Sewage pollution impact on Mediterranean rocky-reef fish assemblages

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Abstract

The effects of sewage outfalls on subtidal fish assemblages were studied along the NW coasts of Malta (Sicily channel, Mediterranean Sea) by means of underwater visual census. The presence of two spatially distinct outfalls discharging untreated wastewaters allowed to use a balanced symmetrical ACI (After Control/Impact) design that consisted of two putatively impacted locations and two controls, with four sites nested in each location. Surveys were performed in 2006 at two random dates. The study highlighted significant changes at both assemblage and individual species levels. Fish assemblages structures were different between controls and sewages, where total abundance of fish were higher. The responses of individual species to sewage pollution were mostly related to an anomalous increase of two small opportunistic species i.e. *Gobius bucchichii* and *Parablennius rouxi* and to a decrease of species of the genus *Symphodus*, particularly *S. roissali* and *S. ocellatus.* Moreover in correspondence of the outfalls, significant changes of the fish size distribution were detected for several species. These results support the use of fish assemblages as biological indicators for marine coastal waters and demonstrated the possibility to obtain sharp signals of environmental impact from some individual fish species.

Keywords: Sewage impact; fish assemblages; opportunistic species; sensitive species; visual census.

1. Introduction

Sewage discharges are a major problem for management of nearshore ecosystems, especially in regions like the Mediterranean Sea, which experienced in the last decades a growing urbanization of coastal areas (Airoldi and Beck, 2007). Residuals waters generated in urban settlements are usually treated in municipal treatment plants to remove fats, settleable solids and floating materials (primary treatment), to degrade the biological content of the sewage (secondary treatment) and finally to raise the effluent quality before being discharged into the receiving water bodies (tertiary treatment) (Stamou and Kamizoulis, 2009). However in some cases, untreated waters rich of particulate organic matter (POM) are discharged into shallow coastal waters with dramatic consequences on water quality, sediments (Martınez and Adarraga, 2003) and biotic communities (Terlizzi et al, 2005 and references therein). Proper treatment and disposal of sewage are necessary to avoid or at least reduce the impact on aquatic environments and associated activities, such as tourism, fishing and aquaculture, that have important socio-economic implications. However, it has been calculated that only 77% of the Mediterranean coastal citiesare served with sewage treatment plants whilst 23% of them discharge untreated waste waters directly into the marine environment (UNEP/MAP, 2004).

The strategy of the European Union (EU) in this respect is to defend and to enhance the quality of water resources (Borja, 2005). The Urban Waste Water Treatment Directive (UWWTD, 1991) of the EU prescribes the required level of treatment prior to discharge into the marine waters. The UWWTD was fully implemented in the EU-15 countries by 2005, while according to the Water Framework Directive, 2000 (WFD, 2000/60/EC) full implementation in the 10 new Member States is expected at latest by 2015.

The raw effluent discharged with sewage outfalls usually includes domestic waters and in some cases also liquid waste from industry and other human activities. The environmental impacts generated by these waste waters is generally localized around the source of discharge (Smith et al., 1999 and references therein) with the spatial scale that may change according to treatments, flow rates, and depth of release (Stamou and Kamizoulis, 2009). Increasing levels of organic matter and pollutants may impact coastal ecosystems at multiple levels of ecological organization (Reopanichkul et al., 2009) with negative socio-economic consequences (Islam and Tanaka, 2004). So far, the effects of the sewage pollutants have been widely documented at biochemical, cellular, organ and organism levels (Porter and Janz, 2003 and references therein included). At the community level (see Johnston and Roberts, 2009 for a review), sewage effects have been traditionally studied on benthic assemblages, especially soft-bottom invertebrates, because these organisms are usually easy to sample quantitatively and show detectable responses to a variety of anthropogenic impacts (Warwick, 1993). Nevertheless, in recent years the effects of sewages have been investigated in different assemblages, such as hard substrate sessile organisms (Terlizzi et al., 2002 and references therein); parasites (Mackenzie, 1999 and references therein); plankton (Bianchi et al., 2003) and fish assemblages (Otway, 1996; Gray, 1997; Smith et al., 1999; Schlacher et al., 2005; Guidetti et al., 2002; 2003; Ribeiro et al., 2008; Reopanichkul et al., 2009). Particularly, the use of fish to reveal both natural and anthropogenic alterations to the environment has received an increasing attention in the last decades (Karr, 1981; Warwick, 1993; Whitfield and Elliott, 2002; Martinho et al., 2008). With respect to benthic invertebrates, fish display a faster response to environmental perturbations, which make them useful to evaluate pulse impacts (Guidetti et al., 2003). In addition, the fish fauna has a pretty simple taxonomy, which is widely recognized as an advantage for bio-monitoring studies (Stephens et al., 1988, Warwick and Clarke, 1993).

In the Mediterranean Sea, only two studies have specifically examined the effects of sewage pollution on littoral fish assemblages (Guidetti et al., 2002; 2003). Both these investigations were conducted by underwater visual census and an ACI (After Control/Impact) asymmetrical design, which basically consists in comparing fish assemblages at one impacted vs multiple control locations. According to these studies, sewage pollution can alter the spatio-temporal distribution of fish assemblages, with both quantitative and structural changes. Planktivorous species and particulate feeders usually respond with increasing densities in correspondence to the outfalls whilst the abundance of other groups of species, such as some sparids and labrids, can be negatively influenced.

The monitoring of biological assemblages has long been used to detect environmental impacts (see Ball and Mudge, 2008 for a complete review) and several studies, carried out in different marine ecosystems and at different latitudes, already evidenced changes of fish assemblages in response to sewage outfalls and organic enrichment (Otway, 1996; Gray, 1997; Smith et al., 1999; Schlacher et al., 2005; Ribeiro et al., 2008; Reopanichkul et al., 2009). Presently, the detection of such impacts at assemblage level has been progressively improved by the evolution of multivariate analyses and calculation systems. Identification and monitoring of single indicator species has long been a practice that is still considered as extremely useful in environmental impact studies (Ball and Mudge, 2008), but this approach, which requires the identification of taxa particularly sensitive to specific sources of pollution, has been rarely applied to fish (Becker et al., 1981; Spies, 1984). Here we studied the effects of sewage pollution on Mediterranean rocky-reef fish by using both multivariate and univariate approaches. Our main aim is to test the hypothesis of an impact on fish assemblages and describe putative sewage-related changes. Special focus was given toward species-specific responses to these sources of pollution.

2. Materials and methods

2.1. Study area

Fish surveys were carried out on rocky bottoms, at two outfall locations and at two control locations along the NW coasts of Malta (Mediterranean Sea; Fig 1). At the time of the study (July-October, 2006), the two outfall locations (‘ic-Cumnija’: 35°58'11"N, 14°20'11"E and ‘Anchor Bay’; 35°57'52"N, 14°20'17"E; Fig. 1) were exposed to discharge of untreated sewage. The sewage network in Malta is a combined system collecting both domestic and industrial wastes, as well as storm water runoffs. During the present study, the northern Malta catchment of wastewater, serving approximately 51,000 population equivalents, was conveyed mainly to the outfalls of ic-Cumnija and Anchor Bay. Both outfalls were not equipped with submarine pipelines and discharged untreated waters directly into the sea waters close to the shoreline. The daily rate of discharge for these outfalls approximately summed up 6700 m^3/day, the outfall at Anchor Bay discharging no more than 10% of this volume (V. Axiak pers. comm.). Control and outfall locations had similar topographic characteristics such as a gently declining slope and heterogeneous substrates composed by a mosaic of rocks and boulders sometimes interrupted by sandy areas.

A clear increase of sedimentary organic materials was observed in the vicinity of the outfalls, where they covered the rocky bottoms as a result of the flocculation of suspended solids in the effluents. The extent of contamination of surface waters by faecal coliforms in the vicinity of both discharge points, assessed in other investigations (V. Axiak, unpublished data), is given in Fig. 1.

2.2. Data collection

For the present study we used a variation of the stationary point count method described by Harmelin-Vivien at al. (1985) and then modified by Bohnsack and Bannerot (1986). Divers took up a position some distance from a semicircular area of 40 m^2 and all fishes that were in or passed through the volume above (3 m high), during a time interval of maximum 5 minutes, were counted. The area was then closely searched for cryptic or small fishes with the help of torchlight. The sampling radius was estimated by laying down a tape measure (of 5.05 m) before each trial. All counts were performed on pure rocks (no sand, no seagrasses) within a depth range of 5-8 m and during day time between 10:00 and 16:00 h. The stationary census method was chosen among other suitable visual techniques, as the most appropriate to the field conditions of the study, i.e. a highly heterogeneous habitat (Harmelin-Vivien *et al.*, 1985; Bohnsack and Bannerot, 1986; Guidetti et al., 2005) and variable levels of water turbidity. The original method of Harmelin-Vivien at al. (1985) was slightly modified so that only the area in front of the observer (a semicircular area instead of a circular one) was censused. This was undertaken to limit the diver's movements that would have caused the re-suspension of the sedimentary materials in proximity of the outfalls. Fish abundance was estimated by counting single specimens to a maximum of 10 individuals, whilst the abundance of schooling species was estimated in abundance classes (11–30, 31–50, 51–100, 101–200, 201–500, >500 individuals), using the mid-point of each category for the data matrix (Harmelin-Vivien *et al.*, 1985; Guidetti et al. (2003). Size of the single individuals was estimated according to three size classes: small (S), medium (M) and large (L), each corresponding to one-third of maximum total length reported in literature (Tortonese, 1975). The stationary method allowed observers to carefully assign each recorded individual to one size class, with the exception of *Chromis chromis, Boops boops* and *Spicara* spp., because of intrinsic difficulties in visual estimation for these schooling species that occupy the water column.

In order to avoid contamination with polluted waters, dives were performed by means of dry suits and helmets (in both impacted and control locations), in accordance to international safety standards (Amson, 1991).

2.3. Experimental design

The BACI (before-after control-impact) approach (Underwood, 2000) provides the ideal system to assess environmental impacts. Unfortunately, 'before data' are seldom available so that ACI (After-Control/Impact) designs and alternative analytical procedures are often applied to carry out post-impact investigations (Underwood, 1991). Here, the presence of two sewage outfalls discharging untreated waters allowed to use a balanced symmetrical ACI design that was hierarchical and spatially replicated. The same experimental design was applied at 2 dates chosen at random across the study year (*Time 1* = July 2006; *Time 2* = October 2006). The factor 'Time' was not included in the design that consisted of three factors: 'Impact versus Control' (IvC), 2 levels, fixed; 'Location', 2 levels nested in 'IvC'; 'Site', 4 levels nested in 'Location'.

Locations putatively impacted by sewages (I1 and I2) were situated at a distance of about 50-100 m from the outfalls. Controls (C1 and C2) were randomly selected about 2 km north (C1) and south (C2) of 'I' locations. Control locations were located on either side of the outfall and were chosen at random from a set of possible locations with comparable topographic conditions to those occurring at the outfall, as mentioned above. Four sites were randomly chosen within each location at about 50m apart from each other. At each site, finally, four replicate counts were performed, during each of the two sampling times, for a total of 128 fish counts.

2.4. Statistical analyses

Statistical analyses were carried out on both multivariate and univariate data sets. Whole assemblage structures (abundance data) were analysed using a three-way permutational multivariate analysis of variance (PERMANOVA; Anderson et al., 2001, 2008), according to the design reported above. In order to reduce the weighting of abundant species (e.g. those forming large schools) and increase that of rare taxa, data were log(x+1)-transformed. When there were not enough permutations, p-values were obtained by Montecarlo tests. The Similarity Percentages procedure (SIMPER) was used to identify the species mostly contributing to the dissimilarity between I and C locations and only those variables whose contribution exceeded an arbitrary chosen threshold value of percent dissimilarity > 3% were shown.

In addition, a one-way distance-based PERMANOVA based on Euclidean distance was used to investigate patterns of distribution of total abundance of fish and species richness, avoiding any assumption about the distribution of the variable. Significance was set at $p = 0.05$, p-values being obtained using 9999 permutations of residuals under a reduced model (Anderson, 2001). Non-metric Multidimensional Scaling (nMDS) (Clarke, 1993) based on the Bray-Curtis similarity matrix was used to visualize the ordination of samples within a two-dimensional space. Bubbleplots, superimposed to the above mentioned nMDS ordinations, were used to visualize the distribution of the species whose abundances were significantly different according to the factor "IvC".

Size structures of the most represented species (i.e., the species whose percent abundances were, in each count, higher than 0.1%) were reported in histograms, as percentages of each size class. This was undertaken in order to account for the relative contribution of each size class regardless of the actual fish population size, according to Guidetti et al. (2002). Differences in the size-frequency distribution among control and impacted sites during each sampling time were tested by a χ^2 test.

To test for differences in total density of relevant (identified by SIMPER) species among impacted and control locations a one-way PERMANOVA based on Euclidean distance measure was performed.

All the statistical analyses were performed with PRIMER 6+PERMANOVA software package from Plymouth Marine Laboratory, UK.

3. Results

3.1. Assemblage level analyses

Overall, 39 fish taxa were identified (Tab. 1), accounting for 9767 individuals. Thirty-five taxa were on the whole recorded at C whereas 36 taxa were registered at I. A number of 31 taxa was common to both Control (C) and impacted (I) locations, whereas 4 and 5 species were exclusive of C or I, respectively. At both control and sewage locations, the most represented families were Labridae and Sparidae (11 and 9 species at C; 10 and 7 species at I, respectively). However, in correspondence of the location I1 (ic-Cumnija, where the largest outfall is located), only 5 species of Labridae were recorded.

PERMANOVAs showed that, both in Time 1 and 2, fish assemblages were significantly different between I and C locations (Tab. 2), and that a significant variability was found at both spatial scales of locations and sites. As a whole, 13 species out of 39 taxa were selected as important in discriminating between fish assemblages at C and I locations, at both sampling times (Table 3). Major contributors to the dissimilarities between C and I were planktivorous fishes such as *C. chromis* and *O. melanura*; the sparids *S. sarpa* and *D. vulgaris*; the cryptobenthic *G. bucchichii* and *P. rouxi* and several labrids: *S. ocellatus, S. roissali, C. julis*, *T. pavo, S. tinca.*

According to one-way PERMANOVA applied to the whole dataset (*Time 1* plus *Time 2*), species richness (S) showed no significant differences between I and C (Pseudo-F = 0.393; p = 0.532), whereas the number of individuals (N) was significantly higher at I than at C (Pseudo-F = 12.57; p = 0.0001), with I > C.

The nMDS ordinations of sites' centroids (Fig. 2a) showed a clear-cut separation among sewage-impacted and control locations during each sampling time. This separation was more pronounced for the largest outfall (I1). The stress of the plots at *Time 1* and at *Time 2* (=0.07 and =0.12, respectively) was low, with no real prospect of a misleading interpretation. Figure 2a also highlighted a coherent separation between all the different locations during each sampling time.

3.2. Species level analyses

The gobid *G. bucchichii* and the blennid *P. rouxi,* were extremely abundant at I locations: as a whole, 996 individuals of *G. bucchichii* were censused in 58 out of 64 fish counts (92.6%) (with a maximum abundance of 100 individuals 40 m^{-2}) and 245 individuals of *P. rouxi* were found in 43 out of 64 fish counts (67.2%) (with maximum abundances of 20 individuals 40 m^{-2}). Conversely, only one individual of *G. bucchichii* and no *P. rouxi* were observed at control locations.

On the contrary, the abundance of some labrids of the genus *Symphodus* was negatively affected by the sewages. Fifteen individuals of *S. ocellatus* (with frequency of occurrence of 10.9% and maximum abundance of 3 individuals 40m^{-2}) and 10 individuals of *S. roissali* (with frequency of occurrence of 14.1% and maximum abundance of 2 individuals 40m^{-2}) were observed at the I locations, whereas 201 individuals of *S. ocellatus* (with frequency of occurrence of 84.4% and maximum abundance of 20 individuals 40m^{-2}) and 85 individuals of *S. roissali* (with frequency of occurrence of 73.4% and maximum abundance of 5 individuals 40m^{2}) were recorded at the C locations. When one-way PERMANOVA was used to compare the abundance of single species, 5 species resulted significantly more abundant at I than at C, i.e. *G. bucchichii* (Pseudo-F = 24.365; p = 0,0001); *P. rouxi* (Pseudo-F = 22.182; p = 0.0001); *B. boops* (Pseudo-F = 11.222; p= 0.018); *C.*

julis (Pseudo-F = 15.261; $p < 0.0001$) and *D. vulgaris* (Pseudo-F = 9.014; $p = 0.0027$) whilst only 2 species resulted significantly less abundant at I than at C, i.e. *S. ocellatus* (Pseudo-F = 11.768; $p = 0.0013$) and *S. roissali* (Pseudo-F = 23.69; $p = 0.0001$).

The distribution of *G. bucchichii* and *P. rouxi* (Fig. 2b), superimposed to the nMDS of Fig. 2a, is clearly skewed to the right side indicating a positive response to the sewage. Conversely, the distribution of *S. roissalii* and *S. ocellatus* were skewed to the right side of the plot, that is towards the C locations (Fig. 2c).

The size structure of the numerically most important species at C and at I during each sampling time is given in Fig. 3. In the impacted locations, some species showed a size distribution significantly skewed toward medium and/or large individuals during one (*S. scriba*, Time 1: χ^2=30.4, P<0.001; *S. cretense*, Time 1: χ^2=38.6, P<0.001; *D. vulgaris*, Time 1: χ^2=30.9, P<0.001) or both sampling periods (*O. melanura*: Time 1: χ^2=132.0, P<0.001, Time 2: χ^2=16.4, P<0.05). Other species showed an opposite pattern, with small and medium sized individuals respectively more and less abundant than expected (*C. julis*, Time 1: χ^2=30.5, P<0.001; *D. sargus*, Time 1: χ^2=14.4, P<0.05; *M. surmuletus*, Time 1: χ^2=60.4, P<0.001; *S. salpa*, Time 1: χ^2=76.6, P<0.001).

4. Discussion

4.1. Assemblage level impact

Multivariate analysis detected a clear difference between fish assemblages at locations at the sewages and those at controls. Significant sewage-related alterations were observed in spite of a significant spatial variability (at location and site scales). Univariate analyses evidenced significant changes in the overall abundance of individuals, but not in the species richness. Species richness, from this perspective, is considered a weak indicator of the effects of sewage pollution (Grigg, 1994) even if, beyond a threshold, strong disturbances may cause the disappearance of sensitive species (Smith et al., 1999). On the contrary, total abundance of fish was significantly higher at sewages than at controls, in agreement with previous environmental impact studies (Grigg, 1994, Hall et al., 1997, Guidetti et al., 2003). This response to sewage pollution is partially caused by the attraction of large schools of planktivorous and particulate organic matter feeders in correspondence of the polluted sites (Guidetti et al., 2003). Overall, the observed changes in both abundance and multivariate structure of sewage impacted fish assemblages in Malta are coherent with previous investigations (Guidetti et al., 2003; 2003).

Effects of sewage discharges on fish assemblages are also presumably associated with profound modifications of other biotic components, such as benthic invertebrates and macroalgae (Fabricius et al., 2005), with serious alterations of the structural and functional attributes of the ecosystem being likely to occur in proximity of sewages (Reopanichkul et al., 2009). Overall, changes in fish assembleages, in other biotic components and ecosystem functions, though spatially localized, are expected to have negative socio-economic effects, especially in countries like Malta where marine-based tourism represents the main economical source of income. It is, therefore, recommended that appropriate treatment and run-off control of these nearshore discharges are set up to avoid ecological impacts and economical losses.

4.2. Species level impact

It is known that organisms may vary in their response to pollution (Ball and Mudge, 2008 for a complete review). Some of them may be exceptionally sensitive and unable to face polluted conditions, others can tolerate various levels of stress and others, indicated as 'opportunistic' or even 'ruderal' species, may flourish in highly impacted areas (Grime, 1977). Changes in the abundance of these species can provide signals of the degree of community change, before the majority of organisms are seriously affected (Mackenzie, 1999). Such information has been frequently provided by environmental impact studies (Mackenzie, 1999; Bustos-Baez and Frid, 2003) and continuous efforts have been devoted for searching the best indicator taxa. Nevertheless, the bulk of these studies focused on benthic sessile invertebrates, which are still considered the most suitable indicator organisms in the marine environment (Wilson, 1994; Jeffrey, 1994; Jones and Kaly, 1996, Rygg, 1985; Occhipinti-Ambrogi and Forni, 2004; Ugland et al., 2008), whereas very few investigations have been carried out on fishes (Becker et al., 1981; Spies, 1984).

In the present study, that used fish monitoring to possibly detect sewage pollution nearshore, individual species displayed quite marked responses. In particular, *G. bucchichii*, and *P. rouxi* were far more abundant at polluted locations, while *S. roissali* and *S. ocellatus* showed an opposite pattern. Both *G. bucchichii* and *P. rouxi* are common but usually not very abundant along the Mediterranean rocky sublittorals (Azzurro et al, 2007; Pais et al., 2005; La Mesa et al, 2006; Guidetti et al., 2005). These two small fish species are easy to recognize in the field and they are not targeted by fishing, both features relevant for an indicator species (Linton and Warner, 2003). Moreover, they are strictly site-attached fish species and the respective families (Gobiidae and Blenniidae) could be useful to monitor point source impacts (Guidetti et al., 2003). Biological communities, including fish assemblages, in polluted sites are frequently dominated by a small number of species displaying opportunistic life-history strategies or high tolerance to stressful conditions (Stephens et al., 1988). Dominant species in polluted conditions may be favored by opportunistic life-history strategies or from high tolerance to a particular environmental parameter. Possibly *G. bucchichii* and *P. rouxi* are capable of tolerating organically enriched conditions and, with respect to the other sympatric cryptobentic species (La Mesa et al., 2006), they might have some particular traits that allowed them to flourish under the environmental conditions generated by the input of untreated sewage waters. Unfortunately, the paucity of fundamental knowledge on the biology and ecology of these species (Miller, 1986; Zander, 1986) and the absence of detailed environmental data in correspondence of the outfalls limit our possibilities to formulate hypothesis regarding the driving mechanisms that are at the basis of their success. Some benefits would be originated from the opportunistic exploitation of some trophic resources associated to the sewage, as in the case of the Dover sole *Microstomus pacificus,* whose abundance was correlated with the organic content of sediments (Becker et al., 1981; Spies, 1984). Considering that wastewaters can modify the rate of sedimentation and generate sediments rich of mud and organic materials (Martinez and Adarraga, 2003), it is possible that *G. bucchichii* and *P. rouxi* would directly benefit from the organic matter input as a food supplement or even feed on opportunistic invertebrates, such as some species of polychaetes like Capitellidae, that usually proliferate in sediments enriched with organic matter (Tomassetti and Porrello, 2005).

A negative effect of organic pollution on fish abundance was observed in the labrids *S. ocellatus* and *S. roissali.* These species are a common component of the Mediterranean rocky-reef fish assemblages all over the Mediterranean coasts (Tortonese, 1975). It is difficult to argue about the causes that are at the basis of the decline of their abundances in proximity of the outfalls. We know, however, that these species have specific ecological requirements and that they use benthic macroalgae to build nests (Tortonese, 1975). Previous investigations documented a negative effect of sewages on the macroalgal communities (Arévalo et al., 2007). The lower abundance of *S. ocellatus* and *S. roissali* in the impacted locations would thus indirectly determined by some sewage-related alterations in the local macroalgal communities, however direct effects of the sewage pollutants cannot be excluded *a priori.*

Finally, the analysis of the fish sizes also showed some effects of the sewage outfalls. At impacted locations, the size distribution of some species (i.e. *S. scriba*, *S. cretense*, *D. vulgaris* and *O. melanura*) resulted to be skewed towards large individuals whilst others (*C. julis*, *D. sargus*, M. *surmuletus*, *S. salpa*) showed an opposite pattern. This only observation is probably weak to infer about the mechanisms that are behind these effects and further studies are certainly needed to clarify this question. Nevertheless, we know that early and young life stages (i.e. larvae, settlers and juveniles) are usually more sensitive to environmental perturbation than adults (Stephens et al., 1988) and that sewage pollution can have drastic consequences on the survivorship of these stages with consequent alteration of the size structure (Kingsford and Gray, 1996). At the same time, the release of particulate organic matter could attract juveniles with opposite effects (Gray, 1996; Gray, 1997).

Future investigation could verify the effects of sewage on the recruitment of Mediterranean littoral fishes and take into account temporal variability, that is know to be a relevant aspect in this kind of studies (Guidetti et al., 2003).

5. Conclusions

The observed changes at both assemblage and single species levels reinforce the importance of fish as biological indicators of anthropogenic changes in marine coastal waters, especially under the condition of organic enrichment. As a matter of fact, the fish component is already considered among the biological quality elements of the Water framework Directive (WFD, 200/60/EC; Ribeiro et al., 2008), and hopefully the present study could contribute to its implementation, that is an ongoing process in time.

The response given by *G. bucchichii* and *P. rouxi* and by *S. roissali* and *S. ocellatus* is tempting in suggesting to look at them as both positive and negative indicators (*sensu* Rygg, 1985) but further efforts are needed to document whether the same response occurs elsewhere, how these species behave along gradients of pollution and to understand the related ecological mechanisms. Moreover, relying on single indicator species can have several disadvantages (Breine et al., 2007) and we cannot neglect multivariate information provided by the whole assemblages, that is essential to obtain an adequate picture of system and the related responses to impacts. Certainly, the evaluation methods for anthropogenic impacts and for assessing the ecological quality status of Mediterranean coastal waters can take an advantage when the fish component is considered and when both communities and individual species are monitored. Beyond this general suggestion, we demonstrated the possibility to obtain sharp signals of change from some individual species, an evidence that strengthen our knowledge on the use of littoral Mediterranean fishes as biological indicators.

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Table captions

Table 1.

Mean abundances and S.E. of the taxa recorded at control (C1, C2) and sewage-impacted (I1, I2) locations throughout the study as calculated from *Time 1* and *Time 2* aggregated data. Species whose distribution was showed in Fig 2b (●) and Fig 2c (○) were pointed out.

Table 2.

Permutational multivariate analysis of variance based on the Bray Curtis dissimilarity measure for Log (x+1) transformed abundance data. The test was done using 9999 permutations under the reduced model. * P<0,05; ** P<0,01; *** P<0,001.

Table 3.

Results of SIMPER analysis showing fish species contributing most (percentage contribution > 3%) to dissimilarity between sewage-impacted and control locations on the two sampling periods (*Time 1* and *2*). Species whose distribution was showed in Fig 2b (●) and Fig 2c (○) were pointed out. Average dissimilarity between impacted and control locations: 74.33 during *Time 1* and 60.19 during *Time* 2.

Figure captions

Fig. 1.

Study area and position of sewage-impacted (I1, I2) and control (C1, C2) locations. Black triangles indicate sewage outfalls (at ic-Cumnija and at Anchor Bay, respectively). The extent of surface waters which are generally exposed to levels of faecal coliforms in excess of 1000 CFU/100mL is also shown (light grey area).

Fig. 2.

(a). Non-metric Multi Dimensional Scaling (nMDS) ordination of site's centroids comparing fish assemblages at sewage-impacted (I1 and I2) and control (C1 and C2) locations, during *Time 1* (July 2007) and *Time 2* (October, 2007). Δ C1; ○ C2; ● I1; ▲ I2

(b) the same nMDS of (a) with circles representing log-transformed number of individuals/40m^2 of *Gobius bucchichii* and *Parablennius rouxi* during *Time 1* and *2*.

(c) the same nMDS of (a) with circles representing log-transformed number of individuals/40m^2 of *Symphodus roissali* and *Symphodus ocellatus* during *Time 1* and *2*.

Fig. 3.

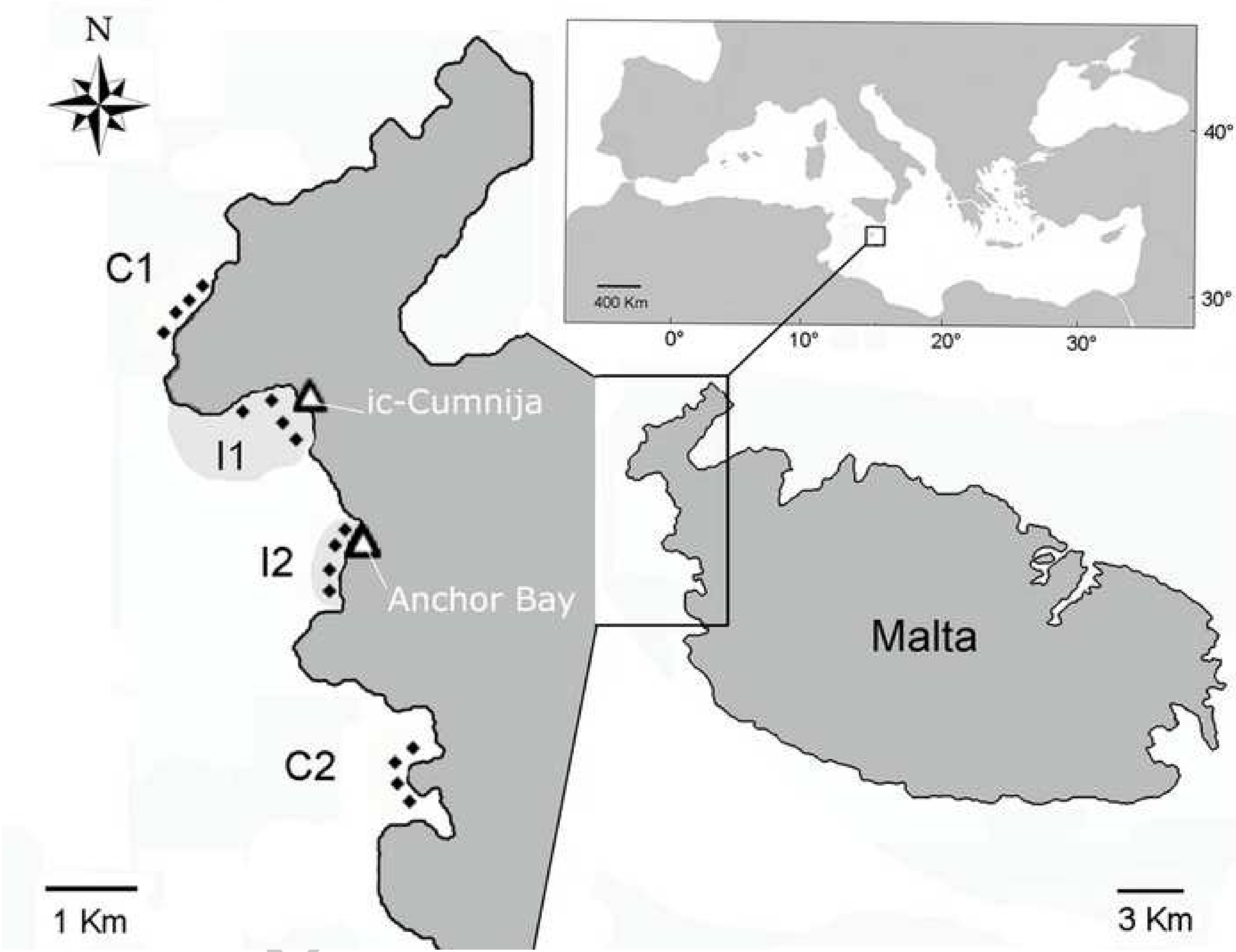
Size structure of the most represented species at control (C) and sewage-impacted (I) locations. Percentages are calculated on the species average values taken for each sampling period (Time 1 = July 2006; Time 2 = October 2006).

□ Large; ▨ Medium; ■ Small

Family Species	C1 Mean	 S.E.	C2 Mean	 S.E.	I1 Mean	 S.E.	I2 Mean	 S.E.
Apogonidae								
Apogon imberbis	0.16	0.08	0.81	0.37	0.80	0.63	0.30	0.15
Blennidae								
● *Parablennius rouxi*	–	–	–	–	5.53	1.00	2.13	0.50
Parablennius zvonimiri	–	–	–	–	0.03	0.03	0.13	0.06
Centracanthidae								
Spicara maena	–	–	0.73	0.63	0.28	0.25	0.11	0.11
Gobiidae								
● *Gobius buchichii*	–	–	0.03	0.03	21.34	4.29	8.83	2.58
Mugilidae								
Mugil sp.	–	–	–	–	0.36	0.27	0.88	0.67
Mullidae								
Mullus surmuletus	0.17	0.12	1.28	0.67	1.59	0.70	0.14	0.11
Labridae								
Coris julis	0.89	0.23	1.28	0.31	2.36	0.49	3.91	0.57
Labrus merula	0.19	0.07	0.09	0.05	–	–	0.09	0.05
Labrus viridis	0.25	0.09	–	–	–	–	0.03	0.03
Symphodus cinereus	–	–	0.09	0.07	–	–	–	–
S. doderleini	–	–	–	–	–	–	0.03	0.03
Symphodus mediterraneus	0.13	0.06	0.09	0.05	0.06	0.04	0.59	0.20
Symphodus melanocercus	0.03	0.03	–	–	–	–	–	–
○ *Symphodus ocellatus*	2.27	0.63	4.00	0.96	0.03	0.03	0.42	0.19
○ *Symphodus roissali*	1.27	0.22	1.39	0.22	–	–	0.31	0.09
Symphodus rostratus	0.16	0.07	0.61	0.19	–	–	0.06	0.04
Symphodus tinca	1.19	0.38	2.64	0.63	0.75	0.19	2.05	0.40
Thalassoma pavo	2.44	0.51	0.63	0.19	0.09	0.05	0.91	0.26
Pomacentridae								
Chromis chromis	26.78	7.25	22.01	5.66	25.97	7.22	37.15	5.38
Serranidae								
Epinephelus marginatus	–	–	0.03	0.03	0.03	0.03	0.22	0.10
Serranus cabrilla	0.09	0.05	0.03	0.03	–	–	0.03	0.03
Serranus scriba	0.53	0.10	0.92	0.20	0.59	0.10	0.66	0.14
Scaridae								
Sparisoma cretense	0.61	0.30	0.69	0.62	0.53	0.29	0.38	0.11
Scorpaenidae								
Scorpaena maderensis	0.19	0.07	0.09	0.07	–	–	0.06	0.04
Scorpaena scrofa	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.04
Siganidae								
Siganus luridus	0.09	0.05	–	–	–	–	0.34	0.26
Sparidae								
Boops boops	1.52	0.71	1.13	0.64	0.66	0.62	–	3.22
Dentex dentex	0.03	0.03	0.03	0.03	–	–	–	–
Diplodus annularis	0.09	0.05	0.19	0.08	0.30	0.12	0.81	0.37
Diplodus puntazzo	–	–	0.14	0.14	–	–	–	–
Diplodus sargus	0.31	0.16	1.02	0.26	0.63	0.29	1.13	0.40
Diplodus vulgaris	1.06	0.65	1.20	0.31	4.31	1.10	1.59	0.42
Oblada melanura	10.08	2.10	10.01	1.50	3.75	1.06	26.56	4.54
Sarpa salpa	3.70	1.29	4.36	1.51	15.27	5.76	7.72	2.93
Spondyliosoma cantharus	0.09	0.05	0.24	0.15	0.03	0.03	0.14	0.11
Sphyraenidae								
Sphyraena viridensis	–	–	–	–	–	–	0.63	0.63
Trypterigidae								
Tripterygion melanurus	0.03	0.03	–	–	0.06	0.04	0.23	0.12
Tripterygion tripteronotus	–	–	0.09	0.01	0.09	0.01	0.34	0.01

Source of variability		*Time 1*		*Time 2*	
	df	MS	Pseudo-F	MS	Pseudo-F
Impact versus Control = IvC	1	40891	4.17*	18208	3.52*
Location (IvC)	2	9798	4.77***	5174.1	2.91**
Site (Location (IvC))	12	2054	1.66**	1778.6	1.62**
Residuals	48	1239		1099.3	
Total	63				

Time 1		*Time 2*	
Species	Contr.%	*Species*	Contr.%
• *Gobius buchichii*	14.54	*Chromis chromis*	10.07
Chromis chromis	9.02	*Sarpa salpa*	9.89
Oblada melanura	8.82	*Oblada melanura*	8.81
• *Parablennius rouxi*	8.24	• *Gobius buchichii*	8.39
Sarpa salpa	5.8	*Boops boops*	6.8
Diplodus vulgaris	5.42	○ *Symphodus ocellatus*	5.82
Coris julis	5.37	*Coris julis*	5.49
○ *Symphodus ocellatus*	5	*Symphodus tinca*	5.17
Thalassoma pavo	4.44	*Diplodus vulgaris*	4.44
○ *Symphodus roissali*	3.92	• *Parablennius rouxi*	4.42
Symphodus tinca	3.76	*Diplodus sargus*	4.16
		○ *Symphodus roissali*	3.77
		Thalassoma pavo	3.41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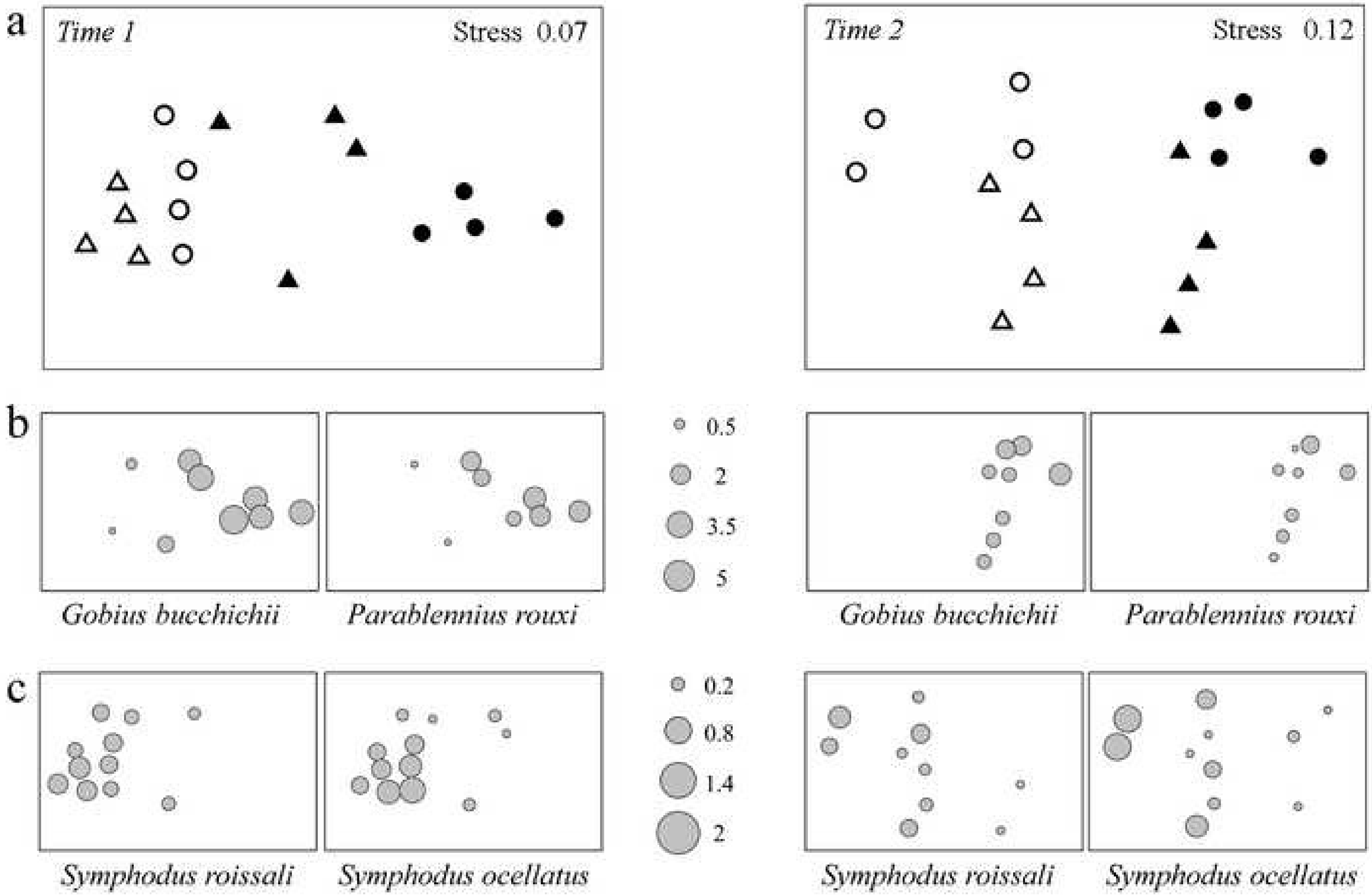
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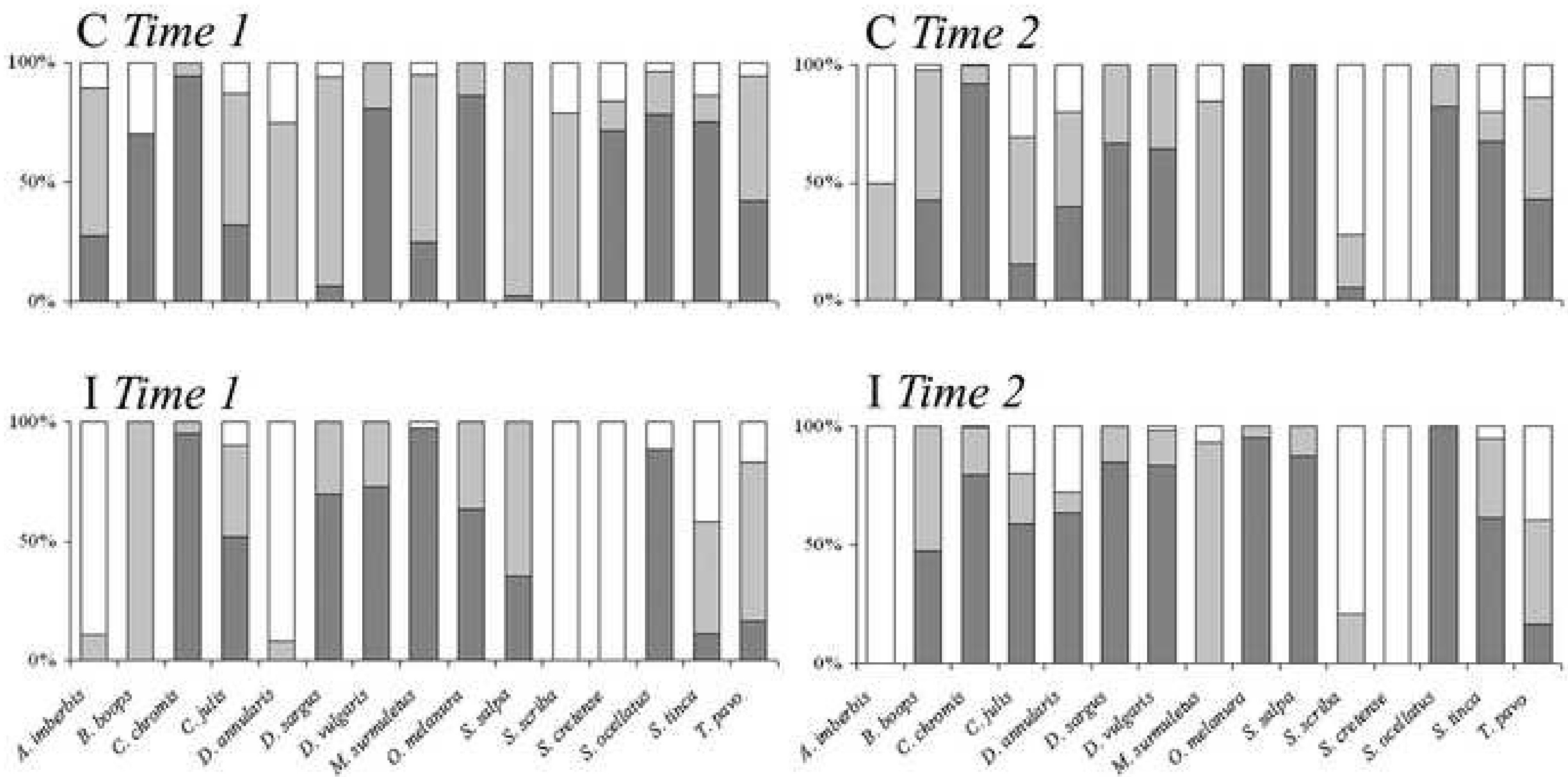
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S. scriba
S. cretense
S. ocellatus
S. tinca
T. pavo