linking the behavioral changes of *L. aurata* to the spatial distribution of the MPB
4 biomass are more complex than previously thought (Polunin and Klumpp, 1992; Smith, 2008). We had
5 predicted that the direct negative effects of grazing on the mean and spatial variance of the MPB
6 biomass would be higher at 20°C than at 10°C because of an increase both in the amount of algae
7 removed and in the amount of variance lost due to grazing by fish at increasing water temperatures, as
8 was found to be the case for herbivorous tropical fish (Polunin and Klumpp, 1992). Our results showed
9 that grazing at 20°C did not induce any change in the mean MPB biomass but it significantly increased
10 the variability in the spatial distribution of the MPB biomass at the smallest spatial scale. This latter
11 result was in accordance with studies showing that grazing can increase the spatial patchiness of
12 resources (Hillebrand, 2008). In our study, it is likely that the enhanced spatial patchiness of MPB due
13 to grazing at 20°C counterbalanced the direct negative effect of algal removal by fish through an
14 enhanced growth rate of algae. Spatial variability at a scale of a few cm is known to favor primary
15 productivity of microalgae (Cardinale et al., 2002) by affecting the flow and uptake of nutrients (Riber
16 and Wetzel, 1987), as well as the ability of small benthic grazers to recognize and select food patches
17 (Kawata and Agawa, 1999). For instance, the small-scale spatial variability of MPB biomass generated
18 by grazing at 20°C may have increased the photosynthetic efficiency of cells and algal growth by
19 preventing nutrient depletion or CO₂ limitation at the base of the biofilm (Riber and Wetzel, 1987). In
20 our mesocosms, it is likely that all these effects yielded a local increase in the mean MPB biomass that
21 counterbalanced the direct negative effects of grazing, resulting in the lack of an overall significant
22 effect (Berlow, 1999).

23 Theoretical and empirical studies have shown that trophic interactions that have weak effects on
24 a resource may enhance the patchiness of that resource, if foraging operates with large spatial or
25 temporal variance (Berlow, 1999; Benedetti-Cecchi, 2000; Sommer, 2000; Adler et al., 2001; Flecker
26 and Taylor, 2004; Hillebrand 2008) and if the amount of variance generated by grazing activity is larger
27 than the residual variability in the resource abundance (Benedetti-Cecchi, 2000). Most of these studies

1 have focused on the grazing pattern of benthic invertebrates while little is known about the ability of
2 epibenthic fish to cause variability in the spatial distribution of the resources (Hillebrand, 2008). Flecker
3 and Taylor (2004) showed that the heterogeneous grazing of the epibenthic fish *Parodon apolinari*
4 significantly increased the spatial variability in biofilm abundance in stream. As with *P. apolinari*, our
5 results provided indications of a heterogeneous grazing of *L. aurata* at 20°C, able to enhance the
6 patchiness of MPB within the mesocosm.
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13 Although previous studies have investigated the effects of grazing on the spatial patchiness of
14 resources (Hillebrand 2008), little attention has been given to the effects of behavioral plasticity of
15 grazers. The effects of temperature-induced behavioral changes of grazing fish on the spatial patchiness
16 of their resources, like those observed in our study, should be taken into account in future ecological
17 studies in natural systems. Indeed, grazing fish such as grey mullets are one of the most abundant fish in
18 coastal areas (Laffaille et al., 2002; Cardona, 2006; Lebreton et al., 2011; Whitfield et al., 2012).
19 Maintaining high level of spatial patchiness of MPB by affecting the behavior of grazing fish may be a
20 way through which high seasonal temperatures favor primary productivity in areas characterized by high
21 biomass or during spring blooms, when nutrient depletion or CO₂ limitation inhibits the photosynthetic
22 efficiency of cells and algal growth (Guarini et al., 1998; Jesus et al., 2005).
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39 **5 Conclusion**

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41 In summary, this study provides the first evidence of clear-cut changes in the feeding behavior
42 of juvenile *L. aurata* at high seasonal temperatures. These changes most likely reflect not only the need
43 to maximize food consumption due to the temperature-induced increases in metabolism, but also the
44 ability of *L. aurata* to modulate individual feeding behavior, in order to save energy for other activities,
45 like locomotion, which are energetically costly.
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53 Our results also provide the first evidence that the temperature-induced changes in the feeding
54 behavior of *L. aurata* influence the spatial distribution of MPB, their main food resource. However, the
55 mechanisms linking the behavioral changes of *L. aurata* to the response of the MPB biomass to grazing
56 are more complex than those expected from a mere increase of algal consumption at higher water
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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.

Source	DF	Time (min)			Events (n. traces)		
		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 Transformation		0.54 (P>0.05) None			0.47(P>0.05) None		

Source	DF	Velocity (cm sec ⁻¹)			Distance (cm)		
		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 Transformation		0.49 (P>0.05) Sqrt(X+1)			0.58(P>0.05) 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; $\mu\text{g cm}^{-2}$) 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.

Source	DF	Mean MPB biomass			CV _{cores}			CV _{plots}			CV _{sites}		
		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 \times 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 \times 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 ($n=6 \pm SE$) 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 ($\pm 1 SE$, $n=2$) of MPB biomass (estimated as chlorophyll-a concentration; $\mu\text{g chl-}a \text{ cm}^{-2}$) 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.

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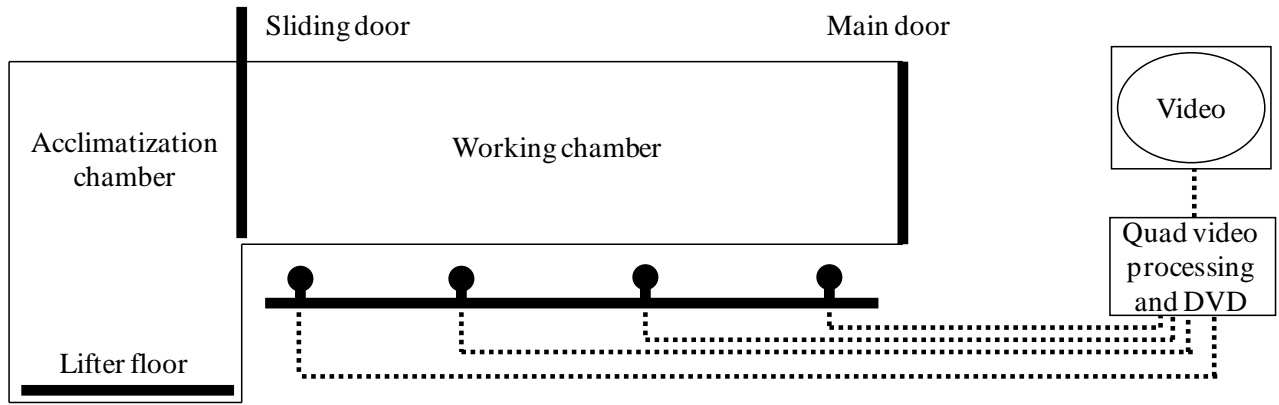


Fig. 1 Como et al.

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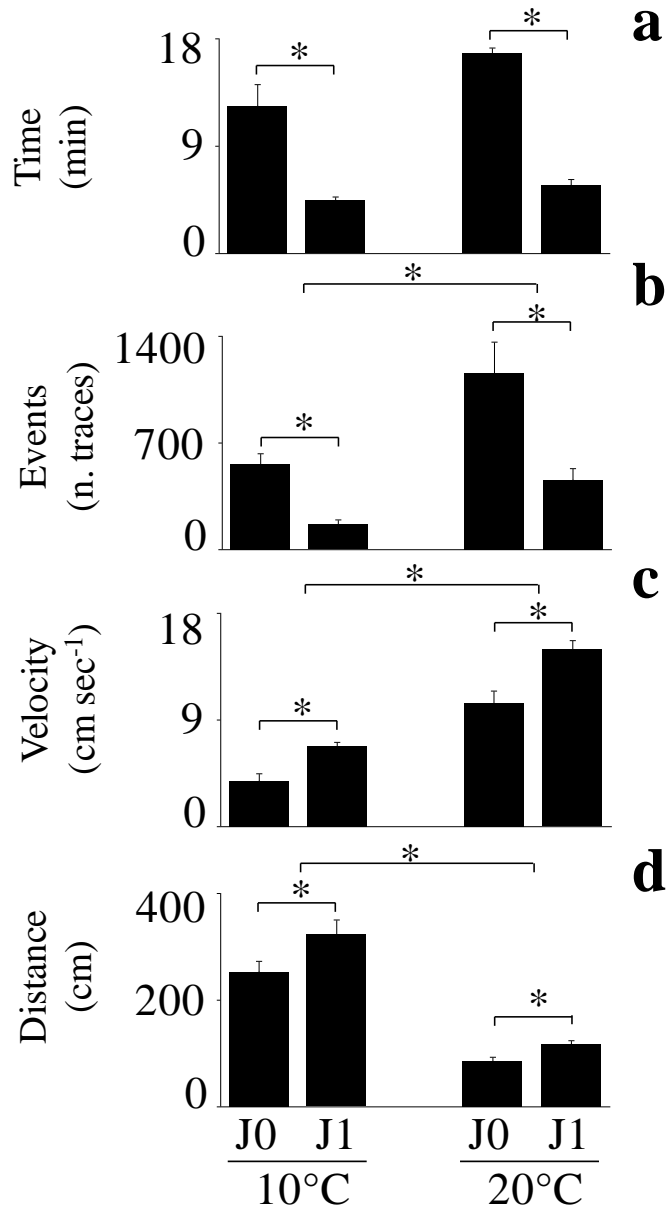


Fig. 2 Como et al.

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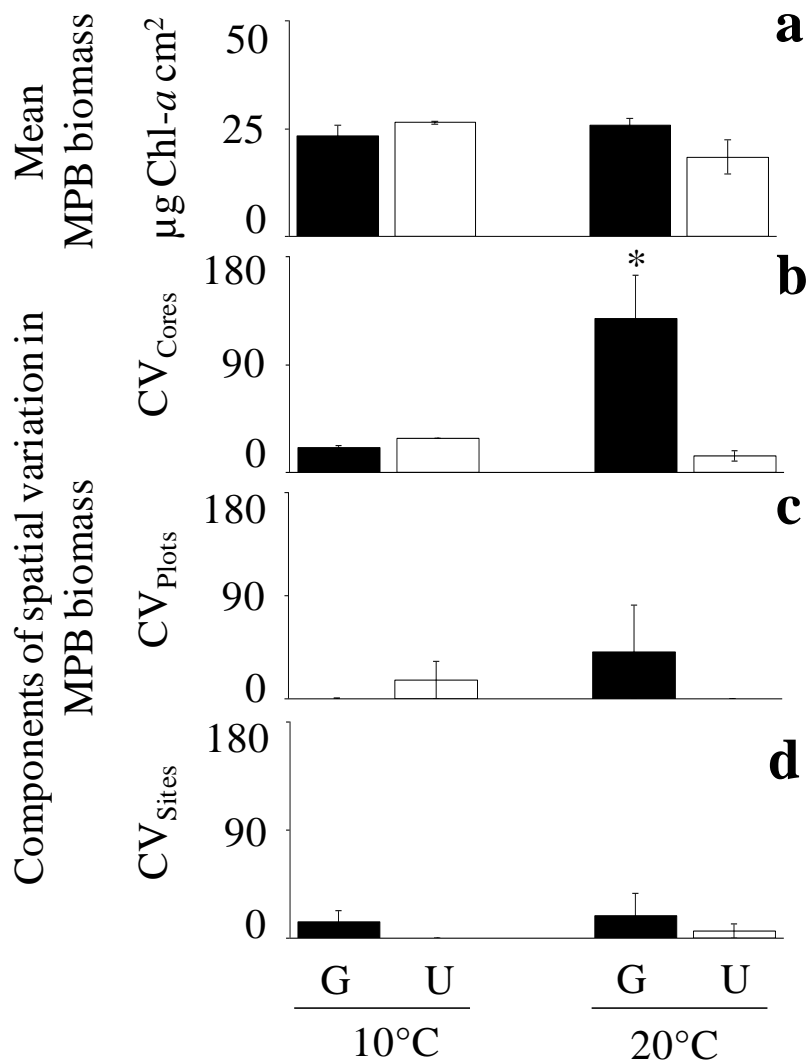


Fig. 3 Como et al.

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; $\mu\text{g cm}^{-2}$). The number of experimental replicates for each time (i.e. each combination of T \times S) was six. Samples were collected before each run.

Source	DF	Mean MPB biomass		
		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, T₁₀ and T₂₀, were run) on mean MPB biomass (estimated as chlorophyll-*a* concentration; $\mu\text{g cm}^{-2}$) 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.

Source	DF	Mean MPB biomass			CV _{cores}			CV _{plots}			CV _{sites}		
		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