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1 **Responses of a Northern Adriatic *Ampelisca-Corbula* community to seasonality**
2 **and short-term hydrological changes in the Po river**

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14
15 **Abstract**

16 During the 20th century, increased nutrient inflow has increased the frequency and
17 intensity of eutrophication events in the North-western Adriatic Sea. More recently,
18 evidence of a reduction in the Po river flow and a recovery of the benthic environment
19 were reported. We examined the role played by Po river regime and seasonality in
20 determining the temporal pattern of macrozoobenthos variation: samples were collected
21 from a site located 10–12 km off Cesenatico during two years from October 2004 to
22 September 2006, when the mean Po discharge remained exceptionally low. 88 taxa were
23 found, and the community was dominated by few species: *Ampelisca diadema*,
24 *Lumbrineris latreillii*, *Corbula gibba*, *Aricidea claudiae*, *Levinsenia gracilis* and *Nucula*
25 *nucleus* account for about 80% of total abundances. The density of these species
26 exhibited a marked seasonal variability. Moreover, the total abundance and the density
27 of the sensitive species, *A. diadema*, were negatively affected by several disturbance
28 events (hypoxic conditions, frequent storms and an anomalous winter flood) that
29 occurred between November 2005 and March 2006. The reduction of *C. gibba* and the
30 increase of *A. diadema* densities observed in the investigated period, and verified
31 recently by other authors, could be related to the reduction in river inputs as a
32 consequence of climatic changes.

33

1 **Keywords**

2 macrozoobenthos; temporal scale; river regime; seasonality; Northern Adriatic Sea

3

4 **1. Introduction**

5 The Northern Adriatic Sea is a semi-enclosed and very shallow marine region. Along
6 the North-western side, particularly near the Emilia-Romagna coastline, the terrigenous
7 inputs of the Po and other rivers and the counter-clockwise circulation of the Adriatic
8 Sea favour the occurrence of significant seasonal haline stratification and a high trophic
9 state (Russo et al., 2002, 2005; Montanari and Pinardi, 2006). Eutrophication of the
10 northern Adriatic Sea, marked by acute events such as red tides, extensive mucus
11 aggregates, anoxic bottom conditions and mass mortalities of benthic animals has been
12 known for centuries (Crema et al., 1991 and reference therein). Yet, after World War II,
13 the Northern Adriatic Sea has been subjected to an increased nutrient inflow from the
14 Po and other rivers, which would have enhanced primary production near the surface
15 and oxygen demand near the bottom. As a consequence, the periodicity and extent of
16 these eutrophication events increased, becoming almost annual between 1970s and
17 1990s (Justic, 1987; Crema et al., 1991; Vollenweider et al., 1992; Bucci et al., 1992;
18 Marchetti and Verna, 1992; Justic et al., 1995). These events badly affected not only
19 tourism and fisheries, but also caused a dramatic change in the macrozoobenthic
20 communities, which are widely-used ecological indicators of the “health” of marine and
21 estuarine ecosystems (e.g. Borja and Dauer, 2008). Comparisons between data from
22 collections carried out off the coast of Ravenna in 1934-1936 (Vatova, 1949) and in
23 1989, revealed that the “historical” fine-grain sand (Sables fins bien calibres, SFBC,
24 Pérès and Picard [1964]) and coastal-terrigenous mud (Vases terrigènes côtières, VTC,
25 Pérès and Picard [1964]) biocenoses had been replaced by assemblages characterized by
26 an abundance of dominant, opportunistic species, such as the bivalve *Corbula gibba*,
27 that are indicators of sediment instability, organic enrichment and anoxic conditions,
28 (Crema, 1991; Cavallini et al., 2005). During the 1990s, it was demonstrated that this
29 change in the structure and composition of the macrozoobenthos was general feature
30 over the whole western coastal area of the Northern Adriatic Sea (Simonini et al., 2004).
31 However, the high biomass, species richness and diversity observed at most sites
32 suggest the occurrence of less extreme conditions, compared with other highly-

1 eutrophicated coastal areas, such as the Baltic Sea, the Gulf of Mexico and the Black
2 Sea (Moodley et al., 1998; Simonini et al., 2004). More recently, it appeared that the
3 communities were evolving towards a higher degree of structural complexity: the
4 establishment of dense populations of the fossorial amphipod *Ampelisca diadema*,
5 which is considered as a bioindicator of environmental recovery, and an increase in
6 diversity and evenness indices were reported (Occhipinti Ambrogi et al., 2005). The
7 considerable temporal variability in composition and density of the community of sandy
8 and muddy bottoms of the Northern Adriatic was attributed to the additive effect of 1)
9 inter-annual variation of the hydrographical regime of the Po river and coastal waters
10 (Occhipinti Ambrogi et al., 2005; Simonini et al., 2005b) and 2) high seasonal variation
11 in temperature and photoperiod (Simonini et al., 2004, 2005a, 2007). These factors can
12 modulate biological events, playing an important role in determining the community
13 structure and composition. In general, in temperate coastal areas, the abundance and/or
14 diversity of benthic communities declines over the autumn and winter period, and
15 recovers in summer, when the reproduction, recruitment and settlement of most species
16 occurs (Dauvin, 1988; Rosenberg et al., 1992; Salen-Picard et al., 2003; Simonini et al.,
17 2005a,b). The seasonal variations in sedimentation rates and freshwater and terrigenous
18 organic inputs caused by floods and droughts can also affect the population dynamics of
19 the different species, with major consequences at the community level (Largier, 1993;
20 Grémare et al., 1998; Salen-Picard et al., 2002, 2003; Occhipinti Ambrogi, 2005;
21 Labrune et al., 2007).

22 These issues have become particularly relevant in recent years when, within a large
23 scale climatic change scenario, an increase in sea water temperature and a drastic
24 reduction in the Po river loads were observed (Russo et al., 2002, 2005; Montanari et al.,
25 2005, 2006, 2007).

26 In this paper we investigated the variation in structure and composition of
27 macrozoobenthic communities sampled from October 2004 to September 2006 in the
28 coastal area of Emilia-Romagna, Italy (Fig. 1). The data were integrated with
29 information on 1) the Po and Northern Adriatic hydrographical regime, and 2) some
30 chemical and physical variables of the water column and sediment properties. The aim
31 was to identify the role played by environmental seasonal changes and the Po river
32 regime in determining the short and medium-term pattern of macrozoobenthos

1 variation, taking into account the community characteristics that had previously been
2 described for the same area.

3 4 **2. Materials and methods**

5 *2.1 Study area and hydrological regime of the Po river during the study period*

6 The sampling activities were performed from October 2004 to September 2006 in the
7 CEBEB (Cesenatico-Bellaria Bianco) area, which is a square 1x1 km sector used as a
8 reference site in a previous investigation on the effects of dumping of dredged harbour
9 sediments on benthic assemblages (Simonini et al., 2005b, Fig. 1). The CEBEB is
10 located 10–12 km off the Cesenatico harbour at a depth of 11–13 m. The sea bottom is
11 composed of infralittoral muds, with a high percentage of silt. The biocenoses living in
12 these bottoms are characterized by high densities of surface-deposit feeder species, such
13 as the ampeliscid amphipod *Ampelisca diadema*, the corbulid bivalve *Corbula gibba*,
14 the lumbrinerid polychaete *Lumbrineris latreillii* and some small-sized spionid and
15 paraonid polychaetes (Simonini et al., 2005b). It represented an ideal site to investigate
16 the natural pattern of temporal variation of benthic communities because of the distance
17 from the coast and from direct source of human disturbance (e.g. aquaculture, drilling
18 platforms, dumping/dredging areas). Moreover, it is representative of the benthic
19 ecosystems of the whole area between the Po river mouth and the promontory of
20 Gabicce at a depth of 8-30 m (Simonini et al, 2005b). This area is strongly influenced
21 by seasonal variation in the main current regime and river runoff (Simonini et al., 2004,
22 2005b; Occhipinti Ambrogi et al., 2005; Russo et al., 2005). The Po regime is highly
23 seasonal, with its maximum flow in spring and autumn and minimum in summer and
24 winter. During the period 1917-2006, the mean Po flow at Pontelagoscuro (44°55'16"
25 N, 11°36'29"E) was about 1472 m³ s⁻¹, with a peak of 9650 m³ s⁻¹ in October 2000.
26 During the study period, the mean annual discharge of the Po river remained low (1st
27 year, October 2004-September 2005: 891 m³ s⁻¹; 2nd year: October 2005-September
28 2006: 756 m³ s⁻¹) with exceptional minima of only 300-350 m³ s⁻¹ during the summers
29 2005 and 2006. The most important flow event was registered just after the beginning of
30 the investigation (November 2004, max. 4600 m³ s⁻¹), and no other significant events
31 were registered during the first year. During the second year, three minor discharge
32 peaks were observed in autumn (October, November and December 2005, max. 2800

1 $\text{m}^3 \text{s}^{-1}$) and there was an anomalous high flow event in winter 2006 (February-March
2 2006, $2400 \text{ m}^3 \text{ s}^{-1}$) (Fig. 1). The peaks in Po river flow often induce a severe reduction
3 in the superficial water salinity, which can fall to less than 30 psu, and can cause water
4 stratification phenomena. The effect of fresh-water inputs decreases with water depth
5 and, at bottom level, the salinity ranged between 33-38 psu (Montanari and Pinardi,
6 2006).

7 The storms driven by the NE and SE winds (Bora and Scirocco), that frequently occur
8 in the Northern Adriatic Sea from December to late March, could cause a significant
9 stress to the benthic ecosystem due to sediment re-suspension and sedimentation
10 phenomenon (Matteucci and Frascari, 1997; Simonini et al., 2007). The storm intensity,
11 estimated using the wave-height recorded at the AGIP (Azienda Generale Italiana
12 Petroli) “GaribaldiC/Cervia B” oil rig (2 km SE to CEBEB), was at a maximum
13 between late autumn and early spring. In fact, between November 2004 and April 2005,
14 three severe storms (waves more than 3 m high) were registered. During the December
15 2005-March 2006 period, five storm events were recorded (Fig. 1). The data on the Po
16 river flow and wave height were obtained from the annual report on the eutrophication
17 of the Emilia-Romagna coastal waters (Montanari et al., 2005, 2006, 2007;
18 <http://www.arpa.emr.it/Daphne/>).

19 20 *2.2 Sampling plan and data acquisition*

21 Taking into account the high heterogeneity of the main biotic and abiotic variables, not
22 only at inter- but also at intra-seasonal level (Montanari and Pinardi, 2006), in order to
23 obtain an homogeneous (but not regular) distribution of sampling dates within seasons
24 and ensure the representativeness of samples, we adopted a two-stage sampling strategy.
25 For each month (of each season) the date of sampling was randomly chosen and the
26 time between two consecutive samplings ranged from 2 to 6 weeks. Because sampling
27 times are not spaced at equal intervals, the inadequate estimation of the intra-season
28 temporal variation should not be a problem (Morrisey et al, 1992; Underwood, 1996).
29 Twenty-four sampling surveys were carried out using the oceanographic M/S “Daphne
30 II” of ARPA (Regional Agency for Prevention and the Environment of Emilia-
31 Romagna). The sampling for macrozoobenthos and sediment was carried out using a
32 Box-Corer with a surface of 200 cm^2 . Seven replicates were collected at each month: six

1 were used for the macrozoobenthos, one for granulometric analysis and Total Organic
2 Carbon (%TOC). Each replicate was subdivided in two layers: superficial (0-5 cm
3 depth) and deep (> 5 cm depth): only the superficial layer was considered because
4 previous investigation had highlighted that the 90-95% of organism, in terms of
5 abundance and biomass, occurred in the upper 5 cm of sediment (Simonini et al., 2004,
6 2005b). The samples for macrozoobenthos analysis were processed through sieves with
7 mesh size of 0.5 mm and the retained material was preserved in 4% CaCO₃ buffered
8 formalin in seawater. The near-bottom chemical and physical data (temperature,
9 salinity, pH, conductivity, dissolved oxygen, *Chl a*) were measured using a Hydronaut,
10 model Ocean Seven316 CTD. Water transparency was obtained using the Secchi disk
11 method (Montanari and Pinardi, 2006). The granulometric and %TOC analyses were
12 performed in accordance with the ICRAM (Istituto Centrale per la Ricerca scientifica e
13 tecnologica Applicata al Mare) guidelines
14 ([http://www.icram.org/Acrobat/Libro/Metodologie_analitiche/metodologie_analitiche.h](http://www.icram.org/Acrobat/Libro/Metodologie_analitiche/metodologie_analitiche.htm)
15 [tm](http://www.icram.org/Acrobat/Libro/Metodologie_analitiche/metodologie_analitiche.htm)). The residual material obtained from the sample sieving was sorted in the
16 laboratory, by separating the organisms according to phylum. The macrofauna was
17 preserved in 70% ethylic alcohol. As a rule, the organisms were identified to species
18 level: in a few cases the identification was limited to genus or family. The data from
19 each sampling month were obtained by the sum of their respective replicates.

20

21 2.3 Data analysis

22 The degree of differentiation of benthic communities on year and seasonal scales was
23 assessed using a two factors nested design. We considered two factors: Year (two
24 levels: first [1st] and second [2nd]); Season (nested in Year, with four levels: Autumn
25 [Au 1st, Au 2nd], Winter [Wi 1st, Wi 2nd], Spring [Sp 1st, Sp 2nd], Summer [Su 1st, Su
26 2nd]). The three samplings performed in each season were considered as replicates
27 (Date). This approach was chosen over an orthogonal Year x Season design because
28 preliminary analyses performed according with the orthogonal design highlighted
29 significant Season and/or Year effects, but no significant interaction between the two
30 factors for any of the investigated variables, suggesting that the season effect was the
31 same in the two years (data not shown). Moreover, the nested design allowed the direct
32 comparison of the magnitude of variation on the two temporal scales (Morrisey et al,

1 1992). The critical values of the F tests performed according with the nested design
2 were: $F_{\text{crit } 1, 6, \alpha < 0.05} = 5.99$ for the factor Year; $F_{\text{crit } 6, 16, \alpha < 0.05} = 2.74$ for the factor Season.
3 Multivariate analyses were performed with 2nd-root transformed abundance data.
4 Outputs from non-metric multidimensional scaling (n-MDS) ordination models of the
5 Bray–Curtis similarity matrix were obtained. The differentiation of communities on
6 inter-annual and season scale was tested using a two-way nested ANOSIM (analysis of
7 similarity) randomisation–permutation test. One-way ANOSIM was also performed to
8 compare the communities from different seasons *a posteriori*. The % similarity or
9 dissimilarity within/between groups (years or season within years), and the species
10 making the greatest contribution to dissimilarity among years or seasons, were
11 investigated using the similarity percentage breakdown procedure (SIMPER). The
12 relationships between multivariate community structure and environmental variables
13 were examined using the BIOENV procedure. The BIOENV compares the (rank)
14 similarity matrices from biotic and abiotic variables and chooses that subset of
15 environmental variables which provides a good match between them, which is
16 expressed in term of a matching coefficient (the Spearman coefficient, ρ_s) (Clarke and
17 Ainsworth, 1993). The multivariate analyses were carried out using PRIMER V6
18 software (Clarke and Warwick, 2001).
19 The two-way nested ANOVA was performed to analyze the inter-annual and seasonal
20 differentiation of several univariate measures: the mean Po river flow observed during
21 the four weeks before the sampling, the chemical and physical variables of the bottom
22 water (temperature, dissolved oxygen, transparency, *Chl a*), %(silt+clay) and % TOC of
23 the sediment, the main ecological indices (number of taxa, abundance, Shannon–Wiener
24 diversity [Log e]), the density of the three dominant species (*Ampelisca diadema*,
25 *Corbula gibba* and *Lumbrineris latreillii*) and three very common species (*Aricidea*
26 *claudiae*, *Levinsenia gracilis* and *Nucula nucleus*). The homogeneity of variances was
27 verified *a priori* using the Cochran C-test, transforming the values (log- or angular-
28 transformation) when necessary.
29 The Spearman rank correlation test ($n=24$, $r_{s \text{ crit}}=0.41$) was used to investigate
30 relationships between the diversity indices, the density of *A. diadema*, *C. gibba*, *L.*
31 *latreillii*, *A. claudiae*, *L. gracilis*, *N. nucleus* and the environmental variables.

1 The multivariate analyses were carried out using PRIMER V6 software (Clarke and
2 Warwick, 2001). The univariate analyses were performed by means of R ([http://www.r-
3 project.org/](http://www.r-project.org/)) and Microsoft Excel software.

5 3. Results

6 3.1. Environmental variables

7 Most of the seawater chemical-physical variables and the Po river discharge (ANOVA,
8 log transformation: Year, $F=0.36$, $p>0.56$; Season, $F=3.4$, $p<0.05$) exhibited significant
9 seasonal and low inter-annual variability (Fig. 1, 2).

10 The sea-bottom temperature showed marked cyclical variation during the year:
11 significant differences were observed only at season level (ANOVA, log
12 transformation: Year, $F<0.01$, $p>0.95$; Season, $F=16.4$, $p<0.001$, Fig. 2). The highest
13 temperatures were observed in summer (July 05-August 05: 24 °C; August 2006,
14 23.8°C); the lowest in winter (January 05-February 05: 6.5°C; February 06: 6.5°C).
15 Anomalous high-water temperature (19.0°C) was observed in November 2005.

16 Bottom water concentrations of dissolved oxygen and % saturation were closely related
17 ($r_s=0.91$): they exhibited significant seasonal variation with the same pattern in the two
18 years (ANOVA on $[O_2]$, log transformation: Year, $F<0.01$, $p>0.95$; Season, $F=2.9$,
19 $p<0.05$, Fig. 2). The dissolved oxygen was inversely correlated with temperature
20 ($r_s=0.87$): in fact, the highest oxygen concentrations (about 10 mg l⁻¹) were observed in
21 winter, the lowest at the end of summer or at the begin of autumn. In particular, short-
22 term hypoxic conditions were observed in the autumn of the 2nd year (November 2005),
23 in correspondence with the occurrence of the anomalous high-water temperature.

24 The pH was correlated with dissolved oxygen ($r_s=0.61$), but did not vary significantly at
25 annual and seasonal level (ANOVA, untransformed data: Year, $F=0.04$, $p>0.83$; Season,
26 $F=1.15$, $p>0.37$, Fig. 2). Most of the time, pH ranged from 8 to 8.25, with the exception
27 of November 2005, when, in correspondence with the minimum of dissolved oxygen,
28 the pH was 7.85.

29 The concentrations of *Chl a*, considered as an estimate of the phytoplankton biomass,
30 varied seasonally but not between the two years (ANOVA, log transformation: Year,
31 $F=0.89$, $p>0.38$; Season, $F=2.9$, $p<0.05$, Fig. 2). *Chl a* concentrations > 10 µg l⁻¹, which

1 indicated eutrophic conditions, were observed during the winter months: in February
2 2006, a maximum value of $32 \mu\text{g l}^{-1}$ *Chl a* was measured.

3 The bottom salinity did not exhibit significant inter-annual or seasonal variation
4 (ANOVA, log transformation: Year, $F=0.31$, $p>0.59$; Season, $F=1.7$, $p>0.18$, Fig. 2). It
5 ranged between 36 and 38 psu, with minima of 35 and 34.5 psu in November 2004 and
6 December 2005, in correspondence with the two peaks of the Po river flow.
7 Nevertheless, the correlation with the Po river flow was rather low ($r_s=-0.34$), probably
8 because of the low discharge observed during the investigated period.

9 The water-transparency also varied at seasonal level (ANOVA, log transformation:
10 Year, $F=0.3$, $p>0.60$; Season, $F=4.3$, $p<0.01$, Fig. 2). During summer, when the meteo-
11 marine conditions are more stable, the Secchi-dish was visible at a depth of up to 8-9 m.
12 Transparency started declining with the first storm events at the end of summer. Lower
13 transparencies were observed during the second year winter (January-March 2006),
14 when values of less than two meters were measured, because of the high concentration
15 of suspended organic and inorganic particles in the water column due to storms and Po
16 river discharge.

17 The sediment composition was dominated by mud and silt (75 to 94 %) throughout the
18 investigation (ANOVA on % mud-silt, angular transformation: Year, $F=2.3$, $p>0.17$;
19 Season, $F=1.1$, $p>0.4$, Fig. 2). The % TOC showed significant seasonal variation: the
20 highest values (1.5-2%) were observed at the end of winter and the begin of spring
21 (ANOVA, angular transformation: Year, $F=1.2$, $p>0.31$; Season, $F=3.2$, $p<0.05$, Fig. 2).
22 The period November 2005-March 2006, has been highlighted in grey in the plots of
23 environmental (Fig. 2) and biotic variables (Fig. 4) because this featured several
24 disturbance events (frequent storms, an anomalous winter flood and, overall, hypoxic
25 events associated with the anomalous high temperatures of November 2005)

27 3.2. *Macrozoobenthos*

28 Throughout the course of the investigation 10988 individuals, belonging to 88 taxa,
29 distributed among 9 phyla were found (in order of abundance: arthropods, annelids,
30 molluscs, nemertines, echinoderms, sipunculids, phoronids, cnidarians, echiurians,
31 ascidians). The polychaete annelids were the most important group in term of taxa (47
32 taxa, 53% of the total), followed by the molluscs (25 species, mainly bivalves) and the

1 arthropods (7 taxa). Yet, arthropods were the most important group in term of
2 abundance with 5465 individuals; most of them (97%) being the amphipod *Ampelisca*
3 *diadema*. The remaining 6 phyla were represented by 9 taxa and contributed marginally
4 to the overall abundances.

5 The multivariate analyses performed on 2nd-root transformed data highlighted that the
6 macrozoobenthic communities varied among season, but most of the variability was
7 observed between years (ANOSIM; Years: $R=0.62$, $p<0.05$; Season (Y) $R=0.51$,
8 $p<0.01$). The Bray-Curtis dissimilarity between the two years was 48%, while the
9 dissimilarity between seasons ranged from 31 to 38% in the first year and from 38 to
10 55% during the second year (SIMPER, Table 1). The MDS ordination plot relating to
11 each sampling date showed 1) two main groups corresponding to the first and the
12 second year, respectively, 2) several sub-groups corresponding to the year-season
13 period of sampling: in particular the point referring to the 2nd year winter, characterized
14 by several disturbance events, segregated apart from the others (Fig. 3).

15 The BIOENV analysis, that relates macrobenthos structural changes to the variability of
16 environmental parameters, highlighted that the correlation between the environmental
17 and biological similarity matrix was rather low. The transparency (related to storm
18 events and Po river flow), temperature (correlated with dissolved oxygen) and mean Po
19 river flow were the combination of variables that gave the highest rank correlation
20 ($\rho=0.421$, 2nd root transformed abundances).

21 The SIMPER analysis highlighted that the community was characterized by the
22 presence of a few dominant species typical of off-shore muddy bottoms. Only 12
23 species contributed at least 3% to the total abundances observed at each season, and
24 they accounted for most of the dissimilarity between season and years. For most of the
25 species, the minimum density was observed during the autumn and winter of the second
26 year, which was the period characterized by the disturbance events, and this feature
27 explains the low similarity between the 2nd year winter and the other sampling times
28 (Table 1-2, Fig. 3-4). Few species, such as the polychaete *Nephtys hystrix*, showed
29 similar density throughout the investigation. Other species, such as the amphipod
30 *Pseudolirius kroyerii* and the bivalve *Nucula nucleus*, were subjected to occasional
31 demographic blooms (Table 2). Yet, most of the inter-annual and season variability of
32 the community could be associated with the oscillation in the abundance of some of the

1 most abundant and characteristic taxa such as *Ampelisca diadema*, *Lumbrineris*
2 *latreillii*, *Corbula gibba*, *Aricidea claudiae*, *Levinsenia gracilis* and *N. nucleus*. The
3 first three species dominated the community and contributed to 65% of total
4 abundances. Overall, the six species account for about 80% of total abundances (Table
5 2).

6 The analysis of the Spearman rank correlation between the main univariate indices, the
7 density of the most important species and the environmental variables that gave the
8 highest correlation between the environmental and biological similarity matrix
9 (BIOENV), highlighted that: 1) the diversity and the number of taxa were not
10 significantly correlated; 2) there were negative correlations between the Shannon
11 Diversity Index, the total abundance and the density of *A. diadema*; 3) the density of *A.*
12 *diadema* was positively correlated with the density of *L. latreillii*, *C. gibba*, *A. claudiae*
13 and *L. gracilis*. With regard to the environmental variables: 1) a positive correlation
14 was found between transparency, temperature and the density of *A. diadema*, *L.*
15 *latreillii* and *C. gibba*; 2) the transparency was negatively related to diversity, oxygen
16 concentration and the Po flow; 3) the dissolved oxygen was inversely correlated with
17 the number of taxa, total abundance and densities of *A. diadema* and *C. gibba* (Tab. 3).

18 The number of taxa and Shannon diversity did not vary significantly between the two
19 years and among the different seasons (ANOVA for the number of taxa, log
20 transformation: Year, $F=1.5$, $p>0.26$; Season, $F=1.5$, $p>0.24$; ANOVA for Shannon
21 diversity: Year, $F=4.6$, $p>0.08$; Season, $F=2.1$, $p>0.11$). Yet, both indices exhibited
22 strong variation on a short temporal scale, among dates of the same season: the number
23 of taxa and diversity ranged from 14-35 and 1.5-2.7, respectively (Fig. 4).

24 The total abundance varied on both the inter-annual and seasonal scales, but most of the
25 variability was found between the two years (ANOVA, log transformation: Year,
26 $F=8.8$, $p<0.05$; Season, $F=3.0$, $p<0.05$). The highest densities were observed during the
27 first year, peaking in July 2005 with 12900 ind.m⁻²; in the second year, the
28 macrozoobenthic assemblages showed lower densities, with a minimum of about 400
29 ind. m⁻² in February 2006 (Fig. 4). Despite the difference in the absolute values, the
30 trends in abundance were similar in the two years: it increased starting from the end of
31 spring, peaking in summer and then declining. These trends can be associated with the

1 population dynamic of the dominant species, which exhibited different patterns of
2 temporal variability.

3 The total abundance was primarily related ($r_s=0.97$, Table 3) to the density of the
4 dominant amphipod *A. diadema* (mean density= 1840 ind. $m^{-2} \pm 2560$ S.D.), which
5 varied on a seasonal level and, overall, between the two years (ANOVA, log
6 transformation: Year, $F=7.9$, $p<0.05$; Season, $F=3.1$, $p<0.05$). During the first year,
7 between October 2004 and April 2005, the density of *A. diadema* population was 600-
8 1800 ind. m^{-2} (Fig. 4). Starting from May 2005, a rapid demographic increase was
9 observed: the density peaked in July 2005, with 11000 ind. m^{-2} . Then the density
10 declined rapidly: in August and September it was about 3200 ind. m^{-2} , and in October
11 2005 it was similar to those observed at the begin of the investigation (1100 ind. m^{-2}).
12 During the second year, from November 2005 and May 2006, a further decrease in the
13 density was observed, with a minimum of 25 ind. m^{-2} in March 2006. Between the end
14 of spring and the begin of summer, new recruitment events were observed. In fact,
15 between May and July 2006, density increased by an order of magnitude, from 270 ind.
16 m^{-2} to more than 2000 ind. m^{-2} . Subsequently, the density of *A. diadema* was reduced to
17 about 500 ind. m^{-2} (Fig. 4).

18 The density of two other dominant species, *C. gibba* (mean density= 334 ind. $m^{-2} \pm 218$
19 SD) and *L. latreillii* (mean density= 230 ind. $m^{-2} \pm 138$ SD) varied only at seasonal
20 level (ANOVA for *C. gibba* density, log transformation: Year, $F=0.5$, $p>0.52$; Season,
21 $F=7.3$, $p<0.05$; ANOVA for *L. latreillii* density, log transformation: Year, $F=0.3$,
22 $p>0.59$; Season, $F=3.6$, $p<0.05$).

23 Between October 2004 and March 2005, the density of *C. gibba* was 250-330 ind. m^{-2}
24 (Fig. 4). Starting from May 2005, a rapid demographic increase was observed that
25 resulted in a peak density in September 2005 (750 ind. m^{-2}). Subsequently, during
26 autumn and winter, density decreased: a minimum of 25 ind. m^{-2} were found in March
27 2006. Starting from April 2006, density increased, peaking in July 2006 with 730
28 ind. m^{-2} , and then declined in the following months (Fig. 4).

29 Also the lumbrinerid polychaete *L. latreillii* exhibited demographical variation with
30 season. The highest densities were observed during summer (400-500 ind. m^{-2}), the
31 lowest in winter (60-180 ind. m^{-2}) (Fig. 4).

1 The pattern of temporal variation of the paraonid polychaete *A. claudiae* was quite
2 unusual (Fig. 4). At the beginning of the investigation, it was one of the most abundant
3 taxa (more than 1000 ind. m⁻²). Subsequently, its density declined and a minimum of
4 less than 20 ind. m⁻² was found in February 2006. Then, the density increased during
5 spring and fall during summer 2005 (ANOVA, log transformation: Year, F=9.5,
6 p<0.05; Season, F=4.3, p<0.01).

7 The dynamic of the other paraonid polychaete *L. gracilis* exhibited seasonal trends with
8 maximum densities during spring and minimum densities during autumn in both years
9 (ANOVA, log transformation: Year, F=2.4, p>0.17; Season, F=3.5, p<0.05; Fig. 4).

10 The ANOVA did not highlight any significant Year and Season effect on the density of
11 the bivalve *N. nucleus* (log transformation: Year, F=1.9, p>0.21; Season, F=1.6,
12 p>0.21). From October 2004 to March 2006, the density of *N. nucleus* ranged from 25
13 to 170 ind. m⁻² (with the exception of February 2006, when no specimens were found).
14 Then a demographic bloom was observed: the density peaked to more than 500 ind. m⁻²
15 in June 2004, then fell to 100 ind. m⁻² in September 2006 (Fig. 4).

16

17 **4. Discussion**

18 Most of the previous investigations on the soft-bottom macrozoobenthos in the North-
19 western Adriatic Sea adopted a sampling strategy with sampling intervals of three
20 months or more (Moodley et al., 1998; Simonini et al., 2001, 2005b). In some cases,
21 this approach can make it difficult to identify the seasonal patterns of variation in the
22 benthic community (Occhipinti et al., 2005). The sampling strategy adopted in the
23 present study provides some insight into the patterns of temporal variability and the role
24 of biotic interactions and abiotic factors in determining community structure and the
25 population densities of the most important species.

26 The macrozoobenthic assemblages at the CEBEB site showed clear seasonal trends.
27 Densities rose sharply at the end of spring/begin of summer, peaked in summer and then
28 strikingly declined, the lowest values being recorded during autumn and winter. Similar
29 seasonal patterns were observed also at other North-western Mediterranean and
30 Northern Adriatic shallow sites, and were relate to seasonal reproductive events, that
31 were driven by the cyclical annual variation of most of the environmental variables
32 (Sardà et al., 1999; Simonini et al., 2005a). Multivariate and univariate analyses also

1 highlighted significant inter-annual variability in the benthic community: in particular,
2 the total abundance and the density of one of the most abundant taxa, *Ampelisca*
3 *diadema*, were higher during the first year relative to the second year. The high inter-
4 annual variability of North-western Adriatic sea benthic assemblages was observed also
5 in previous investigations, and was attributed to several different perturbations, such as
6 Po river floods (Occhipinti Ambrogi et al., 2005), hypoxic/anoxic conditions (Moodley
7 et al, 1998, Simonini et al., 2004), thermal anomalies and severe storms (Simonini et al.,
8 2005 a,b). Findings from a recent 6-years study suggested that the macrobenthic
9 community in the Emilia-Romagna coastal area was mainly determined by the onset of
10 very high Po river discharge values, which caused marked, short-term fall in the
11 macrozoobenthos density (Occhipinti Ambrogi et al., 2005). During the present study,
12 the flow of the Po was very low with respect to historical data (Montanari et al., 2004,
13 2005, 2006). Yet, a series of stress events occurred during the autumn 2005 and winter
14 2006: the hypoxic event associated with the anomalous high temperature of November
15 2005, and the anomalous winter flood of February 2006 could be related to the
16 generalized reduction of density observed in the second year.

17 The most important species exhibited different patterns of temporal variation, in
18 response to seasonality and hydrological stress. The population number of the dominant
19 amphipod *A. diadema* was characterized by marked seasonal and inter-annual
20 variations. The preliminary analyses on the sex- and stage-structure of the population of
21 *A. diadema* at CEBEB highlighted that this species has a bivoltine cycle, with one
22 winter- and one (or two) summer- generations (Grandi et al., 2007). The increased
23 temperature and the high primary productivity at the end of winter promoted the
24 gametogenesis and the reproduction of the winter generation, which occurred between
25 May and June. The descendants build up the short-living summer generation, which
26 matured early and reproduced during the summer, when the maximum density were
27 observed, and gave rise to the winter generation that survived until the next year
28 (Grandi et al., 2007). We have no experimental data on the effect of burial and hypoxia
29 on this species, but some recent LC₅₀ 96h test performed with several inorganic and
30 organic toxicants highlighted that *A. diadema* is one of the most sensitive amphipods to
31 stress and pollution (Lera et al., 2007) and many amphipod species are susceptible to
32 oxygen deficiency (Johansson, 1997; Gray et al., 2002). Moreover, Occhipinti Ambrogi

1 et al. (2005) observed that the density of *A. diadema* declined after severe Po river
2 outflows. It is possible that the stress condition that occurred between the first and
3 second year would have caused a reduction in the number of adults in the winter
4 generation and, subsequently, a lower recruitment into the summer generation in
5 comparison to the previous year.

6 The water temperature and hydrological variables that varied according to season also
7 affected the biological cycle of the bivalve *Corbula gibba* and the polychaetes
8 *Lumbrineris latreillii* and *Levinsenia gracilis*. These species were characterized by
9 seasonal variation with the same magnitude in the two years. Their recruitment took
10 place in spring, probably as a consequence of environmental (temperature-photoperiod)
11 stimuli, and then density declined during autumn. The stress events that took place
12 during autumn 2005 and winter 2006 did not have an appreciable effect on these
13 species, which can tolerate hypoxic conditions and high rates of sedimentation or
14 sediment re-suspension (Jensen, 1990; Diaz and Rosenberg, 1995; Moodley et al, 1988;
15 Makra and Nicolaidou, 2000).

16 The population dynamics of the polychaete *Aricidea claudiae* and the bivalve *Nucula*
17 *nucleus* were quite unusual, because they were very abundant only at the beginning or at
18 the end of the investigation, respectively. Perhaps, the exceptionally low Po river
19 discharge and the lack of severe flood events during the investigation could have had a
20 negative effect on *A. claudiae*: in fact, Salen-Picard et al. (2002) observed that this
21 opportunistic short-lived species exhibited high short-term peaks in density a few
22 months after Rhone flooding events in the Gulf of Lion. Most information on the
23 biology of *N. nucleus* referred to Northern European populations (see Holmes et al.,
24 2002). This species spawned on alternate years and adopted an “exploitative function
25 strategy, i.e. maturation is maximal in the absence of nutritional limits” (Holmes et al.,
26 2002). If we assume that also the Northern Adriatic population exhibits a similar
27 strategy, the peak in the abundance of *N. nucleus* observed in spring and early summer
28 2006 could be associated with a recruitment event that occurred in the second year only,
29 favoured by the peak of primary productivity recorded during winter 2006. Further
30 analyses on the life cycle, population dynamic and secondary productivity will be
31 carried out in order to confirm the speculation and hypotheses on the effects of intra-

1 and inter-annual variability of the abiotic condition on the most abundant and
2 characteristics species at the investigated area.

3 Among the biotic factors, cases of biotic interaction between species have been reported
4 for the North-western Adriatic Sea (Prevedelli et al., 2001). The occurrence of inter-
5 specific competition between *A. diadema* and *C. gibba* was suggested by Moodley et al.
6 (1998), who observed that *C. gibba* abundance may be negatively affected by the
7 presence of large populations of *Ampelisca*. They consider the two species as
8 suspension-feeders, which can compete for food supply; moreover, they suggested that
9 dense stands of *Ampelisca*, which build tubes in the more superficial layers of sediment,
10 would occupy space and influence the structure and density of the community deeper
11 down (Dauvin, 1988; Dauvin & Bellan-Santini, 1990). Also, Occhipinti Ambrogi et al.
12 (2005) reported that *A. diadema* replaced *C. gibba* as the dominant species, especially
13 during and after periods of low river discharge. Our results did not support the existence
14 of negative effects of *A. diadema* on *C. gibba*. In fact we observed a positive correlation
15 between the densities of the two species (Table 3). In both field and laboratory
16 conditions, *A. diadema* and *C. gibba* did not segregate in the space, occurring randomly
17 mixed in the sediment surface, and exhibited generalist trophic behaviour, acting as
18 facultative suspension- or sediment-surface feeders (Simonini et al., 2004; Grandi et al.,
19 2007, unpublished data). Moreover, the density of *Ampelisca diadema* in the North-
20 western Adriatic Sea did not appear to be so high as to allow the bio-construction of the
21 dense carpets observed in the North-eastern Atlantic, where the ampeliscid amphipods
22 can reach density of an order of magnitude greater (Bellan-Santini and Dauvin, 1988;
23 Poggiale and Dauvin, 2001). Manipulative experimentations are required to establish
24 the occurrence and the magnitude of the inter-specific competition between the two
25 species.

26 According with Occhipinti Ambrogi et al. (2005), the incidence of the 1st and 2nd order
27 opportunistic species, which were dominant in the 1980s and early 1990s communities,
28 seemed to be reduced in recent years. During the study period also, the average density
29 of the bivalve *C. gibba* was three times lower relative to those observed from the 1980s
30 until 2000, when dense populations with more than 1000-1500 ind. m⁻² were
31 extensively reported (Crema et al., 1991; Moodley et al., 1998; Prevedelli et al., 2001,
32 2004). On the other hand, the abundance of *A. diadema*, which was rare until the late

1 1980s (<5% of total abundances), seemed to increase starting from the mid 1990s until
2 recent years, when densities of more than 2000-10000 ind.m⁻² were observed (Simonini
3 et al., 2005b; Occhipinti Ambrogi et al., 2005). If we assume that the contribution of
4 inter-specific relationship between *A. diadema* and *C. gibba* is negligible, the decline of
5 the opportunistic species in favour of the sensitive ones could be related to the variation
6 of the Po and Adriatic Sea hydrographical regimes. A tendency towards a reduction in
7 river inputs due to the decreases in rainfall and an increase in sea water temperature has
8 emerged in recent years (Russo, 2002; Montanari et al., 2004, 2005, 2006). This trend,
9 which is generalized for the whole Mediterranean region and should be reinforced in the
10 near future, as a consequence of climatic changes (4th IPCC report,
11 <http://www.ipcc.ch/ipccreports/index.htm>), could lead to two different scenarios. If no
12 eutrophication events and hydrological disturbance occurred, the reduction of river flow
13 could favour the occurrence of more stable physical conditions on the bottom and the
14 establishment of benthic communities with a higher degree of structural complexity
15 and/or a reduction of opportunistic-tolerant species in favour of more sensitive species
16 (Occhipinti et al., 2005). On the other hand, increased surface temperatures, altered
17 circulation, and precipitation changes could increase the thermal stratification and
18 amplify the frequency and extent of bottom water hypoxia or anoxia in late summer and
19 autumn (Russo et al, 2002), with strong effects on community density and structure. If
20 the beneficial impacts of the reduction of river flood prevailed, the establishment of
21 communities at more mature successional stage would be favoured.

22

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28

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15

1 **Table 1.** Within-season Bray-Curtis similarities (diagonals) and between-season dissimilarities (under the diagonals), estimated for the two
 2 years of sampling (2nd root transformed data) by means of SIMPER analysis. The dissimilarity values were given together with the
 3 corresponding R statistic value, obtained from the pair-wise one-way ANOSIM comparison test between season. Significant R values are
 4 highlighted in bold ($p < 0.05$ for the global test, $R_{\text{pairwise test}} < R_{\text{global test}}$ for pair-wise comparison between season of the same year, Clarke
 5 and Warwick, 2001).

6

1 st year (ANOSIM among Seasons, R=0.50)				
	Au 1 st	Wi 1 st	Sp 1 st	Su 1 st
Au 1 st	72			
Wi 1 st	31; R=0.15	71		
Sp 1 st	37; R=0.56	36; R=0.15	65	
Su 1 st	38; R=0.96	38; R=0.74	36; R=0.44	71

2 nd year (ANOSIM among Season, R=0.52)				
	Au 2 nd	Wi 2 nd	Sp 2 nd	Su 2 nd
Au 2 nd	78			
Wi 2 nd	52; R=0.63	50		
Sp 2 nd	38; R=0.56	46; R=0.26	65	
Su 2 nd	45; R=0.93	55; R=0.67	41; R=0.67	64

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1 **Table 2.** Seasonal density (mean± standard deviation) of the species that contributes 1) at least 3% to the total abundances observed at
 2 each season; 2) to more than 60% of the dissimilarity between sampling years and season (SIMPER, 2nd root transformed data). Class (C.):
 3 A = amphipods; B = bivalves; G = gastropods; P = polychaetes.

C. taxa	Au 1 st	Wi 1 st	Sp 1 st	Su 1 st	Au 2 nd	Wi 2 nd	Sp 2 nd	Su 2 nd
P <i>Aricidea claudiae</i>	931±238	581±246	358±36	300±33	239±25	69±71	206±38	69±73
P <i>Euclymene oerstedii</i>	44±41	36±34	22±25	19±13	0±0	11±10	11±13	0±0
P <i>Glycera tridactyla</i>	31±38	11±10	8±8	11±10	3±5	6±10	14±13	8±8
P <i>Lumbrineris latreilli</i>	242±169	192±167	303±46	417±90	78±84	158±93	267±123	331±84
P <i>Magelona alleni</i>	75±43	56±5	31±5	11±13	42±0	17±17	17±14	17±17
P <i>Monticellina dorsobranchialis</i>	331±200	628±427	789±740	142±130	47±10	14±5	56±10	0±0
P <i>Neanthes succinea</i>	0±0	0±0	28±35	0±0	0±0	6±10	0±0	0±0
P <i>Nephtys hystricis</i>	33±14	36±34	56±32	33±17	47±10	25±8	64±38	78±41
P <i>Levinsenia gracilis</i>	106±47	144±167	247±32	147±67	72±13	31±29	203±43	28±13
P <i>Prionospio cirrifera</i>	122±71	119±64	44±55	78±46	33±0	14±24	6±10	0±0
P <i>Sigambra tentaculata</i>	69±70	22±10	19±17	22±13	0±0	8±8	6±5	3±5
A <i>Ampelisca diadema</i>	972±263	1522±211	3961±3755	5747±4362	647±483	125±145	564±551	1186±1007
A <i>Pseudolirius kroyerii</i>	0±0	3±5	0±0	3±5	0±0	14±17	6±5	361±465
B <i>Abra alba</i>	14±13	53±25	3±5	61±63	31±25	47±21	106±67	78±27
B <i>Corbula gibba</i>	361±142	278±49	156±92	700±50	297±100	56±27	361±321	464±64
G <i>Hyala vitrea</i>	22±38	3±5	3±5	14±24	14±10	8±14	6±5	83±117
B <i>Mysella bidentata</i>	131±91	22±38	17±14	119±145	56±59	36±43	3±5	0±0
B <i>Nucula nucleus</i>	56±17	56±38	42±30	33±17	92±71	31±27	225±276	203±142
B <i>Paphia aurea</i>	3±5	3±5	0±0	72±86	44±13	0±0	3±5	3±5
B <i>Scapharca inaequalvis</i>	19±13	0±0	3±5	92±112	192±171	22±32	31±17	25±8

1 **Table 3.** Spearman correlation between the main univariate indices, the density of most important species and the environmental variables
 2 that gave the high correlation between the environmental and biological similarity matrix (BIOENV). Significant correlations were
 3 highlighted in bold (n=24, $r_{s \text{ crit}}=0.41$). Diversity: Shannon Index; Tot Ab: Total Abundance; Density of 6 main species: *Ampelisca*
 4 *diadema*, *Corbula gibba*, *Lumbrineris latreilli*, *Aricidea assimilis*, *Levinsenia gracilis*, *Nucula nucleus*; Transparency: Secchi disk.

	Taxa	Tot Ab	Diversity	<i>A. diadema</i>	<i>C. gibba</i>	<i>L. latreilli</i>	<i>A. assimilis</i>	<i>L. gracilis</i>	<i>N. nucleus</i>	Transparency	Temperature	[O ₂]
Total Abundance	0.36											
Diversity	0.23	-0.71										
<i>A. diadema</i>	0.24	0.97	-0.83									
<i>C. gibba</i>	0.34	0.61	-0.37	0.56								
<i>L. latreilli</i>	0.18	0.66	-0.39	0.55	0.60							
<i>A. assimilis</i>	0.54	0.61	-0.15	0.53	0.13	0.13						
<i>L. gracilis</i>	0.41	0.55	-0.13	0.41	0.11	0.49	0.58					
<i>N. nucleus</i>	0.38	0.08	0.15	0.02	0.48	0.27	-0.09	0.01				
Transparency	0.24	0.58	-0.41	0.54	0.52	0.36	0.23	0.41	0.21			
Temperature	0.28	0.50	-0.37	0.46	0.77	0.51	0.01	0.07	0.32	0.58		
[O ₂]	-0.42	-0.47	0.20	-0.48	-0.67	-0.37	-0.25	-0.09	-0.13	-0.42	-0.87	
Po Flow	0.09	-0.31	0.38	-0.37	-0.32	-0.39	0.27	-0.12	-0.20	-0.41	-0.29	0.01

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1 **Legends of figures**

2

3 **Fig. 1.** Location of the sampling station CEBEB (Cesenatico-Bellaria Bianco) (from
4 Simonini et al., 2005b, modified) and Po river discharge in the period October 2004 -
5 September 2006 (from Montanari et al, 2004, 2005, 2006). In the Po river flow plot, the
6 main storm events (wave height > 3 m) and the occurrence of hypoxic conditions are
7 indicated by the grey bars and the asterisk, respectively.

8

9 **Fig. 2.** Chemical-physical variables of bottom sea water, % (silt+clay) and %TOC of
10 sediment from CEBEB in the period October 2004 - September 2006. Several
11 disturbance events during November 2005-March 2006 are highlighted in grey.

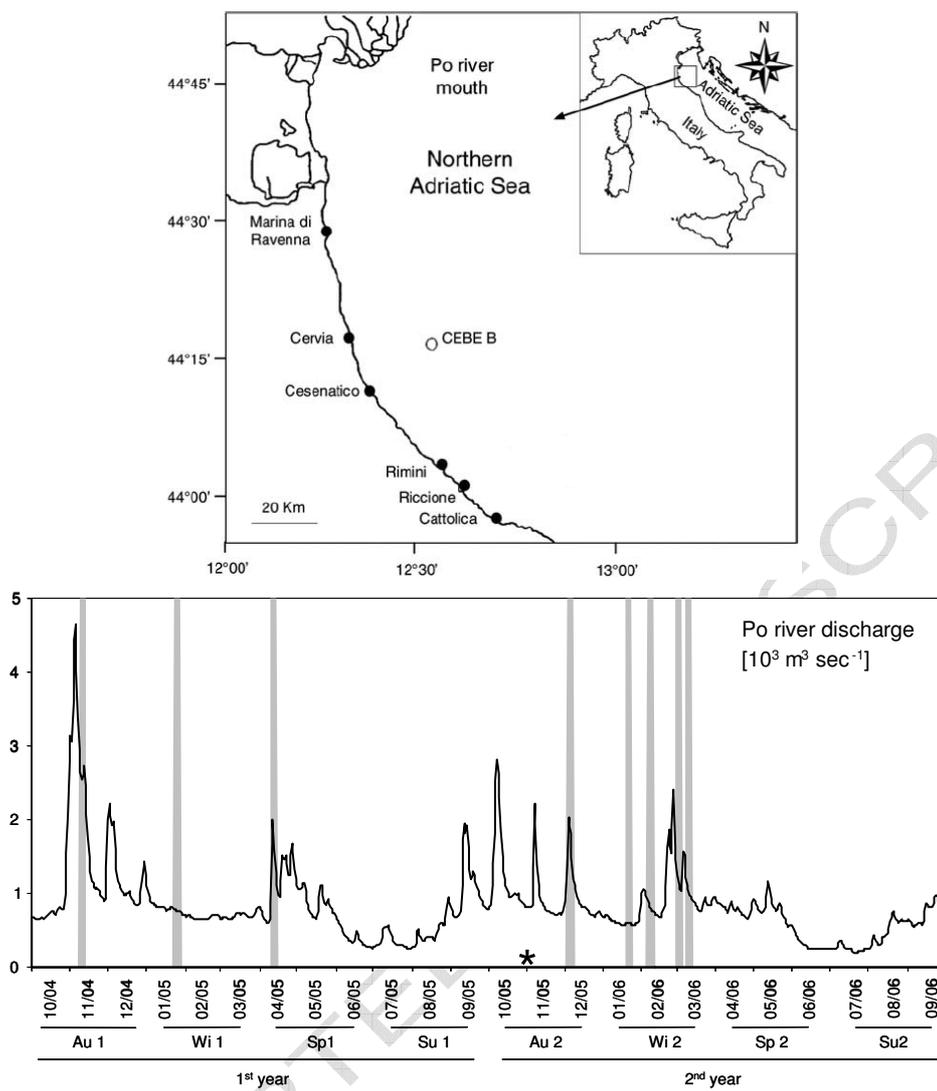
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13 **Fig. 3.** 2D-nMDS ordinations of macrozoobenthic communities at CEBEB site during
14 the period October 2004 - September 2006 (2^{2d} -root transformed abundance data).
15 Symbols: Sampling year (circles=1st year; Triangles=2nd year); Season (white=winter;
16 light grey=spring; black=summer; dark grey=autumn).

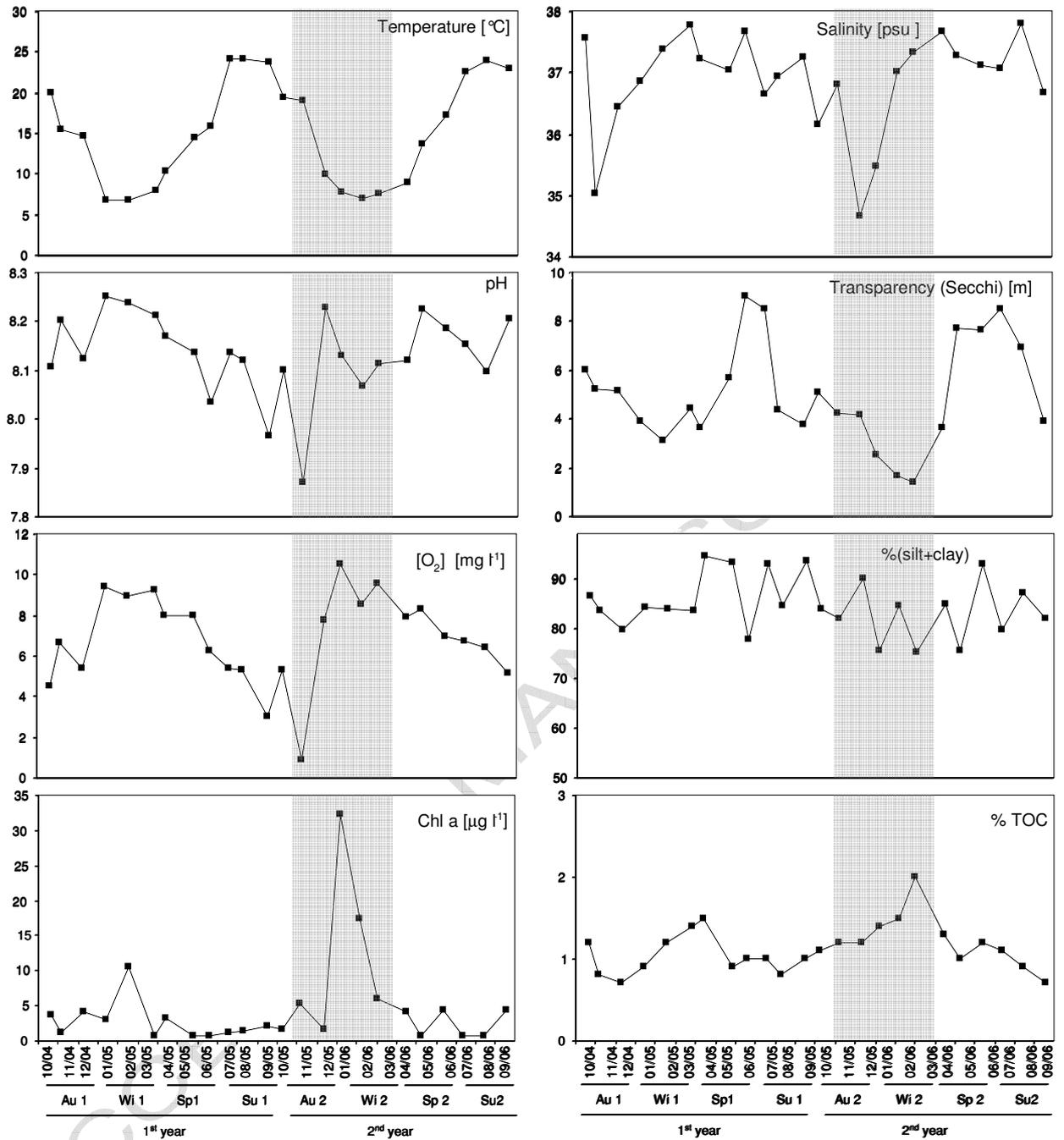
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18 **Fig. 4.** Main ecological indices (total abundances, number of taxa and Shannon
19 diversity) and density of the dominant species *Ampelisca diadema*, *Corbula gibba* and
20 *Lumbrineris latreillii*, and the three common species *Aricidea claudiae*, *Levinsenia*
21 *gracilis* and *Nucula nucleus* from CEBEB during the period October 2004 - September
22 2006. Note the different scales for the total abundance and species density plots. Several
23 disturbance events during November 2005 - March 2006 are highlighted in grey.

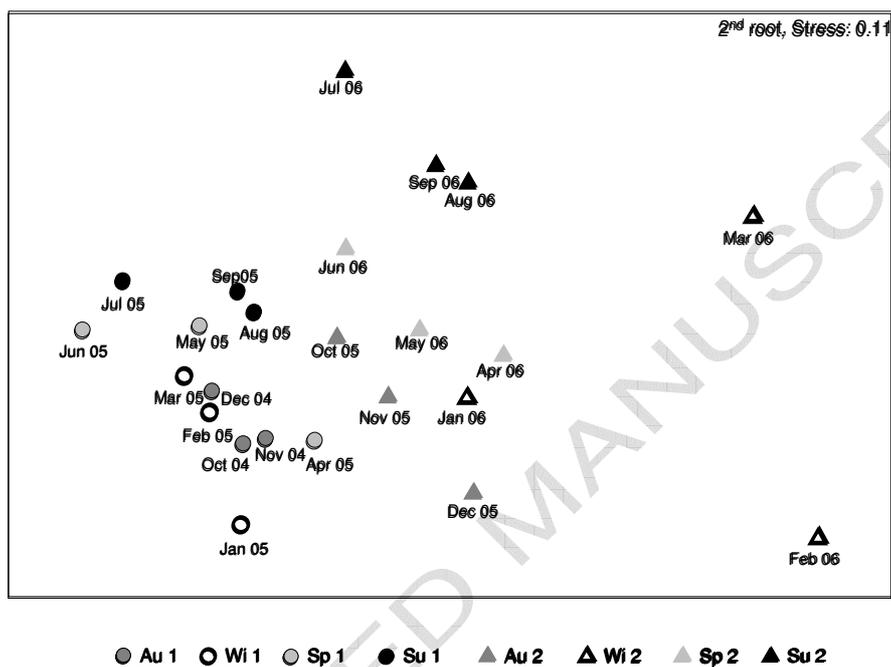
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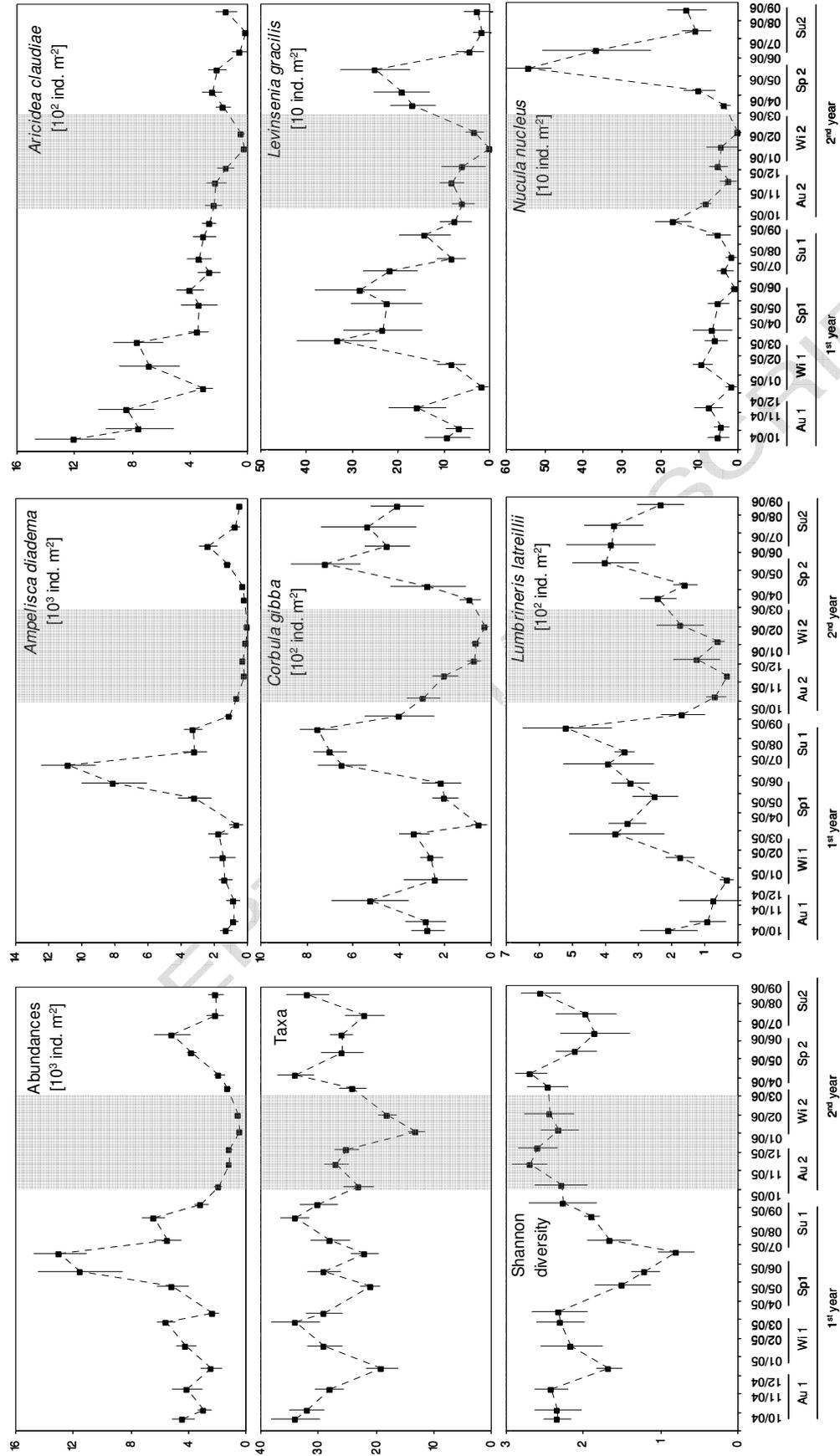
Massamba N'Siala et al. FIGURE 1



Massamba N'Siala et al. FIGURE 2



Massamba N'Siala et al., FIGURE 3



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Massamba N'Siala et al., FIGURE 4