

RESEARCH ARTICLE

Size variations of the amphipod crustacean *Melita palmata* in two Adriatic lagoons:

Goro and Lesina

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Abstract

- 1 - Body length of the peracarid crustacean *Melita palmata* (Montagu, 1804) was measured in two Adriatic lagoons, Goro and Lesina, in order to verify the relationships between body size and ecological conditions.
- 2 - The size class distribution was unequal in the two lagoons. Two distinct generations were observed at Goro, one including small or medium sized juveniles and the other medium or large sized adults; most of the Lesina specimens, instead, had medium-sized bodies and the population size dispersion was lower.
- 3 - Mean adult sizes were significantly different among stations and between lagoons.
- 4 - Larger sizes were observed in stations at higher salinity, under direct seawater influence, whereas adult individuals of smaller size were present in more confined stations.

Keywords: Adriatic sea, lagoons, *Melita palmata*, body size, environmental gradient, image analysis.

Introduction

Within the scope of a research project dealing with ecological indicators, two Italian lagoons, Sacca di Goro and Lesina, were considered for sampling surveys of biological communities, including the zoobenthos of artificial hard bottoms. The Sacca di Goro (northern Adriatic sea), an embayment part of the delta system of the river Po, and the coastal Lesina lagoon (southern Adriatic sea), display very different conditions as regards climate, tidal level, continental input, sediment composition (Viaroli and Christian, 2003; Nonnis Marzano et al, 2003). Therefore, they were chosen as a pair of test sites to identify and test different biological indicators of environmental conditions.

Body size descriptors have repeatedly been proposed for monitoring benthic communities (Basset et al, 2004; Mouillot et al, 2006; Reizopoulou and Nicolaidou, 2007), because size is a trait easy to measure and to compare

across taxa, guilds and sites, and it is expected to vary along disturbance gradients (Basset et al, 2004). Most researches on body size distribution investigate whole communities, whereas intra-guild or intra-population variations have generally been disregarded (Durou et al, 2008). In this paper we investigate the size distribution of a peracarid crustacean species, *Melita palmata* (Montagu, 1804), whose populations are abundant in the two lagoons over a range of different substrata. The observed size spectra are then related to key environmental variables.

Peracarid crustaceans are used as biomonitors in various regions (Gómez-Gesteira and Dauvin, 2000; Guerra-García and García-Gómez, 2001; Chintiroglou et al, 2004) because they are considered very sensitive environmental indicators (Conradi et al, 1997). We focused our attention on the gammaridean amphipod *M. palmata* because

it is easy to identify and to collect and it is typical of brackish waters (Bellan-Santini, 1998). Furthermore, its typical laterally compressed body shape allows an easy measure of its total length, which is an appropriate metric to quantify the body size of peracarids living on hard bottoms, as it is related with the ability of the animal in hiding in crevices or among algae (Robson et al, 2005).

Materials and Methods

Description of Melita palmata

Melita palmata (Montagu, 1804) is a gammaridean amphipod belonging to the family of Melitidae (Lincoln, 1979). This peracarid is easily recognizable by the presence of only one dorsal tooth on the segment 1 of the urosome, by its laterally compressed body and its pale yellowish colour (Lincoln, 1979; Karaman, 1982). Sexual dimorphism is clearly visible; in males gnathopod 2 propodus is very large with a quite characteristic trapeziform shape, whereas in females it is not so conspicuous. Moreover, males are generally bigger than females, with an average total length of 11mm, reported for Mediterranean specimens (Karaman, 1982).

M. palmata is a common and abundant inhabitant of lagoons, estuaries and all brackish environments along the European coasts of the Atlantic, the Baltic, the Mediterranean and the Black Sea (Lincoln, 1979) and it is also present as a non-indigenous species in the SW-Atlantic (Alonso, 1997). It is usually observed where the influence of freshwater is stronger, for example in lagoons or near river mouths, due to its tolerance to a very wide range of salinity rates (Karaman, 1982; Sconfiatti, 1989). It lives on sandy and muddy sediments, under stones or cobbles and among algae and seagrass on which it feeds (Lincoln, 1979). Furthermore it can be easily found on submerged structures like wooden poles, which are abundant

in the Adriatic lagoons (Sconfiatti, 1989). In the Adriatic it has been recorded in the lagoons of Grado (Gemelli et al, 2001), Caorle (unpublished data), Venezia (Sconfiatti, 1989), Lesina (Nonnis Marzano et al, 2003) and in the brackish environments of the river Po (Relini et al, 1985; Mistri et al, 2000; Marchini et al, 2004).

Its life cycle is strictly related to the one of macroalgae: it can reproduce all over the year, but the population generally declines in winter (Cunha et al, 1999), showing peaks of reproduction in spring and autumn.

Study area

The Sacca di Goro is a shallow coastal lagoon located in the Po river delta (Fig. 1a), with a surface of 26km² and an average depth of 1.5m. The mean water temperature measured hourly between may 2003 and may 2004 was $17.5 \pm 8.5^{\circ}\text{C}$, with minimum and maximum values of 1.1 and 32.3°C respectively. A southern sand bar separates the lagoon from the North-Adriatic Sea, and a branch of the river Po supplies freshwater to the north-western part of the basin. The Sacca di Goro is affected both by chronic and acute disturbances such as eutrophication, heavy metals contamination, clam cultivation, siltation, introduction of exotic species and algal blooms (Viaroli et al, 1992; Bartoli et al, 1997; Viaroli et al, 2001, Bartoli et al, 2001; Locatelli and Torsi, 2002); therefore its macrozoobenthos is typical of heavily impacted coastal environments, with a poor fauna dominated by a few opportunistic species (Reizopoulou et al, 1996; Mistri et al, 2001; Marchini et al, 2004); seagrass meadows are completely absent (Fano et al, 2003).

The Lesina coastal lake (Fig. 1b), has a surface of about 53km² and an average depth of 0.8 m. The mean water temperature measured monthly in 2003-04 was $17.8 \pm 7.6^{\circ}\text{C}$, with minimum of 7.2°C and maximum of 28.4°C .

Lesina lagoon communicates with the South-Adriatic Sea by means of two artificial outlets, one located eastward, sometimes occluded, and one westward, longer and narrower (Nonnis-Marzano et al, 2003).

the available hard substrata consisting mainly of wooden poles in both lagoons and, in some cases, in Lesina lagoon, of *Ficopomatus enigmaticus* reefs. Methods for collecting and preserving samples are

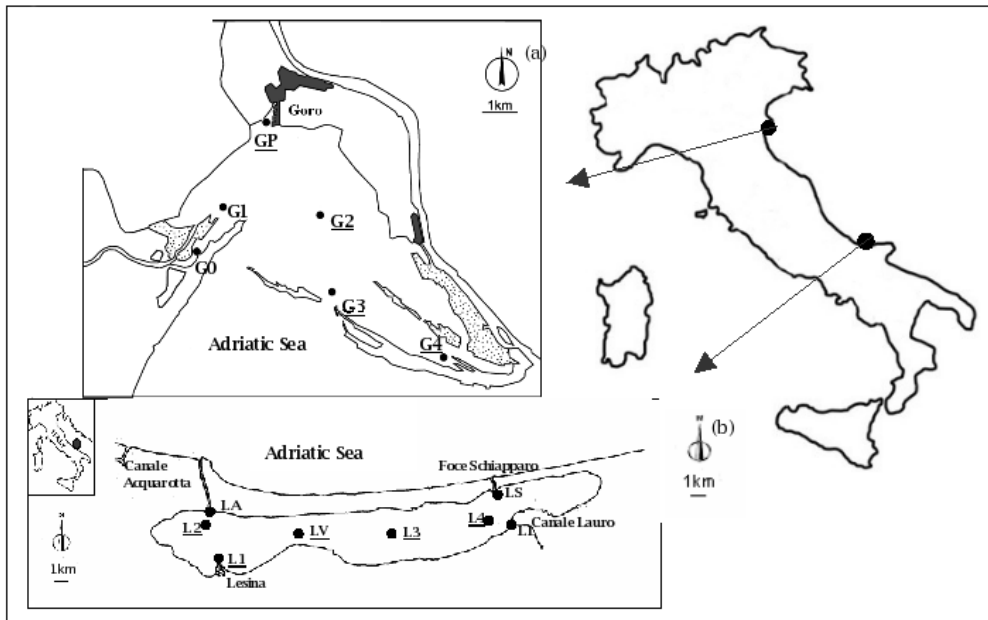


Figure 1. The Goro (a) and the Lesina (b) lagoons. Stations with *M. palmata* are underlined.

Seasonal streams like the Lauro, located in the eastern area of the lagoon, supply freshwater inflow, thus the salinity gradient decreases from west (values similar to the sea) to east (more brackish values). The catchment area is mostly exploited for agriculture and zooculture; three towns, with a total of about 30,000 inhabitants, are located nearby (Fabbrocini et al, 2005). Wide patches of *Ruppia cirrosa* and *Zostera noltii*, and of *Cladophora prolifera*, *Gracilaria confervoides* and *Valonia aegagrophila* cover the bottom (Cozzolino, 1995; Nonnis-Marzano et al, 2003); in the central part of the lagoon biogenic reefs, built by the serpulid polychaete *Ficopomatus enigmaticus*, provide additional substrata for hard-bottom zoobenthos.

Sampling and data analysis

The benthic macrofauna was collected from

described in Marchini et al (2004). Within the framework of a national research project, two sampling surveys were performed in 6 stations in the Goro lagoon, and 8 stations in the Lesina lagoon, in May and July 2004. However, *M. palmata* was found only in the following stations (Tab. 1): 4 in Goro (G2, G3, G4, GP) and 5 in Lesina (L1, L2, L3, L4, LV); moreover, the July samples were not analysed because amphipods were in short supply. Temperature, salinity and dissolved oxygen were measured by the colleagues of the Research Unit of Parma University.

Individuals of *M. palmata* were sorted under the stereoscope, and divided into three categories: juveniles (immature individuals without clearly recognizable sexual characters), mature females (ovigerous individuals or with well-developed oostegites), and mature males (individuals with well-developed gnatopod 2 propodus).

Table 1. Results of the *M. palmata* population analysis in each station: L_t : total body length; M: number of males; F: number of females; J: number of juveniles.

	Goro				Lesina				
	G2	G3	G4	GP	L1	L2	L3	L4	LV
N.o of specimens	48	224	320	252	60	27	77	227	13
L_t mean (mm)	6.5±2.0	7.5±2.1	7.1±2.8	5.1±2.7	6.8±1.5	7.4±1.9	6.3±1.6	5.6±1.3	5.7±1.9
L_t min (mm)	3.5	1.9	1.4	1.4	3.5	4.3	2.6	2.2	2.7
L_t max (mm)	10.9	11.8	12.6	13.9	9.8	10.1	10.4	8.8	9.6
M	18	67	106	51	18	9	26	65	1
F	18	111	101	33	32	11	32	87	3
J	12	46	113	168	10	7	19	75	9

The total length of each specimen was measured by means of Image Pro-plus (4.5) software package for image analysis; notably, the semi-circular dorsal side of the specimens was approximated to a straight line by means of the “trace” tool of the software.

Total lengths were grouped into 17 size classes (interval 0.75mm), ranging from a minimum of 1.25mm to a maximum of 14mm, in order to produce length-frequency histograms. Measures of juveniles were not considered when comparing sizes in the various stations, in order to exclude the influence of recruitment.

The following statistical tests were carried out with MINITAB Student Release 12 in order to compare the population size structure among stations and between lagoons: the non-parametric Kruskal-Wallis test was performed to compare sample medians among stations and replicates within each station, as the ANOVA test could not be used because the data did not satisfy the condition of homogeneous variances (after Bartlett’s and Levene’s tests). The one-tailed unpaired t-test (not assuming equal variances) was then employed to order the stations according to the increasing size of their *M. palmata* population, and to compare the whole populations of Goro and Lesina lagoon. Pearson correlations were calculated between mean adult size and temperature, salinity and dissolved oxygen percent saturation. The combinations of

environmental variables best explaining size patterns were identified with the BIO-ENV analysis (Clarke and Ainsworth, 1993) of PRIMER software package, by maximizing the rank correlation between the matrix of environmental data and the matrix of adult size classes frequency. Stations GP and LV were excluded from these last analyses because of lack of environmental data.

Results

Melita palmata was found very abundant in the stations G3, G4 and GP of Goro lagoon, and in the station L4 of Lesina lagoon. On the contrary, in Goro lagoon it was absent in stations G0 and G1, where the river Po influence is stronger; the same could be observed in Lesina lagoon: *M. palmata* was absent in station LL, near the Lauro river mouth, and in stations LA and LS, located in front of the two artificial channels. The mean temperature and salinity values, measured during the biological samplings in the stations where *M. palmata* was actually found, were respectively: $15.4 \pm 0.5^\circ\text{C}$ and 21.3 ± 4.8 at Goro, and $21.1 \pm 0.7^\circ\text{C}$ and 17.1 ± 3.1 at Lesina.

1248 individuals were considered for total length measurement, 844 from Goro lagoon and 404 from Lesina lagoon. Table 1 shows the total number of measured specimens for each station, the mean, minimum and maximum total body length (L_t) of *M. palmata*, and the

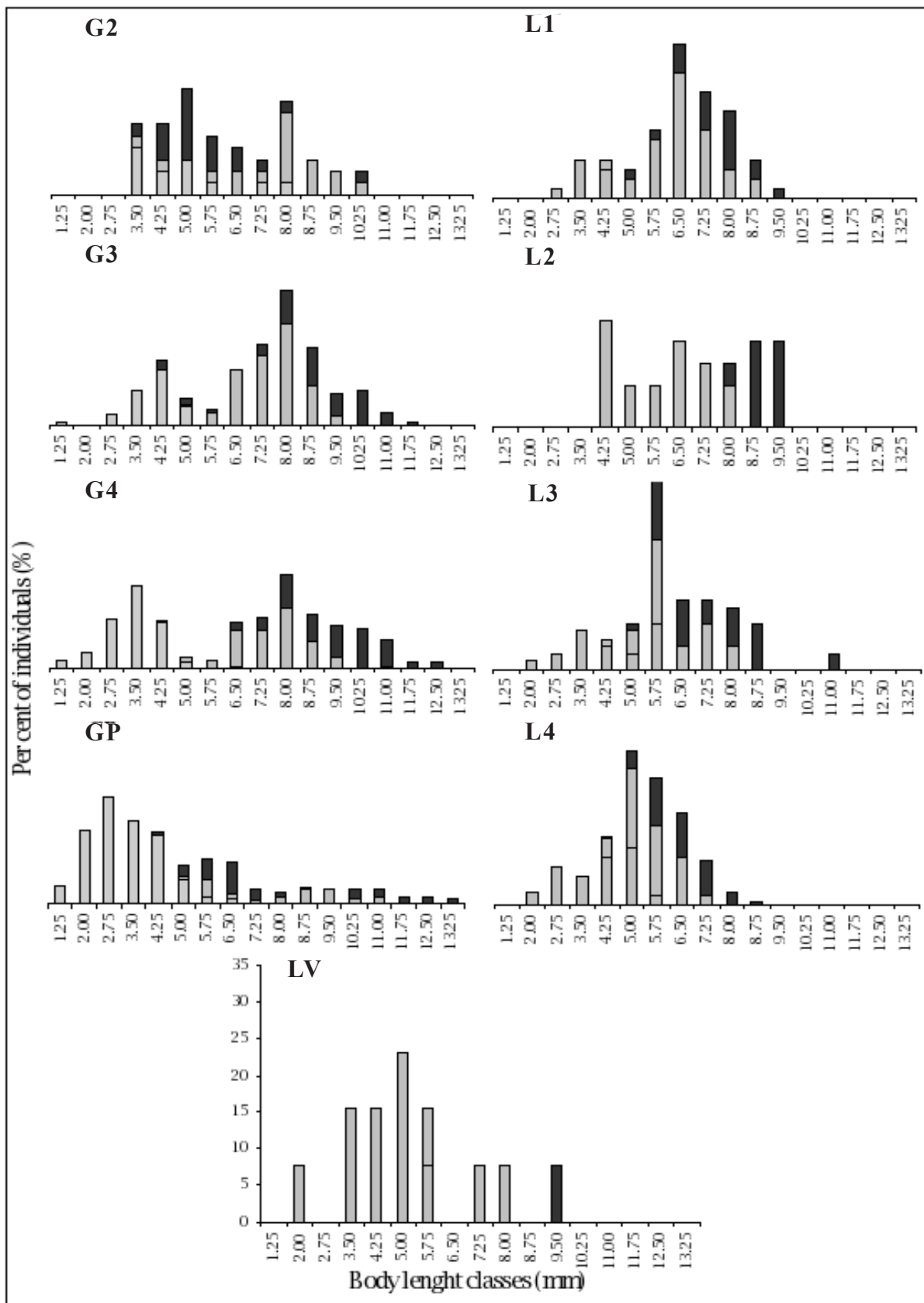


Figure 2. Length frequency histograms of each station of the two lagoons, with indication of total number of measured individuals (N), and percentage of males (M), females (F) and juveniles (J) in the population.

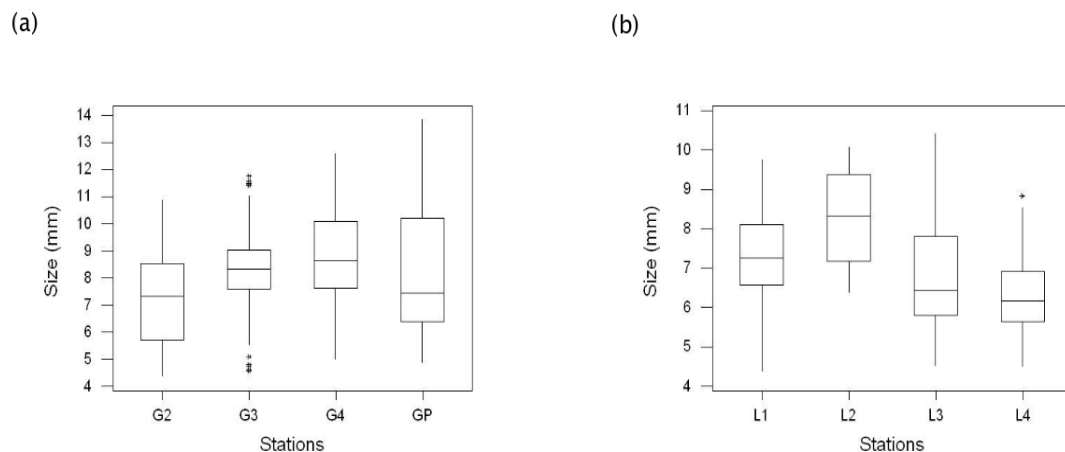


Figure 3. “Box and whiskers” plots of total size (body length) of *M. palmata* in each station of the two lagoons.

subdivision in males, females and juveniles. In each station juvenile, male and female specimens were present: juveniles were dominant in stations GP and LV, and females in stations G3 and L1, while the three groups were equally represented in the remaining stations in both lagoons. The minimum body size was observed in specimens collected in station G4 (1.4mm) of Goro lagoon and in station L4 (2.2mm) of Lesina lagoon, whereas the maximum body size, reached by male individuals, was observed in stations GP (13.9mm) and L3 (10.4mm).

The length-frequency histograms of the four Goro lagoon stations (Fig. 2) allow the distinction of two cohorts: one composed by small juveniles with a body length of modal size of 3mm and one comprising adult specimens of varying size. The latter includes females with body lengths ranging from about 5 to 10mm, and males often bigger than 10mm. This condition was evident in station G4, where a clear bi-modal distribution could be observed. Stations G3 and GP differed: there was a relatively low percentage of juveniles in G3 and of adults in GP, respectively. In station G2, the intermediate length classes (5-8mm) predominated, while smaller and larger sized individuals were scarce.

In Lesina lagoon, *M. palmata* size was unimodally distributed (Fig. 2): juveniles smaller than 2.2mm and adult males longer than 10.4mm were not recorded. This was more evident in stations L1, L3, L4 than in stations L2 and LV, where the number of collected individuals was scarce. For the same reason, we could not evaluate whether the differences within replicates of stations L2 and LV were statistically relevant. These differences were not significant in all the other stations of both lagoons, with the exception of station L3 in Lesina lagoon (Kruskal-Wallis test: $H = 6.47$, $df = 2$, $p < 0.05$).

For the Goro lagoon, (Fig. 3a) shows the distribution of adult *M. palmata* sizes in the four stations. The Kruskal-Wallis test ($H=30.15$, $df=3$, $p < 0.001$) proved a very highly statistically significant difference among stations and the unpaired t-test between couples of stations allowed to order the populations in the following sequence: $G2 < GP = G3 < G4$ (Tab. 2a).

In Lesina lagoon statistical analyses were also performed to confirm the differences shown by the “Box and Whiskers” plots (Fig. 3b), excluding station LV where adult individuals were in short supply. The Kruskal-Wallis test demonstrated the very high statistical

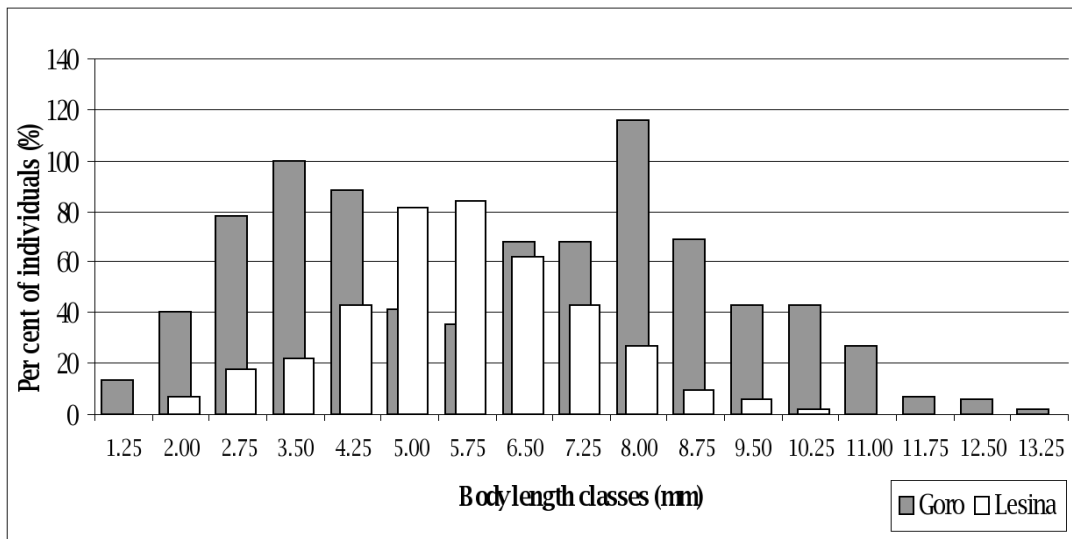


Figure 4. Length-frequency histograms of the two lagoons.

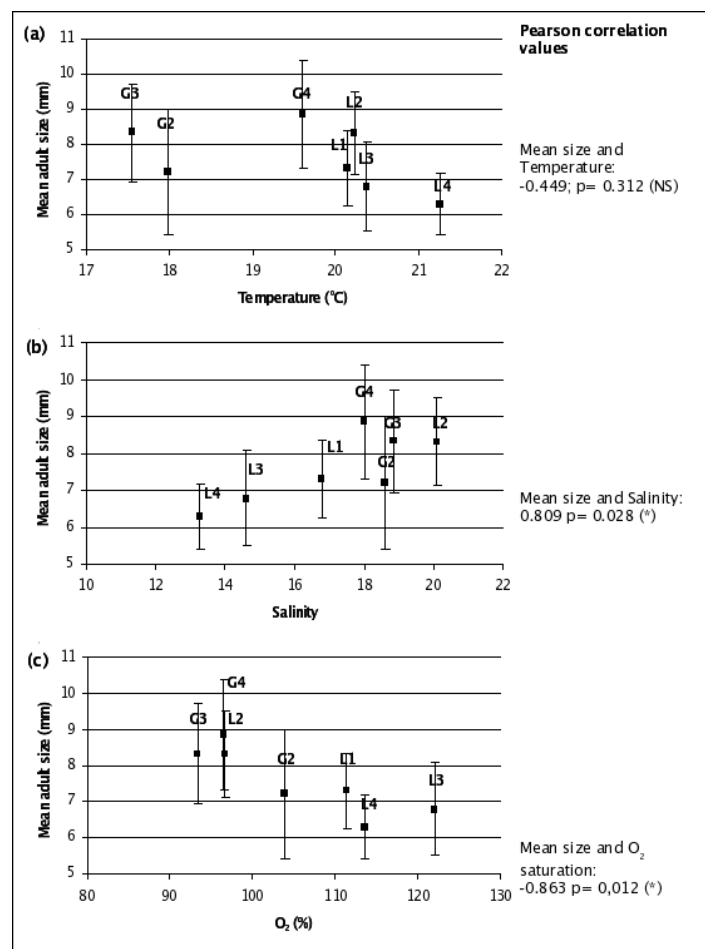


Figure 5. Relationship between mean size (body length) of *M. palmata* adults and values of temperature (a), salinity (b) and dissolved oxygen percent saturation (c) in the investigated stations of Goro and Lesina lagoons. Values of Pearson correlation are shown on the right.

Tab.2 – Results of the unpaired comparisons between *M. palmata* adult populations. Station LV was excluded from this analysis as only four adult specimens were present.

	<i>T</i>	<i>df</i>	<i>p</i>
(2a)			
G2<GP	-2.94	78	**
GP<G3	-0.18	111	NS
GP<G4	-2.08	113	*
G3<G4	-3.50	382	***
(2b)			
L4<L3	-2.74	78	**
L3<L1	-2.29	105	*
L1<L2	-3.31	31	**

Tab. 3. Results of the *BIO-ENV* analysis: combination of K variables giving the highest rank correlations (*Q*) between similarity matrices of size classes and environmental data.

K	Best variables combination			<i>ρ</i>
1	Salinity	-	-	0.579
2	Salinity	Temperature	-	0.602
2	Salinity	O ₂ saturation %	-	0.541
2	Temperature	O ₂ saturation %	-	0.520
3	Salinity	Temperature	O ₂ saturation %	0.551

significance of the observed differences in adult body lengths ($H=62.63$, $df= 4$, $p<0.001$). The unpaired t-test between couples of stations ordered *M. palmata* populations as:L4<L3<L1<L2 (Tab. 2b).

If the two lagoons are compared, pooling the measurements of their various stations, length-frequency histograms confirm the above results (station by station), showing that the Goro population followed a bi-modal distribution, with two clearly recognizable cohorts, while in Lesina lagoon the dispersion of *M. palmata* size was lower and the population unimodal (Fig. 4). An unpaired t-test confirmed the significance of the different adult population size between Goro and Lesina (unpaired t-test: $T =16.53$, $df =753$, $p<0.001$).

Mean adult size of *M. palmata* populations was negatively related with temperature (Fig. 5a) and positively related with salinity (Fig. 5b), but Pearson correlation with temperature was weak and not statistically significant ($p>0.05$). The relationship between size and dissolved

oxygen percent saturation resulted negative (Fig. 5c): smaller mean sizes were observed at stations where oxygen saturation exceeded the 100% value. This could be related to the high primary production in such stations, which in turn might result in dystrophic crises during the night or in other periods. Table 3 shows the results of the *BIO-ENV* analysis: salinity alone, and salinity combined with temperature, determined the highest rank correlations with size patterns ($ρ=0.579$ and $ρ=0.602$, respectively). The limited number of samples, reducing the power of correlation techniques, could be responsible for such relatively low values of correlation.

Discussion and conclusions

Melita palmata is known to cope well with different ranges of tide, salinity and temperature. For this reason, it is common and abundant along Atlantic and Mediterranean European coasts (Sconfiatti, 1989; Marques and Bellan-Santini, 1990; Catrijsse et al, 1993; Bachelet et al, 1996; Conradi and López-González, 1999; Cunha et al, 1999; Węśławski and Legeżyńska, 2002; Kirkim et al, 2005). The literature provides some values of *M. palmata* maximum size, according to the geographical location of the collected specimens (Tab. 4). These locations broadly differ in temperature, which is considered an important factor affecting adult size (Sibly and Atkinson, 1994). Generally, larger maximum sizes correspond to latitudes with lower temperature, and the Goro and Lesina populations fit in this trend. In Lesina lagoon none of the specimens reached the four largest size classes (11-14mm) observed at Goro stations. Apart from general climatic factors, the influence of various physical-chemical variables has been hypothesized. In our data, temperature provided a rather weak relationship with *M. palmata* mean adult size. Temperature combined with salinity obtained the highest rank correlation with the distribution of size classes in the Goro and Lesina stations; salinity

Table 4. Maximum *M. palmata* size (body length) reported in literature from different geographical areas and results of the present study.

Source	Lincoln (1979)	Petrescu (1989)	Petrescu (1989)	Alonso (1997)	Present study	Present study
Ocean/Sea	NE Atlantic	NE Atlantic	Black Sea	SW Atlantic	Mediterranean	Mediterranean
Country	Britain	France	Romania	Argentina	Italy (Goro)	Italy (Lesina)
<i>M. palmata</i> max size (mm)	16.0	9.8	9.7	8.0	13.9	10.4

and oxygen displayed a high correlation with mean adult size values. Salinity and oxygen availability, in fact, can also influence the size spectrum of amphipods (Chapelle and Peck, 2004). However, the naturally high variability of these variables, typical of lagoon ecosystems (Elliott and Quintino, 2007), prevents from detecting precise trends, if lacking intensive ad-hoc schemes.

More generally, temperature, salinity and dissolved oxygen are indicators of the conditions of confinement, which are in turn related to environmental stress. Confined lagoon areas are, in fact, less resilient to potential impacts than areas exposed to marine vivification (Marchini and Occhipinti-Ambrogi, 2007). Therefore, low or high confinement can be reasonably associated with low or high stress, respectively.

Body size is often smaller in stressed environments (Barnes, 2005). For this reason, size class distribution of biological communities has recently been used as a novel quality descriptor in transitional waters (Alexandrova et al, 2007; Reizopoulou and Nicolaidou, 2007): small size classes have resulted dominant at the disturbed sites.

Within single species such as *M. palmata* we have shown highly significant differences both between and within the two lagoons. Larger sizes were observed where salinity was higher, temperature was lower and oxygen saturation was a little below 100%. Although a strict definition of confinement gradients, based on the concomitant analysis performed by the other groups engaged in the collaborative research, is out of the scope of the present paper, we have found that mean adult size was larger in stations where sea water inflow

was more abundant and steadier (Goro: G3, G4; Lesina: L2), while smaller size classes dominated in more confined stations (Goro: GP; Lesina: L3, L4). Station G2 (where the collected individuals were few) deviated from this pattern: located in an area of high seawater exchange, it displayed small adult specimens. In conclusion, our study on the size variations of the amphipod *M. palmata* suggests that size differences of a single species can be interpreted in terms of ecological conditions, assuming that the presence of many large-sized adults can be considered as indicator of persistently favourable environmental conditions. However, our field observations should be confirmed by manipulation experiments, in order to separate the effects of the specific environmental factors that can affect the population size distribution.

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