JTI000010

Detecting changes resulting from human pressure in a naturally quick-changing and heterogeneous environment: Spatial and temporal scales of variability in coastal lagoons

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Abstract

To detect changes in ecosystems due to human impact, experimental designs must include replicates at the appropriate scale to avoid pseudoreplication. Although coastal lagoons, with their highly variable environmental factors and biological assemblages, are relatively well-studied systems, very little is known about their natural scales of variation. In this study, we investigate the spatio-temporal scales of variability in the Mar Menor coastal lagoon (SE Spain) using structured hierarchical sampling designs, mixed and permutational multi-variate analyses of variance, and ordination multi-variate analyses applied to hydrographical parameters, nutrients, chlorophyll a and ichthyoplankton in the water column, and to macrophyte and fish benthic assemblages.

Lagoon processes in the Mar Menor show heterogeneous patterns at different temporal and spatial scales. The water column characteristics (including nutrient concentration) showed small-scale spatio-temporal variability, from 10^0 to 10^1 km and from fortnightly to seasonally. Biological features (chlorophyll a concentration and ichthyoplankton assemblage descriptors) showed monthly changes and spatial patterns at the scale of 10^0 (chlorophyll a) − 10^1 km (ichthyoplankton). Benthic assemblages (macrophytes and fishes) showed significant differences between types of substrates in the same locality and between localities, according to horizontal gradients related with confinement in the lagoon, at the scale of 10^0−10^1 km. The vertical zonation of macrophyte assemblages (at scales of 10^1−10^2 cm) overlaps changes in substrata and horizontal gradients. Seasonal patterns in vegetation biomass were not significant, but the significant interaction between Locality and Season indicated that the seasons of maximum and minimum biomass depend on local environmental conditions. Benthic fish assemblages showed no significant patterns at the monthly scale but did show seasonal patterns.
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Keywords: spatio-temporal scales; coastal lagoons; transitional waters; EC Water Framework Directive; environmental impact assessment; Mar Menor lagoon

1. Introduction

Patchiness in the distribution of marine organisms is widespread in all environments and is present at all spatial scales, from the distribution of individuals of a population in their habitat to the mosaics of faunal benthic communities described by Petersen (1918), Thorson (1951) or Peres and Picard (1964), and to the faunal provinces and zones at a biogeographical level (Briggs, 1996). From a macrofaunal perspective, marine coastal 'landscapes' can be viewed as a set of patches hierarchically arranged in space over different spatial scales, ranging from 10^{-3} to 10^4 m (García-Charton and Pérez-Ruzafa, 1998; García-Charton et al., 2000; Benedetti-Cecchi et al., 2003).

Knowledge of the scales at which changes in the abundance of organisms can be detected may help identify the ecological

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processes that determine the observed patterns of distribution (Underwood and Chapman, 1996). This knowledge is therefore essential for developing and testing hypotheses about processes and when designing sampling strategies for environmental impact assessment in which the changes produced by human activities need to be differentiated from the sources of natural variability. For example, the effects of disturbances or protection from fishing detected at a small spatial scale may be lost at a larger-scale of sampling since the inherent spatial variability may mask subtler small-scale patterns (Langlois et al., 2006).

As our understanding of spatial patterns of variation depends on and is constrained by the scale at which we observe them, there is an increasing need to test the existence of general patterns using coherent sampling protocols that span the potentially relevant range of space and time scales (Anderson et al., 2005). Furthermore, to detect changes in ecosystems due to human impact, sampling experimental designs must include replicates. Without such replication, it can not be demonstrated that statistically significant differences between experimental treatments are due to the investigated factor and not simply due to the chance variation among the units measured and to the intrinsic variability of the system (Underwood, 1997). Furthermore, replicates must be selected at the appropriate scale to avoid "pseudoreplication" *sensu* Hulbert (1984) or "confounding" *sensu* Underwood (1997).

However, this fact is rarely taken into account when studying lagoon assemblages. These considerations take on special relevance in the European Union (EU), since the European Water Framework Directive-WFD tries to ensure the highest ecological and chemical status possible for water bodies within its borders (European Union, 2000). This directive establishes that the development in water status should be monitored by Member States on a systematic and comparable basis throughout the Community using standardized methods of monitoring, sampling and analysis.

In practice, although coastal lagoons, with their highly variable environmental factors and biological assemblages, are relatively well-studied systems, very little is known about their natural spatial or temporal scales of variation. Some authors assume a relatively uniform assemblage, as in the euritherm and eurihaline lagoon assemblage *sensu* Peres and Picard (1964) or Augier (1982). Others admit biological gradients related to a particular parameter such as salinity or confinement (Guelorget and Perthuisot, 1983). For others, lagoon assemblages are heterogeneous and patchily distributed, so that they can only be explained from a multi-factorial perspective in which the confinement concept will be mainly related to species colonization rates (Pérez-Ruzafa and Marcos, 1992, 1993).

It is especially important to know the factors responsible for the possible sources of heterogeneity on different spatio-temporal scales in coastal lagoons since, although all coastal lagoons can be defined by some particular features, such as shallowness or relative isolation from the open sea, these characteristics lead to the presence of boundaries with strong physical and ecological gradients. This means that the coastal lagoons are dynamic and naturally stressed systems suffering frequent environmental disturbances and fluctuations. Therefore, a high diversity of environments is included in the term lagoon, with marked differences in size, morphology, trophic status and salinity which condition their biological assemblage structure, species composition and fishing yield (Pérez-Ruzafa et al., 2007). Variations in salinity and other environmental factors may also be observed within a lagoon, both spatially and temporally. As a consequence, biological assemblages will also show high variability both in response to environmental conditions and in their intrinsic dynamic (including life cycles and migrations).

In several respects, this environmental and biological variability may mask the impact produced by human activity. In this work, we analyze the main sources and the spatio-temporal scales of variability in coastal lagoons and discuss their implications for detecting human impact.

2. Material and methods

To illustrate the possible sources of variability in coastal lagoons some examples taken from different studies developed during the last 20 years in the Mar Menor lagoon have been used. Some of them (hydrographical, ichthyoplankton and part of the benthic fish assemblage data) correspond to published works (Pérez-Ruzafa et al., 2004, 2005a,b, 2006), others (algal assemblages and benthic fish assemblage temporal dynamic) remain unpublished. As a whole, they cover a range of spatial scales from 10^1 to 10^2 cm in the case of vertical zonation in macrophytes, 10^2 m in the case of macrophyte and fish assemblages on different types of substrates, and medium spatial scales, from $<10^0$ km (named Sectors in this work) to 10^0-10^1 km (Zones) and $>10^1$ km (main hydrographical basins) for hydrography, ichthyoplankton and macrophyte and fish assemblage horizontal distribution. The temporal scales analyzed ranged from fortnightly (in the pelagic system) to monthly and seasonally (in all the studies).

2.1. Study area

The Mar Menor is a restricted hypersaline coastal lagoon, with an area of 135 km^2 and a mean depth of 3.6 m. It is located on the SE Mediterranean coast of Spain (Fig. 1), and it supports, as many others all over the world, a wide range of uses that have led to great changes in recent decades with a detrimental impact on its assemblage structure and dynamics. Some of the changes are the result of coastal works to develop tourism facilities (land reclamation, the opening, deepening or extension of channels, urban development and associated wastes, marinas, artificial beaches, etc.), while others are related with agricultural practices in the watershed, which have changed from extensive dry crop farming to the cultivation of intensively irrigated crops, with the subsequent increase in the amount of agricultural wastes and nutrients received by the lagoon (Pérez-Ruzafa et al., 1991, 2000, 2005b).
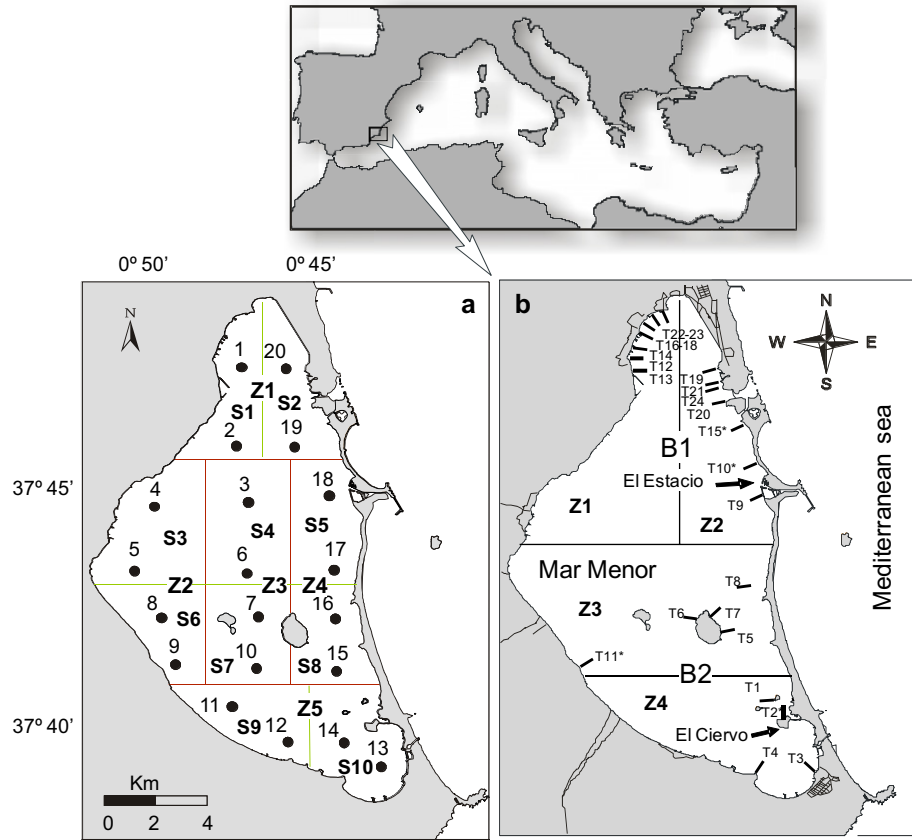
Fig. 1. Location of the Mar Menor lagoon and distribution of sample sites of the nested design in the corresponding sectors (S), zones (Z) and basins (B). (a) Sectorization for the analyses performed on the water column parameters, (b) sectorization for the analyses performed on benthic assemblages.

2.2. Data analyses

To quantify the multi-scale spatio-temporal variation in the pelagic system (hydrological characteristics, trophic conditions and ichthyoplankton), data on nutrient concentration, salinity, temperature, chlorophyll a and ichthyoplankton total abundance and species richness were analyzed using mixed analysis of variance (Underwood, 1997). Samples were taken weekly in 1997 from 20 sampling stations distributed throughout the lagoon (Pérez-Ruzafa et al., 2005a) (Fig. 1a). Medium- and small-scales of spatial variations were introduced in the linear model as successively nested fixed factors, Sectors (S) and Zones (Z). For time scale variations the nested factors Fortnight (F), Month (M) and Season (Se) were considered. The resulting linear model, under the null hypothesis that each variable tested is homogeneous across the considered spatial factors and time scales, is:

$$X_{ijklm} = \mu + Z_i + \mathrm{Se}_j + Z_i S_j + S_k(Z_i) + M_l(\mathrm{Se}_j) + Z_i M_l(\mathrm{Se}_j)$$
$$+ S_k(Z_i)\mathrm{Se}_j + S_k(Z_i)M_l(\mathrm{Se}_j) + F_m(M_l(\mathrm{Se}_j))$$
$$+ Z_i F_m(M_l(\mathrm{Se}_j)) + S_k(Z_i)F_m(M_l(\mathrm{Se}_j)) + \mathrm{Error}_{ijklm}$$

where X_{ijklm} is each individual value of the dependent variable, μ is the overall mean, Z_i is the effect of the ith Zone (with five levels), Se_j is the effect of the jth Season (with four levels),

$S_k(Z_i)$ is the effect of the kth Sector, $M_l(\mathrm{Se}_j)$ is the effect of the lth Month, $F_m(M_l(\mathrm{Se}_j))$ is the effect of the mth Fortnight and Error_{ijklm} is the random error term (weekly samples). Due to missing data, to balance the analyses we took 640 samples for each variable from a total data set of 700 (20 sampling stations × 4 Seasons × 2 Months per Season × 2 Fortnights per Month × 2 weeks per Fortnight). Prior to analyses, homogeneity of variances was checked using Cochran's test, and heterogeneity was eliminated by square-root transformation of the data. In some cases, transformations did not remove heterogeneity, but we performed the analyses anyway, since analysis of variance is quite robust in the face of departures from the underlying assumptions, especially when the design contains a large number of samples or treatments (Underwood, 1997).

To visualize spatial patterns in ichthyoplankton species composition Principal Component Analyses (PCA), non-metric multi-dimensional scaling (MDS) and analyses of similarities (ANOSIM) were performed (Clarke and Warwick, 2001) on a Bray−Curtis similarity matrix considering the factors Zone and Sector. In both cases, the data were previously transformed using $\ln(x+1)$.

Spatio-temporal variability in benthic assemblages was analyzed for macrophytes and fishes. For macrophyte assemblages, two localities were sampled monthly from June 1995 to October 1996 (Fig. 1b), one in the northern basin close to

El Estacio, the main channel through which water exchange takes place, and the other in the southern basin, which is considered the most isolated and confined area of the lagoon (Pérez-Ruzafa et al., 2005a,b). Macrophyte assemblages were hand-collected in an area of $400 \, cm^2$ ($20 \times 20 \, cm$), which is considered representative for midlittoral and infralittoral Mediterranean macrophyte communities (Ballesteros, 1991, 1992). Rocky and mud substrates were sampled. On rocky substrates, samples were taken at two depths (midlittoral: $0−25 \, cm$ and infralittoral: $1.5 \, m$). On mud substrates a *Caulerpa prolifera* meadow was present in both localities.

Spatial and temporal variations in the composition and structure of the macrophyte assemblages were assessed by non-metric multi-dimensional scaling (MDS) with ANOSIM, using similarity matrices based on the Bray−Curtis similarity coefficient generated with biomass (DW) data for macrophytes. Values were square-root transformed before the analyses so that each species contributed fairly evenly to each analysis (Clarke and Green, 1988). Pair-wise ANOSIM comparisons were made between the defined groups, using 10,000 simulations.

Furthermore, to quantify the spatial and temporal variations in macrophyte assemblage descriptors, data of total biomass, species richness and H' Shannon diversity were analyzed using a three-factor analysis of variance (Underwood, 1997), under the null hypothesis that each variable tested is homogeneous across the different localities, assemblages and time. Monthly data were considered as replicates in each season. All factors, Assemblage (As = 3, photophilous midlittoral on rock, photophilous infralitoral on rock and *Caulerpa prolifera* meadow), Locality (L = 2, El Estacio and El Ciervo islands) and Season (Se = 5, from summer 1995 to summer 1996, inclusive) were fixed orthogonals. The localities represent the two main hydrographical basins and extremes of confinement in the Mar Menor (Fig. 1b). A total of 90 samples (3 Assemblages $\times$ 2 Localities $\times$ 5 Seasons $\times$ 3 Months per Season) were included in the analyses. Prior to analyses, homogeneity of variances was checked using Cochran's test. If the analyses were significant, Tukey's Honestly Significant Difference (HSD) tests were performed to identify those means that were significantly different.

Fish assemblages were sampled by underwater visual census in 24 sampling stations on three types of substrata: rock, sand and the *Caulerpa prolifera* meadow on mud. In each community, censuses were performed along transects $50−100 \, m$ long and $1 \, m$ wide. The length of the transects was limited in each census by the size of the community patch, avoiding the boundaries. The censuses were performed at random times from 1985 to 1992 (Fig. 1b). A total of 46 species were included in the analyses.

Spatial variations in the composition and structure of the benthic fish assemblages were assessed by non-metric multi-dimensional scaling (MDS) with ANOSIM, using similarity matrices based on the Bray−Curtis similarity coefficient generated on abundance data (number of individuals per $100 \, m^2$). Values were log $(x + 1)$ transformed before the analyses so that each species contributed fairly evenly to each analysis

(Clarke and Green, 1988). Pair-wise ANOSIM comparisons were made between the defined groups, using 10,000 simulations. Of the 60 visual censuses performed at the 24 sampling stations as shown in Fig. 1b, 18 were performed on rock (9 in each basin), 19 on *Caulerpa* meadows (11 in the north basin and 8 in the south), and 23 on sand (12 in the north and 8 in the south). A total of 46 species were included in the analyses.

As the number of transects and censuses for each of the considered factors was unbalanced, the small and medium spatial variation in benthic fish assemblage descriptors (total abundance, species richness and Shannon diversity) was analyzed using permutational multi-variate analysis of variance (PERMANOVA) on Euclidean distances (Anderson, 2001, 2005). By using permutations, the test requires no specific assumption concerning the number of variables or the nature of their individual distributions or correlations (Anderson, 2001). A random subset of 9999 permutations was used. The experimental design consisted of three factors: Substrate (Sb) (fixed), with three levels (rock, sand and *Caulerpa* meadow on mud); Basin (B), with two levels (north and south), considered fixed according to the main hydrographical basins and the greater (north) or lesser (south) influence of the Mediterranean waters; and Zone (Z), with two levels (random), nested in Basin and representing potential spatial variability within each basin. Significant terms were investigated using *a posteriori* pair-wise comparisons with the PERMANOVA *t*-statistic and 9999 permutations.

The combination of temporal and spatial variability was analyzed by means of a three-factor analysis of variance using monthly censuses performed from November 1989 to December 1991 at four selected sites, two in the less confined areas of the lagoon influenced by the main inlets in the north basin, and two in the more confined areas of the south basin (Fig. 1b).

All factors, Substrate (Sb = 3, rock, sand and *Caulerpa* meadow on mud), Season (Se = 4, spring, summer, autumn and winter) and Basin (B = 2, northern, southern) were fixed orthogonals. As in the case of macrophyte assemblages, north and south basins were defined in accordance with the hydrographical characteristics of the lagoon and marine influence. A total of 72 censuses ($3 \times 4 \times 2 \times 3$ times per season) were included in the analyses. Prior to the analyses, homogeneity of variances was checked using Cochran's test and heterogeneity was removed by transformation of the abundance data (individuals per $100 \, m^2$) to $\ln(x + 1)$. If the analyses were significant, Tukey's Honestly Significant Difference (HSD) tests were performed to identify significantly different means.

3. Results

3.1. Multi-scaled spatio-temporal variability in the water column

The results of the nested analyses of variance performed are shown in Table 1. Hydrographical conditions (water temperature and salinity), nutrient concentration, chlorophyll *a* and

Table 1

Results (*F* values and significant level) of the mixed analyses of variance performed on hydrographical characteristics, nutrient concentration, chlorophyll *a* and ichthyoplankton abundance and species richness in the water column in the Mar Menor lagoon, considering the spatial factors Zone (with five levels) and Sector nested in Zone and the temporal factors Season (with four levels, spring, summer, autumn and winter), Month nested in Season and Fortnight nested in Month(Season). Significant level: ****$p < 0.001$; ***$p < 0.005$; **$p < 0.01$; *$p < 0.05$; ns, non significant. Dark cells: significant spatial variation; medium grey cells: significant temporal variability; light grey cells: significant spatio-temporal interaction

Source	df	Temperature	Salinity	Suspended solids	Nitrate	Nitrite	Ammonia	Phosphate	N:P ratio	Chlorophyll *a*	Ichthyoplankton Abundance	Richness
Zone	4	3.030*	22.861****	0.417 ns	3.317*	1.923 ns	3.858***	3.361*	0.406 ns	24.057****	10.286****	1.482 ns
Season	3	3798.539****	416.758****	103.208****	23.724****	177.842****	57.448****	198.693****	14.998****	315.219****	208.557****	143.954****
Zone × Season	12	1.089 ns	0.663 ns	0.489 ns	1.762 ns	1.221 ns	0.550 ns	1.867*	1.309 ns	7.010****	6.071****	0.566 ns
Sector(Zone)	5	0.653 ns	6.721****	0.207 ns	3.300**	1.520 ns	2.080 ns	1.221 ns	1.653 ns	1.510 ns	1.121 ns	1.288 ns
Month(Season)	4	413.632****	146.184****	80.950****	5.755****	51.773****	9.867****	315.805****	13.795****	100.821****	67.033****	47.268****
Zone × Month(Season)	16	0.282 ns	2.251***	0.862 ns	0.864 ns	1.570 ns	0.639 ns	1.941*	1.606 ns	4.713****	3.047****	0.646 ns
Sector(Zone) × Season	15	0.713 ns	1.841*	0.422 ns	1.096 ns	1.068 ns	2.299***	0.603 ns	0.576 ns	2.700***	2.006*	1.299 ns
Fortnight(Month(Season))	8	100.156****	52.925****	14.877****	7.563****	5.568****	18.468****	46.053****	22.753****	11.934****	11.687****	5.958****
Sector(Zone) × Month(Season)	20	0.386 ns	2.564****	0.360 ns	1.010 ns	1.477 ns	1.427 ns	0.262 ns	0.894 ns	1.217 ns	2.053**	0.763 ns
Zone × Fortnight(Month(Season))	32	0.290 ns	1.316 ns	0.359 ns	1.329 ns	0.722 ns	0.623 ns	0.662 ns	1.111 ns	1.520*	1.680*	0.847 ns
Sector(Zone) × (Fortnight(Month(Season)))	40	0.128 ns	2.187****	0.249 ns	1.068 ns	0.631 ns	1.272 ns	0.707 ns	0.917 ns	0.822 ns	0.826 ns	0.682 ns
Error	480											

ichthyoplankton showed significant variability at all the temporal scales analyzed (Fortnight, Month and Season). Water temperature showed spatial patterns at Zone scale ($p < 0.05$) (10^0– 10^1 km), while salinity showed strong differentiation at different spatio-temporal scales and was significant for the interaction Sector(Zone × Fortnight(Month(Season))) ($p < 0.001$), Sector(Zone) × Month(Season) ($p < 0.001$), Sector(Zone) × Season ($p < 0.05$) and Zone × Month(Season) ($p < 0.001$). Salinity showed significant variability at smaller spatial scales than temperature, indicating the influence of the open sea (through inlets) and fresh water inputs (through main watercourses).

All nutrients showed similar temporal patterns, with significant differences at Fortnight(Month(Season)), Month(Season) and Seasonal scales ($p < 0.001$). On the other hand, small-scale spatial patterns differed among nutrients. Except for nitrite, which did not show spatial patterns at any spatial scale, all showed significant variation at Zone scale. Nitrate showed the highest variability at Sector(Zone) scale ($p < 0.001$). Phosphate showed significant variation in the interaction Zone × Season ($p < 0.05$) and Zone × Month(Season) ($p < 0.05$). Finally, ammonia showed significant variation in the interaction Sector(Zone) × Season ($p < 0.001$).

Chlorophyll *a* concentration in the water column showed significant variability for the interactions Zone × Fortnight (Month(Season)) ($p < 0.05$), Sector(Zone) × Season ($p < 0.001$), and Zone × Month(Season) ($p < 0.001$).

Finally, ichthyoplankton abundance dynamics responded to the same spatio-temporal scales as chlorophyll *a* plus for the interaction Sector(Zone) × Month(Season) ($p < 0.01$). In contrast, species richness showed significant variability at the three temporal scales of Fortnight, Month and Season ($p < 0.001$), but did not show any spatial pattern at the scales considered in this work, despite the fact that species richness shows a decreasing gradient from the inlets' mouth to the more confined areas (Pérez-Ruzafa et al., 2004).

The first two axes of the principal component analysis (PCA) performed on the ichthyoplankton mean annual abundance matrix explained 62.4% of the total variance and showed a spatial distribution of the sampling stations according to three well-differentiated areas (Fig. 2a): the north basin (including sectors S1, S2 and S5) influenced strongly by Mediterranean waters, an area of transitional water still influenced by the Mediterranean (S3, S8 and sampling station six), and the southern basin in the more confined part of the lagoon (Fig. 2b).

The MDS analyses with ANOSIM confirmed this result. The stress value was 0.11 and the Global Test showed significant differences between Zones (Global *R* statistic = 0.613, $p = 0$). Pair-wise comparisons between zones (Table 2) showed significant differences for all groups except Z3–Z5 and Z3–Z4, and marginally significant for Z2–Z5 and Z5–Z4. The two-way nested analysis with the factor Sector nested in Zone showed no significant differences for the tests between Sector groups averaged across all Zone groups, and marginal significance (Global *R* statistic = 0.22, $p = 0.075$) for the test between Zone groups using Sector groups as samples.
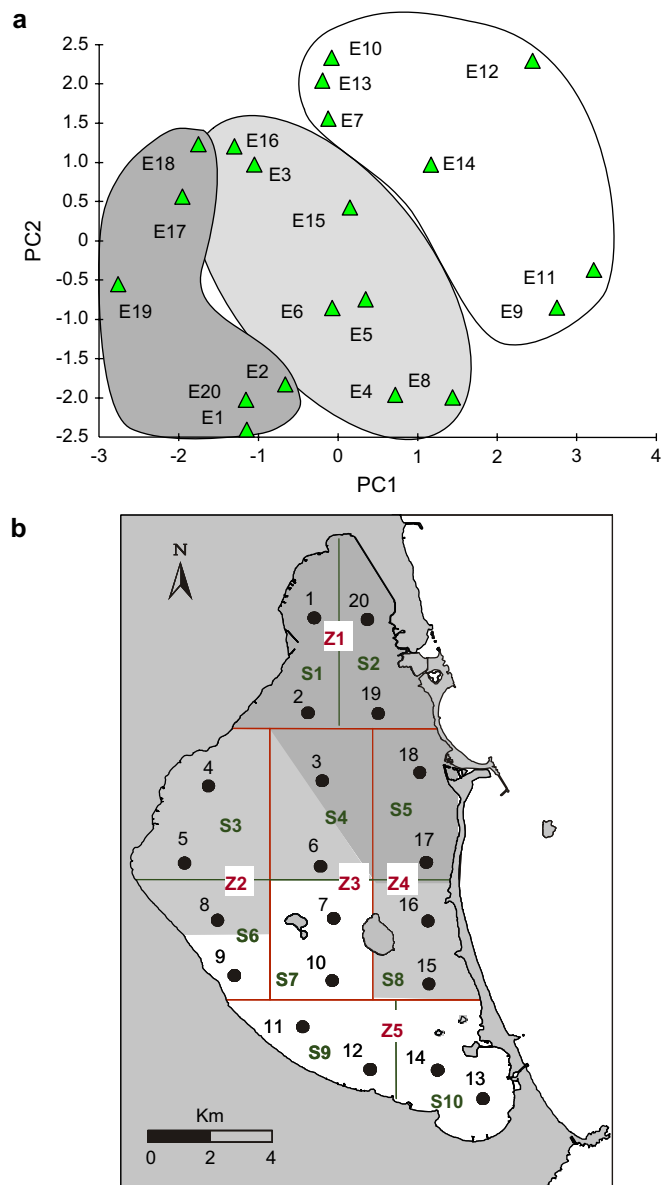
Fig. 2. (a) Representation of the first two axes of the analyses of principal components performed on ichthyoplankton mean annual abundance matrix. Sample stations are distributed according to the spatial localization represented in (b).

Table 2
Results of the pair-wise tests for the factor zone performed using ANOSIM on the ichthyoplankton annual means matrix at the Mar Menor

Groups (Zones)	R statistic	Significance level
1 vs 3	0.729	0.029
1 vs 2	0.813	0.029
1 vs 5	0.927	0.029
1 vs 4	0.76	0.029
3 vs 2	0.698	0.029
3 vs 5	0.125	ns
3 vs 4	0.094	ns
2 vs 5	0.625	0.057
2 vs 4	0.823	0.029
5 vs 4	0.542	0.057

3.2. Benthic macrophyte variability

The MDS ordination plot based on macrophyte species composition (DW biomass) pointed to a separation between depths and assemblages from different substrates and localities (stress value = 0.1) (Fig. 3). Different assemblages were characterized by well-defined groups of species, including midlittoral and infralittoral photophylic assemblages on rocky substrata and *Caulerpa prolifera* meadows in both localities. ANOSIM indicated that both localities and these macrophyte assemblages differed significantly (Table 3). However, no significant differences were found between seasons (Global $R = -0.35$, $p = 0.99$).

The seasonal variation in vegetation composition and structure of the assemblages and in the biomass of individual species showed a different pattern, depending on the assemblage and locality. The three-way analyses of variance performed considering the factors Season, Locality and Assemblage found significant differences between assemblages but did not show significant differences in biomass between seasons or localities. However, there were significant differences for the interaction Locality × Season ($P < 0.05$) (Table 4) indicating that the seasonal pattern differs in both localities. This suggests that the different assemblages show low seasonal changes in their total biomass and that these small changes are dependent on differences in the environmental conditions in the two localities that condition the biomass assemblage.

The spatial and seasonal variability in species richness and diversity was greater than the corresponding variation in total biomass. Species richness and diversity showed strongly significant differences between assemblages, with the infralittoral photophilous communities being the richest and more diverse and *Caulerpa prolifera* meadows being poorer and less diverse. Seasonal variations showed similar patterns for species richness and diversity. In both cases the highest values were reached at El Ciervo Island, in the more confined part of the
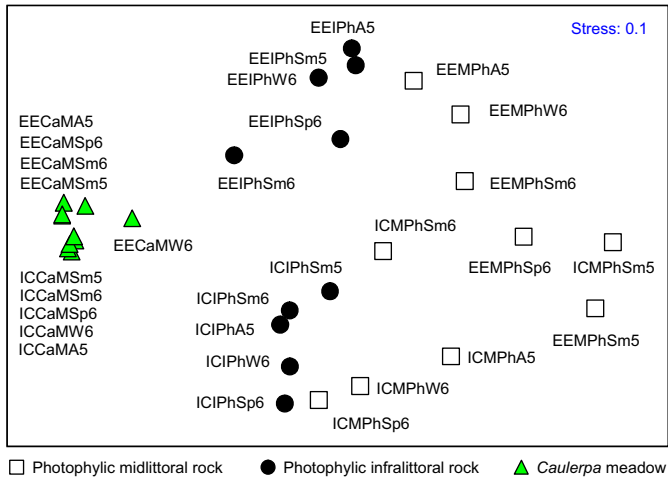

Fig. 3. MDS ordination plot based on macrophyte species composition (DW biomass) showing a separation among depths and assemblages from different substrates and localities.

Table 3
R statistic values and their significance levels (p) for pair-wise comparisons of macrophyte species composition based on the biomass using ANOSIM two-way cross-tests for differences between Locality groups (averaged across all Assemblage groups) and assemblages (averaged across all Locality groups)

Localities			Assemblages	Global R = 0.789, p = 0.000	
				R Statistic	p
					0.000
El Estacio vs El Ciervo Island	Global R = 0.789	p = 0		0.435	
			Photophytic midlittoral rock vs Photophytic infralittoral rock	0.925	0.000
			Photophytic midlittoral rock vs Caulerpa meadow	0.976	0.000

Mar Menor, and the lowest at El Estacio. Species richness showed significant seasonal variations at $p < 0.001$, with the highest number of species in spring and the lowest in autumn. Diversity also showed significant seasonal differences ($p < 0.005$) but the Tukey's test identified no significant groups. However, the fact that the interaction Locality × Assemblage × Season was also significant ($p = 0.000$) indicates that seasonal patterns in species richness and diversity were not the same at all the Localities and Assemblages (Fig. 4).

3.3. Benthic fish assemblage variability

The MDS ordination plot based on fish species abundance showed a clear separation of assemblages according to the type of substrata (Fig. 5). ANOSIM indicated that these assemblages differed significantly.

The global test in the two-way nested analysis performed for the factors Zones (Z1–Z4) in Basin (confined vs non-confined) did not identify significant differences between Zone groups (averaged across all Basin groups) (Global $R = 0.046$, $p = 0.114$) or between Basin groups (using Sector groups as samples) (Global $R = -0.5$, $p = 1$).

However, the pair-wise comparisons of species composition based on the species abundance using ANOSIM two-way cross-tests for differences between Basin groups (confined vs non-confined) (averaged across all Substrata groups) and substrata (averaged across all Basin groups) showed significant differences between basins and between all classes of substrata (Table 5). Furthermore, the pair-wise comparisons of species composition based on the biomass using two-way cross-tests for differences between Zone groups (averaged across all Substrata groups) and substrata (averaged across all Basin groups) maintained the significant differences between all substrata types and showed significant differences, especially for Z1 vs Z3 and Z2 vs Z3 (Table 6).

The results of the permutational analyses of variance performed on fish assemblage descriptors considering the factors Substrate, with three levels (rock, sand and Caulerpa meadows on mud), Basin, with two levels (north and south corresponding to non-confined and confined areas of the lagoon, respectively), and Zone (nested in Basin) confirmed the differences between the different substrata ($p < 0.001$) (Table 7). Assemblages on sand and rock showed significantly higher abundance than the assemblages of the C. prolifera meadow. At the same time, abundance showed significant differences

Table 4
Results of three-way analysis of variance performed on macrophyte assemblages at two localities in the Mar Menor, showing the effects of the factors Season, Locality, Assemblage and their interaction (Season × Locality; Season × Assemblage; Locality × Assemblage and Season × Locality × Assemblage) on the biomass, species richness and H' Shannon index of diversity, indicating the factors at which significant variation exists. Results of Tukey's (HSD) test for individual factors are also shown indicating the homogeneous groups identified (nd: non-differentiated groups)

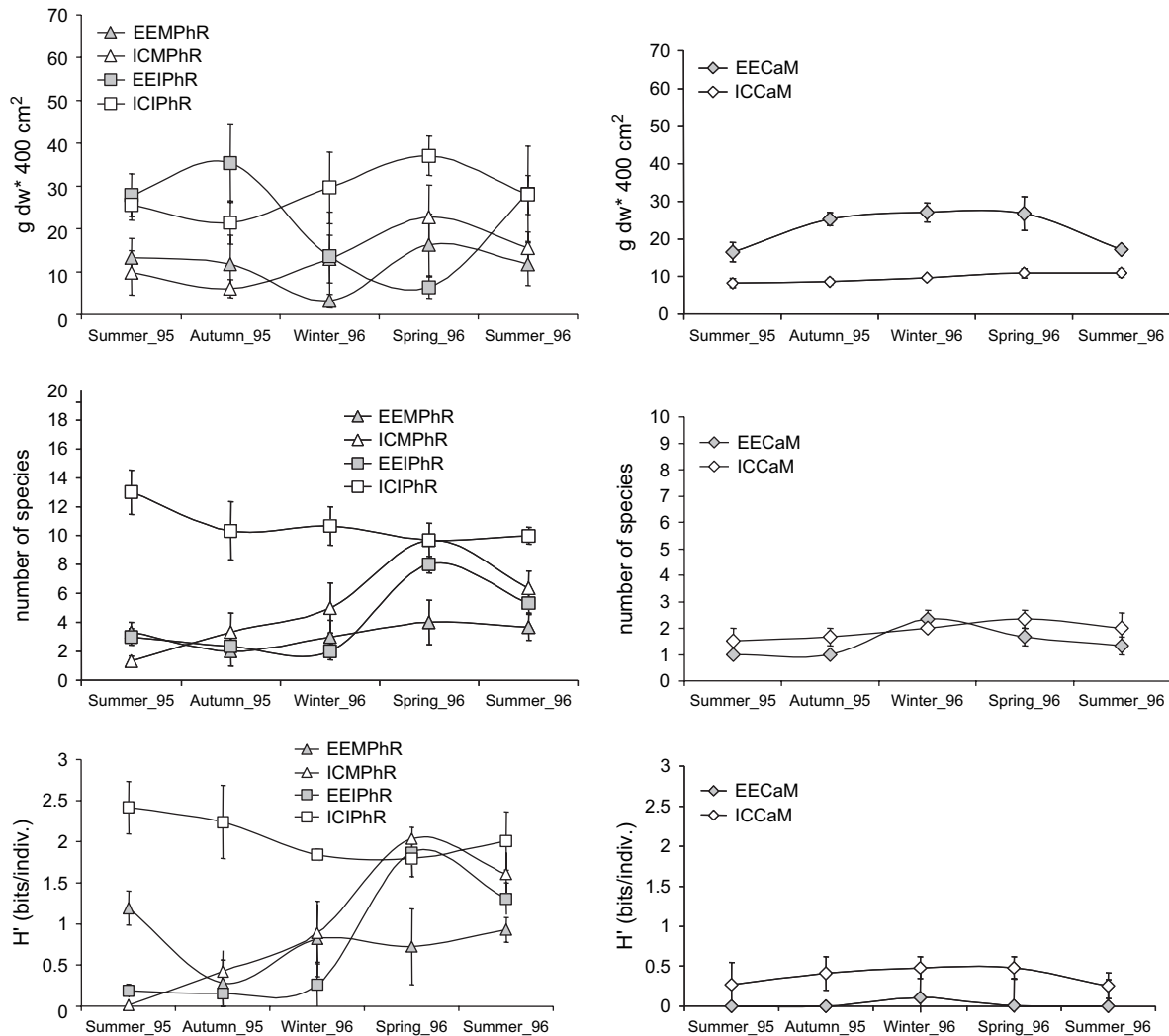| Source	df	Biomass (n = 90)		Tukey (HSD)	Species richness (n = 90)		Tukey (HSD)	H' (n = 90)		Tukey (HSD)
		F-ratio	P		F-ratio	P		F-ratio	P	
Locality	1	0.047	0.83		52.843	**0**	El Ciervo is. > El Estacio	31.107	**0**	El Ciervo is. > El Estacio
Assemblage	2	14.728	**0**	IPhR = Ca > MPhR	96.373	**0**	IPhR > MPhR > Ca	76.445	**0**	IPhR > MPhR > Ca
Season	4	0.576	0.681		5.324	**0.001**	Spring96 > summer96 = winter96 = summer95 > autumn95	4.41	**0.003**	nd
Locality × Assemblage	2	7.497	**0.001**		21.217	**0**		10.412	**0**	
Locality × Season	4	3.066	**0.023**		0.713	0.586		1.15	0.342	
Assemblage × Season	8	1.809	0.093		2.835	**0.01**		3.34	**0.003**	
Locality × Assemblage × Season	8	1.641	0.132		4.994	**0**		7.09	**0**	
Error	60									

Fig. 4. Seasonal variation of total biomass (up), species richness (middle) and Shannon *H′* diversity (down) in the macrophyte assemblages at the two localities studied in the Mar Menor lagoon. EE: El Estacio; IC: El Ciervo Island; MPhR: midlittoral photophilous assemblage on rock; IPhR: infralittoral photophilous assemblage on rock; CaM: *Caulerpa prolifera* meadow.
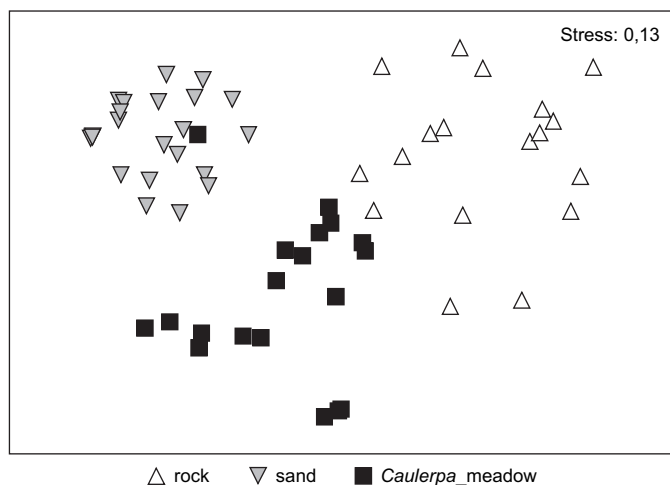

Fig. 5. MDS ordination plot based on fish species abundance showing the separation of assemblages according to the type of substrata.

between Basins ($p < 0.005$), with a higher abundance in the north basin (the less confined). On the other hand, species richness and diversity showed variability at smaller spatial scales, for the interaction Zone(Basin) $\times$ Substrate.

The results of the three-way analysis of variance showing the effects of the factors Basin, Substrate and their interaction (Basin $\times$ Substrate) and Season on fish assemblage descriptors (total abundance, species richness and *H′* diversity) confirmed the differentiation between assemblages from the three types of substrata as regards abundance and in species richness and diversity (Table 8). The less confined zone showed higher abundance, species richness and diversity than the southern confined zone. No significant monthly patterns were detected in abundance (Fig. 6) or in diversity, although seasonal patterns were significant (Fig. 7). The species richness was significantly higher in summer and lower in winter. Fish abundance showed different seasonal patterns in the different substrates and species richness also showed significant Substrate $\times$ Basin and Basin $\times$ Season interactions.

Table 5
R statistic values and their significance levels (p) for pair-wise comparisons of fish species composition based on abundance data using ANOSIM two-way cross-tests for differences between Basin groups (southern or confined vs northern or non-confined) (averaged across all Substrata groups) and Substrata (averaged across all Basin groups)

Zones			Substrata	Global $R = 0.8$, $p = 0.000$	
				R Statistic	p
Confined vs non-Confined	Global $R = 0.196$	$p = 0$	Rock vs sand	0.964	0.000
			Rock vs *Caulerpa* meadow	0.636	0.000
			Sand vs *Caulerpa* meadow	0.717	0.000

4. Discussion

To analyze the significant scales of variation in environmental or biological processes, a useful approach is to apply structured hierarchical sampling designs, where smaller-scaled phenomena or sampling structures are nested within larger-scaled ones (Anderson et al., 2005; Dethier and Schoch, 2005; Moranta et al., 2006; Moore and Fairweather, 2006). In this work we have combined this approach with multi-factorial analyses, obtaining coincident and complementary results. According to these results, coastal lagoon processes in the Mar Menor show highly heterogeneous patterns at different temporal and spatial scales.

In general, water column characteristics (including nutrient concentration) show small-scale spatio-temporal variability, from 10^0 to 10^1 km and from fortnightly to seasonally. Only suspended solids, nitrite concentration and the nitrogen:phosphorus ratio did not show any spatial pattern. Temperature showed a uniform distribution at the smallest spatial scales considered but showed differences at Zone scale (10^1 km). For salinity, ammonia and phosphate, the spatial patterns change temporally. All the parameters showed relevant patterns at all temporal scales, from fortnight to season, indicating that comparisons to detect human-influenced differences must consider natural variability, sampling over the same period at lower temporal scales or including a sufficient number

Table 6
R statistic values and their significance levels (p) for pair-wise comparisons of fish species composition based on abundance data using ANOSIM two-way cross-tests for differences between Zone groups (Z1–Z4) (averaged across all Substrata groups) and Substrata (averaged across all Zone groups)

Groups	Global $R = 0.159$, $P = 0.006$		Groups	Global $R = 0.826$, $P = 0.000$	
	R Statistic	p		R Statistic	p
Z1 vs Z2	0.107	0.096	Rock vs sand	0.973	0.000
Z1 vs Z4	0.046	0.328	Rock vs *Caulerpa* meadow	0.674	0.000
Z1 vs Z3	0.443	0.000	Sand vs *Caulerpa* meadow	0.785	0.000
Z2 vs Z4	0.091	0.238			
Z2 vs Z3	0.177	0.018			
Z4 vs Z3	−0.143	0.748			

Table 7
Results of PERMANOVA on Euclidean distances for fish assemblage descriptors (total abundance, species richness and H' Shannon diversity) showing the effects across the Mar Menor lagoon of the factors Basin, Substrate and their interaction (Basin × Substrate) and Zone (nested in Basin), indicating the factors at which significant variation exists (bold figures). Results of pair-wise *a posteriori* tests among treatments are also shown for total abundance. Basins have two levels (north and south corresponding to non-confined and confined areas of the lagoon, respectively), and Substrates have three levels (rock, sand and *Caulerpa* meadows on mud). Bold figures indicate significant values

Source	df	Total abundance				Species richness				H' diversity			
		SS	MS	F	P	SS	MS	F	P	SS	MS	F	P
Substrate	2	8.31E+10	4.16E+10	12.154	**0.0011**	2.27E+10	1.13E+10	10.448	0.4339	5.23E+10	2.62E+10	24.978	0.1455
Basin	1	3.11E+10	3.11E+10	20.074	**0.0044**	1.87E+09	1.87E+09	0.12874	0.9153	35286	35286	3.49E+02	0.9799
Zone(Basin)	2	3.01E+09	1.51E+09	0.48171	0.7508	2.96E+10	1.48E+10	35.363	**0.0151**	2.06E+10	1.03E+10	27.404	**0.0448**
Substrate × Basin	2	1.77E+10	8.84E+09	25.831	0.1015	8.24E+09	4.12E+09	0.37992	0.818	7.69E+09	3.85E+09	0.36728	0.8049
Substrate × Zone(Basin)	4	1.37E+10	3.43E+09	10.965	0.3608	4.42E+10	1.11E+10	26.393	**0.0168**	4.28E+10	1.07E+10	28.489	**0.0167**
Res	48	1.50E+11	3.13E+09			2.01E+11	4.19E+09			1.80E+11	3.75E+09		
Total	59	3.08E+11				3.07E+11				3.08E+10			

Comparison		t											P
Groups													
Rock vs sand		0.94249											0.4779
Rock vs *Caulerpa* meadow		41.273											**0.0098**
Sand vs *Caulerpa* meadow		36.156											**0.0094**

Table 8
Results of three-way analysis of variance showing the effects of the factors Basin, Substrate and their interaction (Basin × Substrate) and Season on fish assemblage descriptors (total abundance, species richness and H' diversity), indicating the factors at which significant variation exists (bold figures). Results of Tukey (HSD) test are also shown. Basins have two levels (north and south corresponding to non-confined and confined areas of the lagoon, respectively), and Seasons have four levels (spring, summer, autumn and winter) and Substrates have three levels (rock, sand and *Caulerpa* meadows on mud)

Source	df	Abundance (n = 72)			Species richness (n = 72)			H' diversity (n = 72)		
		F-ratio	P		F-ratio	P		F-ratio	P	
Substrate	2	109.530	**0.000**	Rock > sand = *Caulerpa*	13.686	**0.000**	Rock > sand > *Caulerpa*	42.15	**0.000**	Rock = *Caulerpa* > sand
Basin	1	4.176	**0.047**	North > south	47.472	**0.000**	North > south	19.25	**0.000**	North > south
Season	3	3.572	**0.021**	nd	3.537	**0.021**	Summer > autumn = spring > winter	2.481	0.072	nd
Substrate × Basin	2	1.811	0.175		6.411	**0.003**		2.838	0.068	
Substrate × Season	6	5.369	**0.000**		3.107	**0.012**		1.991	0.085	
Basin × Season	3	0.509	0.678		0.42	0.739		0.758	0.523	
Substrate × Basin × Season	6	1.779	0.123		0.21	0.972		0.802	0.573	
Error	48									
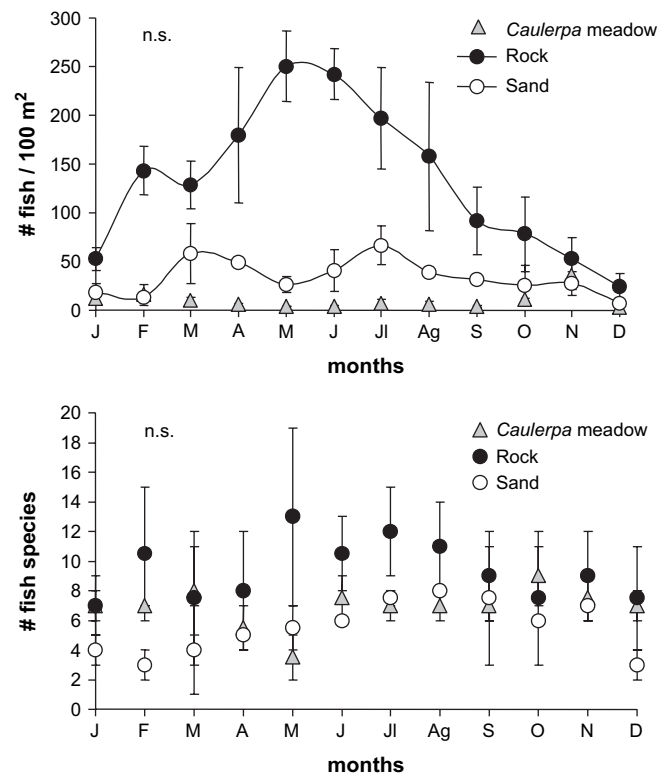
Fig. 6. Monthly variation in total abundance (a) and species richness (b) of fish assemblage in the three types of substrates sampled. Although seasonal trends are showed, differences between months are not significant at $p < 0.05$.

of random independent replications when monthly or seasonal comparisons are made.

Biological features (such as chlorophyll *a* concentration and ichthyoplankton assemblage descriptors) showed changes at all temporal scales. Chlorophyll *a* concentration and ichthyoplankton abundance showed spatial patterns at $10^0–10^1$ km, changing fortnightly, monthly and seasonally. The nearly identical scales of response of chlorophyll *a* and ichthyoplankton abundance confirms previous observations about the regular cycles in phyto- and zooplankton assemblages, despite the high degree of variability in the planktonic habitat (Smayda, 1998; Pérez-Ruzafa et al., 2005a).

Sessile benthic assemblages (macrophytes) showed significant differences between types of substrates in the same locality, and between localities according to horizontal gradients related with the degree of confinement in the lagoon, at the scale of $10^0–10^1$ km. Seasonal patterns in biomass were not significant, but the significant interaction Locality × Season ($p < 0.05$) indicates that the seasons of maximum and minimum biomass are not the same in the compared localities, but depend on local environmental conditions and not on assemblages. This coincides with the findings in the seasonal patterns in productivity in the water column, in which the interaction Sector(Zone) × Season was significant for chlorophyll *a* concentration and for phosphate concentration (Table 1). It also agrees with previous works, in which a clearer zonal pattern with a seasonal lag in maximum chlorophyll *a* concentration emerged when plurianual data were included in the
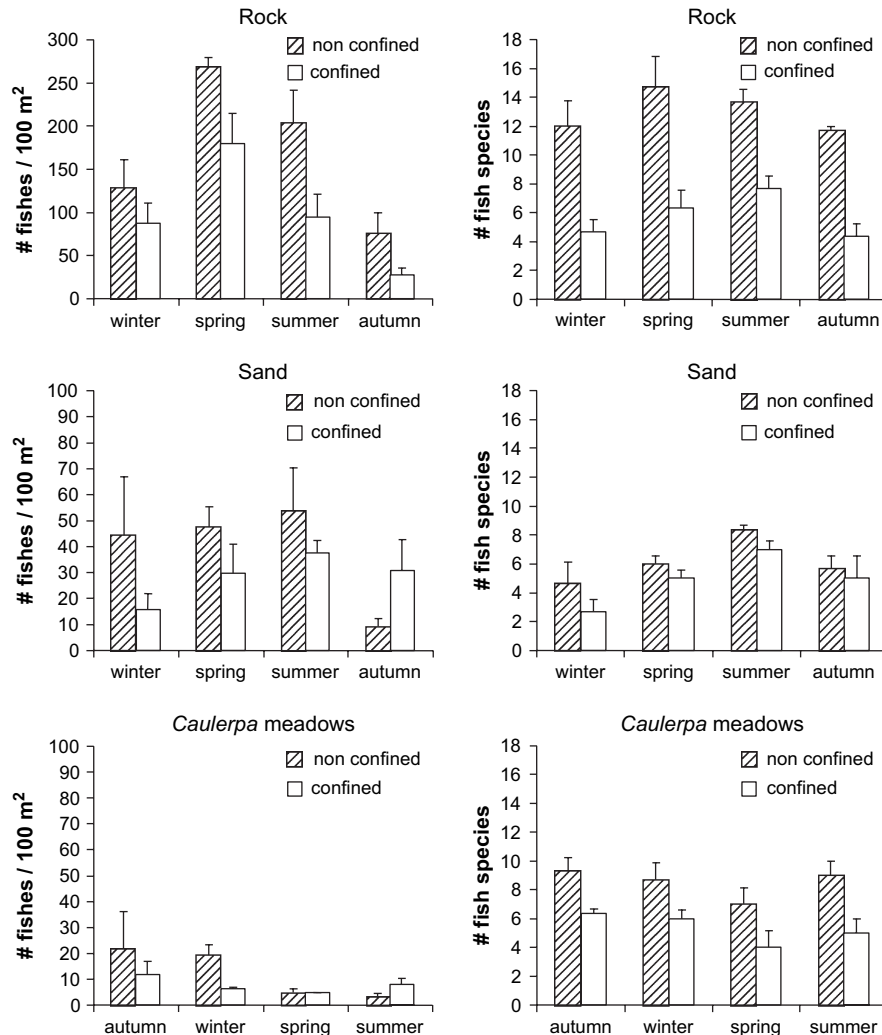
Fig. 7. Spatio-temporal variability in total abundance, species richness and diversity of fish assemblage in the three types of substrates sampled. Abundance showed significant differences between the confined and non-confined basin ($p < 0.005$) and between substrata types ($p = 0$) and seasonal patterns which differ between substrata types ($p = 0$). Species richness and diversity showed significant differences for the interaction Zone(Basin) × Substrate ($p < 0.05$). Species richness also show seasonal patterns which differ between substrata types ($p < 0.05$).

analyses (Pérez-Ruzafa et al., 2005a). Species richness and diversity showed significant variability between localities, assemblages and seasons, the different seasonal patterns depending on the assemblages (which behaved differently at each locality). According to the confinement theory, a decreasing gradient in species richness and diversity should be expected from the less confined to more confined zones in a lagoon (Guelorget and Perthuisot, 1983; Pérez-Ruzafa and Marcos, 1992). Indeed, this has been confirmed in macrozoobenthic (Pérez-Ruzafa and Marcos, 1993) and ichthyoplankton assemblages (Pérez-Ruzafa et al., 2004), and in benthic fishes (Mariani, 2001; this work), although macrophyte assemblages did not show this pattern. The finding that species richness and diversity were highest in the more confined locality and in each locality in the infralittoral rocky assemblage suggests that environmental stability is probably more important in the case of algal assemblages than colonization processes.

Vertical zonation (at scales of $10^1−10^2$ cm), which was related, as usual in marine benthic communities, with vertical

gradients in light and hydrodynamism, overlapped changes in substrata and confinement-related horizontal gradients.

Fish assemblages were highly sensitive to changes in substrate characteristics, coinciding with the findings of previous works (Pérez-Ruzafa et al., 2006; Ribeiro et al., 2006) and showed significant variability at Basin scale (10^1 km), which was related to the degree of lagoon confinement. This spatial scale agrees with the scale of variation found in other lagoons (Mariani, 2001; Ecoutin et al., 2005), although these works made no discrimination between possible differences in substrata. This could explain the high within-sector spatial heterogeneity that Ecoutin et al. (2005) found, and the differences and the low percentage values of total inertia explained by the first axis of the Correspondence Analyses in the respective works.

With respect to temporal variability, fish assemblages showed no significant patterns at monthly scale but did show seasonal patterns, suggesting that seasonal sampling or comparing samples in the same season could be sufficient for

Table 9

Relevant spatio-temporal scales in coastal lagoons according to the results of this work and other bibliographic sources (see Section 4) and main driving forces operating at each scale. Dark grey cells represent significant scales ($p < 0.05$) according to the present work; light grey cells represent scales of variability according to the bibliography but not tested in the present work; * represents the interaction between the spatial and the specified temporal scales; ? is used for proposed scales when no evidence is available

Spatial scales	Main driving forces	Water column						Benthic assemblages	
		Temperature	Salinity	Suspended solids	Nutrients and N:P ratio	Chlorophyll a	Ichthyoplankton	Macrophytes	Fishes
Biogeographic regions (10^2–10^3 km)	Climate						Species composition?	Species composition?	Species composition?
Lagoons (10^1–10^2 km)	Geomorphologic features (size, coastal development, openness, sea/freshwater influence), water balance, tidal range								
Basins (10^1 km)	Confinement: colonisation rates (faunal assemblages)/ Environmental stability (macrophytes)								
Zones (10^0–10^1 km)	Sea/fresh water influence; Seasonal weather				(Phosphate: *Month)	*Month	Abundance*Month		Species richness; H'
Sectors ($<10^0$ km)	Sea/fresh water influence; Seasonal weather; Wind and circulatory patterns		*Fortnight		(Ammonia/Phosphate: *Season)	*Season	Abundance*Season		
Substrate (10^2 m)	Substrate nature and habitat complexity								
Vertical zonation ($<10^0$ m)	Vertical gradients in light and hydrodynamic								

Temporal scales (years)	Main driving forces	Water column						Benthic assemblages	
		Temperature	Salinity	Suspended solids	Nutrients	Chlorophyll a	Ichthyoplankton	Macrophytes	Fishes
Decades (10^1–10^2)	Coastal works: inlets modifications, land claiming, river divergence/changes in agricultural practices in the watershed, urban and industrial development, waste inputs								
Years (10^0)	Interannual variation in weather and water balance								
Seasons (10^{-1}–10^0)	Seasonal weather/biological cycles and life stories								
Months (10^{-2}–10^{-1})	Seasonal weather/biological cycles and life stories								
Fortnight ($<10^{-2}$)	Wind and circulatory patterns		*Sector			*Zone	*Zone		

monitoring human induced changes in benthic fish assemblages. However, control and impact sites should be located at basin scale (closer than 10^1 km) according to the scale of influence of the open sea. Such scales may differ depending on the number and size of inlets and the water renewal rate in each particular lagoon. Long term colonization processes (Pérez-Ruzafa et al., 1991, 2006) must also be taken into account when there are modifications in the interchange of waters through inlets.

Our results imply that, in general, low scale spatial (10^0 km) and temporal (fortnight) variability must be considered for monitoring purposes and impact assessment, while monthly sampling could be sufficient to monitor the mean annual dynamic and to detect changes in pelagic biological assemblages if factor and control samples and all the replicates are taken in the same week. In the case of benthic assemblages, seasonality must be understood and low scale spatial variability (between substrate types, vertical zonation in macrophytes and between Zones (10^1 km)) may mask the detection of human impact.

The variability in these spatial scales and the effect of depth coincide with the results of Balata et al. (2006) for low rocky subtidal assemblages in the north-west Mediterranean and Dethier and Schoch (2005) in an estuarine fjord. Armonies (2000) found lower spatio-temporal scales of variability in North Sea coastal benthos assemblages, and, although they might be affected by the translocation of organisms by currents, experimental designs considering fine grain sampling could be interesting in coastal lagoons.

At geographical scales, variability between lagoons has been analyzed in 28 English coastal water bodies by Joyce et al. (2005), who found a high degree of heterogeneity in the hydrographic characteristics, vegetation and invertebrate assemblage composition, which were mainly determined by differences in salinity, sea influence, bank slope and parameters describing habitat heterogeneity. For Atlanto-Mediterranean coastal lagoons, Pérez-Ruzafa et al. (2007) studied those factors, of a set of hydrological, trophic or geomorphologic variables and indices, which best explained the composition and species richness of fish assemblages and the fishing yield. According to this work, species composition was highly variable and only six species were common to at least 70% of the 40 studied lagoons. Most of the variability (77.8%) could be explained by the differences in hydrographical and trophic variables but, at the same time, these factors were strongly correlated with geomorphological features, so that the variation due exclusively to hydrographical conditions in the lagoons was only 3.2%. The geomorphological features of the lagoons (22.2%) and the covariation between hydrographical variables and geomorphological features (74.6%) explain most of the total variation. This means that when comparing human impact in different lagoons, this large scale variability must be removed by carefully selecting lagoons with similar characteristics.

Therefore, the main conclusion is that coastal lagoon assemblages show high spatio-temporal variability (Table 9), similar to or higher than that expected in open coastal assemblages. This means that the patterns in species and community distribution, and the sources of such variability, must be taken into account when designing sampling strategies to evaluate human impact. Only if this is done, can the changes in communities caused by human pressure be differentiated from natural variability. Sources of variability should be controlled by sampling replicates at the lowest significant scale, and maintaining impacted and control sites within a suitable spatio-temporal scale.

Although we have not considered macro-invertebrate or infaunal assemblages in this work, higher variability at all scales is presumably to be expected, at least in the case of infauna (Norén and Lindegarth, 2005).

Further research should be directed at comparing different lagoon systems to determine whether the detected patterns of variability are lagoon-specific and controlled by external factors or are inherent to lagoon complex functioning and top-bottom processes determined by biological components.

Acknowledgements

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