PROPAGULE DISPERSAL AND LARVAL PATCH COHESIVENESS IN A MEDITERRANEAN COASTAL FISH

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ABSTRACT

The assessment of fish dispersal has a fundamental importance for both conservation and management of fisheries resources as it can provide crucial information for the establishment of effective marine protected areas (MPAs) and networks of MPAs. In this study we investigated otolith elemental composition in early life stages of the saddled sea bream *Obalda melanura* (Linnaeus, 1758) (Perciformes: Sparidae) in order to obtain information on its propagule (egg - larva) dispersal in the south-western Mediterranean Sea. Specifically, considering late stage larvae and early post-settlers, we investigated: (1) number of potential natal sources; (2) propagule dispersal distances and (3) larval patch cohesiveness during the last phase of the larval life. Seven natal sources were found to replenish, with different proportions, almost all the sampling sites along a stretch of coastline of ~180 km. This outcome suggests that propagule dispersal can take place at least up to ~90 km. We show also that different larval patches can merge in the pelagic environment after having travelled separately for some days. This information can provide important insights for

the understanding of fish dispersal process and support the correct establishment of spatially explicit conservation strategies such as MPAs and MPA networks in the south-western Mediterranean sea.

KEYWORDS: propagule dispersal, natal origins, patch cohesiveness, saddled sea bream, Mediterranean Sea

INTRODUCTION

Dispersal is the process by which organisms expand actively or passively the space where they live affecting patterns of connectivity (i.e. the link between sub-populations through the exchange of individuals), meta-population dynamics and ultimately species persistence (Shima & Swearer 2010) (Cote et al. 2010). The study of dispersal patterns is thus of paramount importance for marine biodiversity conservation and fisheries management as it provides fundamental information for the establishment of effective marine protected areas (MPAs) and networks of MPAs (Grüss et al. 2011) (Green et al. 2014). In many coastal fish with a bipartite life cycle (i.e. composed by a larval and a juvenile/adult phase), the life stages after settlement (i.e. when the pelagic larval phase ends with the metamorphosis into juvenile) are relatively sedentary, so that species dispersal potential is mostly determined by the propagule (i.e. egg and larva) phase (Leis 2015). Despite the huge effort being currently accomplished, the empirical measure of propagule dispersal is still extremely challenging for fish ecologists due to several issues, such as the difficulty to mark small planktonic larvae and/or to recapture them (given the high mortality rates) (Barbee & Swearer 2007, Fontes et al. 2009).

In recent years, a number of studies have been conducted using natural environmental markers for investigating fish natal origins and evaluate species dispersal capacity (Leis et al. 2011, Calò et al. 2013 for reviews). Otoliths are particularly useful for their unique feature of recording the chemical characteristics of the surrounding environment experienced by fishes at all the life stages (Barbee & Swearer 2007). Otoliths grow by a continuous deposition of layers of calcium carbonate, mainly aragonite, in a protein matrix (Campana 1999). During the deposition, trace-elements from the ambient water can substitute for calcium remaining permanently in the matrix (Campana 1999). The physical and chemical characteristics of the environment (primarily temperature, salinity and trace-elements concentration) influence the incorporation rates of some specific elements (e.g. Sr and Ba)

into the otolith matrix (Campana 1999, Walther & Thorrold 2006, Barnett-Johnson et al. 2008, Muhlfeld et al. 2012). Variations in otolith elemental composition can be used to discriminate larval groups that experienced different environmental conditions and obtain information on important early life history traits such as the number of potential natal sources, larval dispersal distances and pathways (Hamilton et al. 2008, Fontes et al. 2009, Di Franco et al. 2012, Shima & Swearer 2010, Miller et al. 2014, Shima et al. 2015, but see also Berumen et al. 2010). The analysis of the chemical composition of otolith cores (i.e. the inner portion of otoliths that forms during the embryogenesis) allows to assess the number of potential sources of propagules that supply different settlement sites along a stretch of coastline (Ruttenberg et al. 2008). From this information it is possible to infer the spatial scale over which propagule dispersal takes place, considering the distance between settlement sites replenished by the same natal sources (*sensu* Di Franco et al. 2012). The analysis of the chemical profile from the otolith edge toward its core provides information on the different environmental conditions faced by a larva backwards in time (Sandin et al. 2005). The comparison of otolith chemical profiles of larvae coming from the same patch (i.e. an aggregation of larvae) can be used to investigate the consistency of the patch during the pelagic phase (i.e. cohesiveness), that is, if larvae travelled together to the sampling site or experienced different dispersal pathways, that would result in different individual otolith chemical profiles (Shima & Swearer 2009, Ben-Tzvi et al. 2012). The knowledge of the ontogenetic cohesiveness of a larval patch can provide important insights on post-settlement demographic dynamics (Shima et al. 2015) as different larval trajectories, potentially shaped by a variable dispersal environment, can have crucial implications on the post-settlement persistence of the larval patch (Shima & Swearer 2009, Shima & Swearer 2010, Shima et al. 2015).

In this study we investigated geochemical signatures in the otoliths of early life stages of the saddled sea bream *Obalda melanura* (Linnaeus, 1758) (Perciformes: Sparidae) in order to obtain information on its propagule dispersal characteristics in the south-western Mediterranean Sea. This

fish was chosen as a model species as it is widely distributed and generally abundant in Mediterranean coastal ecosystems (Bauchot & Hureau 1986, García-Charton et al. 2004) and for its relatively high commercial value for artisanal and recreational fisheries (Harmelin-Vivien et al. 1995, Lloret et al. 2008, Félix-Hackradt et al. 2014). Specifically, considering early post-settlers and late stage larvae of *O. melanura*, we investigated: (1) the number of potential natal sources replenishing our study area; (2) propagule dispersal distances and (3) the cohesiveness of larval patches during the last phase of the larval life. This information, can provide important insights for the understanding of fish dispersal process and support the correct establishment of spatially explicit conservation strategies such as MPAs and MPA networks.

MATERIALS AND METHODS

Study area and sampling design

The study was conducted along the south-east Spanish coast (SW Mediterranean Sea). This sector is particularly relevant from a conservation perspective hosting an important biodiversity hotspot (Calvin Calvo et al. 1999).

With the aim to investigate the number of potential natal sources and propagule dispersal distances of *O. melanura* in the study area, early post-settlement individuals were sampled in July 2013 after the peak of settlement (Calò et al. submitted). Post-settlers were collected in 17 sites (stretches of 1 km of coastline), scattered within 3 zones (stretches of 30 km of coastline), as follows, from North to South: southern Alicante province (SAP, 6 sites), central Murcia region (CMR, 5 sites) and western Murcia region (WMR, 6 sites) (Fig 1). SAP and CMR host 2 of the most effective MPAs of the Western Mediterranean Sea, Tabarca MPA and Cabo de Palos e Islas Hormigas MPA (Di Franco et al. 2014a) (Fig 1). In each site 11 to 15 post-settlers (i.e. individuals of 1.1-1.8 cm in total length) were collected during snorkeling using a hand net. Specimens were sampled in shallow water habitats (i.e. <2m of depth) characterized by shadowed overhangs and steep rocks, as these are considered to be the main habitat requirements of *O. melanura* early post-settlers (Bussotti & Guidetti 2011, Félix-Hackradt et al. 2014) for evidence from our study area). All the specimens were firstly euthanized, by immersing them in a sea water solution with few drops of 96% alcohol for minimizing their suffering (Leary et al. 2013) and, after cessation of opercular movements, preserved in 70% ethanol.

Larval patch cohesiveness was investigated considering late stage larvae (pre-settlers) of *O. melanura*. Specimens were caught using light-traps that were deployed during the period of larval supply to the coast in July 2013 (Calò et al. submitted). Six light-traps were deployed in 2 sites (S1 in the SAP zone and S2 in the WMR zone, Fig. 1) and placed on an imaginary line parallel to the

coast using a buoy moored at 20 –30 m depth, depending on how close the sampling site was to the coast (distance ranging 0.3 –0.8 km). Three light-trap samples from site S1 (here named T1, T2 and T3), sampled during the same night, and one light-trap sample from site S2 (T4) contained from 8 to 11 pre-settlers each, and thus were processed in the laboratory. All the other light-trap samples were not included in the successive analyses, because they contained only 1-2 individuals and thus it was impossible to investigate any inter-individual variability in dispersal, in order to assess patch cohesiveness.

Otolith preparation and analysis

In the laboratory, the right sagittal otolith was removed from each pre-settler and post-settler, cleaned of soft tissue using plastic pins and rinsed with ultra-pure water (18.2MΩcm^{-1}). Then, otoliths were mounted sulcus side up onto a glass slide using Crystalbond^{TM} thermoplastic adhesive. Otoliths were polished with 3 µm and 1 µm Imperial lapping film through the nucleus. After polishing, otoliths were rinsed with an ultrasonic bath for 10 minutes in ultra-pure water in order to remove surface contamination.

Elemental analysis was performed using a Thermo Elemental X7 inductively coupled plasma mass spectrometer (ICP-MS) coupled to a New-Wave Research UP213 with aperture imaging laser ablation (LA) system. Otolith sections were viewed remotely on a computer screen where the area for ablation was selected. The laser was focused on the sample surface and fired through the microscope objective lens. Helium gas was flushed into the ablation cell to reduce the deposition of ablated aerosols and to improve signal intensities. The ablated aerosol was then mixed with Argon before entering the ICP torch.

Otoliths of post-settlers were used for obtaining information on the number of potential natal origins and larval dispersal distances of *O. melanura*. Post-settler otoliths were analysed in 2 different regions: the core and the edge. The core elemental composition was investigated to obtain

information about natal origins and was analysed through three vertical pits with a diameter of 30 µm (approximate size of the core) and 10 µm deep each. The otolith edge was investigated to quantify recent elemental incorporation (i.e. material laid down just before capture) that was later tested for site discrimination (see *Data analysis*) and to account for within otolith variability (Di Franco et al. 2012, Di Franco et al. 2014b). Edge portion was analysed by three horizontal pits using the same laser spot dimensions as specified before. Each spot run consisted of 62 s acquisition: 25 s blank to correct for background which was subtracted from each sample, 2 second of pre-ablation to remove surface contamination (laser at 30% power), 10 s ablation and 25 s for washout. Otoliths were placed in the ablation chamber in groups of 6, randomly selected from the 17 sampling sites, to prevent sample batch bias.

Otoliths of pre-settlers were analysed for the investigation of larval patch cohesiveness. Specifically, we compared the variability of otolith chemical profiles among individuals coming from the same light-trap sample (here considered as a larval patch). Pre-settlers otoliths were analysed through a series of 7 laser spots, following the major axis of the sagitta, and positioning each spot on a different daily growth ring from the edge of the otolith (corresponding to the otolith ring laid down during the sampling day) to the seventh last growth ring. In this way, for each pre-settler, we obtained the chemical profile of the last 7 days of larval life. We chose not to analyse the first days after hatching to avoid biased interpretation of chemical composition variability. In fact, the consideration of the earliest larval stages could have added an additional source of variation (ontogenetic) hiding or confounding the spatial variability of otolith chemical signatures, on which the patch cohesiveness analysis is based (see *Data analysis* for the complete rationale of the statistical method). The mean pelagic larval duration of *O. melanura* individuals sampled by light traps is ~11 days (Calò et al. submitted), thus we reasonably excluded from the analysis the first 3-4 days after hatching, corresponding to the yolk sack phase of the species (Antolović et al. 2010). Spot size was set to 15 µm in order to fit it with the daily rings width. Each spot run consisted of 42

s acquisition: 10 s blank to correct for background, 2 second of pre-ablation, 10 s ablation and 20 s for washout.

For both post-settlers' and pre-settlers' otoliths, instrumental precision was maintained by analysing solid glass standard material from the National Institute of Standards and Technology (NIST 610 and NIST 612) every 6 samples, carrying out a linear interpolation between the 2 consecutive sets of standards. Calcium was used as internal standard to take into account variation in ablation and aerosol efficiency. All the 7 elements analysed (^{7}Li, ^{24}Mg, ^{45}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb) were expressed as ratios relative to ^{44}Ca. Detection limits were calculated from the concentration of analyte yielding a signal equivalent to 3× the standard deviation of the blank signal for each of the elements (Tab. 1). Recoded values of Li, Mn, Zn and Pb were consistently below the detection limits and thus were excluded from the analysis.

Data analysis

Otolith elemental concentration data were converted to molar concentrations and log (x+1) transformed.

The number of post-settler potential natal origins was investigated analysing otolith core elemental concentrations (as a proxy for identifying the existence of single or multiple areas of origin (Di Franco et al. 2012) for a total of 230 individuals. No Mn:Ca spike, generally considered as an indicator of the core location (Brophy et al. 2004, Ruttenberg et al. 2005), was recorded in almost all the otolith cores analysed. For this reason the centroid of the three core pits for each otolith was considered for the subsequent analysis, as it has been done in other species where no Mn:Ca spike seems to not be an effective “core localizer” (Papetti et al. 2013, Guidetti et al. 2013). The SIMPROF ('clustsig' package, R software) test was carried out on the output of a cluster analysis (based on Euclidean distance) to determine which clusters were significantly different at the 5% level. Similarity percentage (SIMPER, 'vegan' package, R software) was used to assess which

Fish propagule dispersal and patch cohesiveness

Elemental/Ca ratios mainly contributed to the differences among the significant clusters identified by the SIMPROF. Because homogeneity in otolith chemical composition may simply reflect environmental similarity, we used permutational multivariate analysis of variance (PERMANOVA) to test for differences between the 17 sampling sites by analysing the otolith edge of post-settlers (i.e. post-settlement portion laid down just before capture) (Di Franco et al. 2012). 'Site' (Si) was treated as a random factor (17 levels), ‘Otolith’ (Ot) was treated as a random factor nested in (Si) (11-15 levels). There were three replicate ablations for each otolith (total n=690). Once different natal origins were identified (see Results), we tested for possible differences in zone and site replenishment for each identified natal source with a univariate two-way PERMANOVA using natal origin number as a variable. 'Zone' (Zn) was treated as a random factor (3 levels); 'Site' (Si) was treated as a random factor (5 to 6 levels) nested in (Zn). Finally, a Mantel test (based on 10^4 permutations, 'ade4' package, R software) was performed to assess if closer sites were more likely to be replenished by the same natal origin. Thus, we measure the correlation between the abundance of post-settlers coming from the same natal origin and the geographic distance between pairs of sites. The distance matrix of abundances was created considering the 17 sites as observations and the major natal sources identified as variables. Thus, each site was associated to n values of abundance, corresponding to the number of post-settlers coming from each of the n major natal sources.

Larval patch cohesiveness was investigated analysing the within patch (i.e. light-trap) variability of pre-settler otolith chemical profiles. In particular we compared the chemical composition of otoliths day by day, grouping together chemical data from chronologically homologous rings of different otoliths (i.e. rings corresponding to the same day of life for all the fish). Based on the proviso that the chemical composition of otoliths is similar for individuals that experienced the same environmental conditions, we assumed that for individuals caught in the same light-trap, the variability of the chemical composition would have increased moving from the first ring analysed

(i.e. the ring laid down immediately before the sampling) toward the last ring analysed, in the case in which fish travelled different pathways (i.e. starting from different sites and then reaching the same light-trap). Otherwise, if pre-settlers travelled together to the site where we collected them (light-trap), a similar variability would have been found between edge and the previous rings (days of larval life) of the otolith. In order to perform the analysis, otolith chemical data were normalised and grouped by ring analysed (i.e. day of life) from the last (1, the edge of the otolith) to the seventh last (7) and considering the different light-traps samples separately. Then, individual deviations from the centroids for each otolith ring, across individuals coming from the same light-trap, were calculated using PERMDISP. Centroid distances were finally analysed through a PERMANOVA in which 'light-trap' (LT) was treated as a random factor with 4 levels, 'specimen' (SP) was treated as random factor with 8-11 levels, nested within LT and 'otolith ring' (OR) was treated as a fixed factor with 7 levels, crossed to SP. This model, considering OR as a factor crossed to SP, was adopted as a solution to repeated measures (the repeated measure effect is removed by fitting it as a factor). For each light-trap, a means plot of distance from centroid per each otolith ring was used to show the variation in fingerprints variability passing from day 1 to day 7. PERMANOVA analyses were run using Primer 6 PERMANOVA+ software package. All the other analyses were run using R software (R core development team).

234 RESULTS

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236 SIMPROF test segregated 7 statistically different clusters, i.e. core chemical 'finger-prints'
237 potentially corresponding to seven different post-settler natal sources (Fig. 2). The five major natal
238 sources (A, C, D, E and G) accounted together for the 92.3% (212 individuals) of all the post-
239 settlers with single contribution ranging from 12.2% to 32.3%. The remaining natal sources, B and
240 F, consisted, respectively of 5 (2.1%) and 13 (5.6%) individuals. SIMPER analysis indicated that
241 Mg:Ca ratio contributed, on average, for ~50% to the total dissimilarity in pairwise comparisons
242 among five major natal sources identified, while Ba:Ca and Sr:Ca contributed with similar
243 proportion to the remaining 50% of dissimilarity. Cluster 'G' (that included 1/3 of all the post-
244 settlers) was characterized by a 2 times higher concentration of Mg:Ca than the remaining clusters.
245 Cluster 'A' and 'D' showed lower values of Ba:Ca than C, E and G. Sr:Ca ratios resulted different in
246 all the 5 groups (Fig. 3).
247 Post-settlement replenishment from the natal origins identified was found to be statistically different
248 among the 3 zones (PERMANOVA $p<0.01$), with the southernmost zone (WMR) highly different
249 from the northernmost zone (SAP) (pairwise comparison $p<0.01$) and weakly different from the
250 central zone (CMR) (pairwise comparison $p<0.05$). No significant differences were found between
251 SAP and CMR. No differences were detected at the scale of site. The 5 major natal sources
252 replenished, with different proportions, almost all the sampling sites. Post-settlers from source 'G'
253 were mainly found in the WMR zone, with similar, lower, abundances recorded in the other two
254 zones. Source 'A' similarly replenished north (SAP) and central (CMR) sites. Source 'C' was mainly
255 composed by individuals from sites of SAP (north of the study area). Individuals from source 'E'
256 were mainly sampled in CMR, while a comparable number of post-settlers coming from the source
257 'D' was found in each site (Fig. 1 and 4). Regarding the last two natal sources, individuals from 'F'
258 were found in WMR, CMR and the southernmost site of SAP. Source 'B' replenished five sites (1, 2,

5, 13 and 14) with one individual. The abundance of post-settlers sharing the same natal origin and the geographic distance between sites were significantly positively correlated (Mantel test, r=0.38 and p=0.0001), this indicating that geographically closer sites had, on average, higher abundances of post-settlers coming from the same natal origin, than distant sites. Focusing on natal sources G, A and C (the three major natal sources clustered, representing more than 2/3 of all post-settlers), the two higher values of abundance were recorded in pairs of adjacent and less distant sites (17-16 for source 'G', 7-8 for source 'A' and 3-4 for source 'C' (Fig. 4). The chemical composition of the juvenile portion of post-settlers was significantly different among the sampling sites (PERMANOVA, $p<0.01$). Significant differences among otoliths were also found ($p<0.01$) suggesting within-site differences among individuals.

Regarding the analysis of pre-settlers' patch cohesiveness, a significant difference between otolith rings (days of life) was recorded (PERMANOVA, $p<0.01$), with an increasing variability moving from day 1 to day 7 (Fig. 5) for all the 4 light-trap samples. Pairwise comparisons between days indicated that statistically significant increments in chemical fingerprint variability were not simultaneous across light-traps samples (i.e. did not happen between the same pair of days across light-traps), resulting in a significant interaction Lt x OR ($p<0.05$): for pre-settlers from light-trap T4 the first significant increment was recorded between the 2nd and the 3rd ring, in T1 between the 4th and the 5th ring, while in T2 and T3 a significant increment was recorded between the 1st and the 2nd ring (Fig. 5).

278 DISCUSSION

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280 For the most of marine fish, the pelagic phase before settlement represents a fundamental period of
281 dispersion shaping connectivity patterns between populations (Burgess et al. 2014). Knowledge on
282 species dispersal characteristics (e.g. number of natal origins, dispersal distances) is crucial for
283 understanding fish populations dynamics and connectivity and ultimately helping the design of
284 effective marine protection strategies (Green et al. 2014). In this study we analysed otolith chemical
285 composition of early life stages of *O. melanura* in order to provide information on the dispersal
286 characteristics of its propagule pelagic phase in the south-western Mediterranean Sea.
287 The analysis of post-settler core elemental composition indicate the presence of multiple sources of
288 propagules, replenishing several sampling sites with different proportions along the south-eastern
289 coast of Spain. In recent works carried out in the Eastern Atlantic and in the Mediterranean Sea
290 similar results were recorded, with a high diversification of natal sources in early post-settler
291 individuals sampled in coastal sites (Fontes et al. 2009, Di Franco et al. 2012). The significant
292 spatial variability in the chemical composition of the juvenile portion (i.e. otolith edge) of post-
293 settler otoliths highlight the ability of the method to discriminate spatially distinct chemical
294 signatures, allowing us to reasonably draw inferences on the core portion. The segregation of
295 different core chemical signatures is very likely to be related to spatial differences in chemical-
296 physical characteristics of the water masses where the spawning took place. Different chemical
297 fingerprints in otolith cores could be associated also to a temporal variability in water masses
298 characteristics associated to temporally distinct spawning events. In 2013 the study area was
299 replenished by simultaneous spawning events that took place in a temporal window of 2 weeks
300 (Calò et al. submitted), so we can reasonably assume that the different chemical fingerprints
301 recorded are unlikely to be a consequence of a temporal variation.
302 The methodology we used does not allow to geographically locate the natal origins identified,

impeding to have the exact range and the frequency distribution (i.e. kernel) of larval dispersal distances. On the other hand, we can have an estimation of the spatial scale over which dispersal can take place in the area. All the sites were supplied by more than one natal source. Two sources replenished the extreme sites of the study area, spaced ~180 km from each other. This information allows us to infer that the most conservative value of maximum dispersal distance is ~90 km (i.e. half of the study area). In fact, supposing the case in which two of the natal sources identified were adjacent and in the centre of the study area, propagules would have travelled at least 90 km. In all the other cases, the dispersal distance would be greater. This estimate of propagule dispersal distance is in accordance with the results of other studies conducted in the Mediterranean Sea on other sparid species, using otolith natural tags (Di Franco et al. 2012, Di Franco et al. submitted) and, in general, in the range of propagule dispersal suggested for other temperate fish species (Anadón et al. 2013).

Even though it is not possible to locate the natal sources, the results suggest that the pattern of larval supply to the sampling sites has a certain spatial dependence. Post-settlers from the same natal origin were more likely to be found in closer sites with the highest abundances recorded in pairs of adjacent sites and lower values both in northernmost and southernmost sites. This output was consistent among almost all the major natal origins identified. Moreover, although the major natal origins supply almost all the sampling sites, significant differences in post-settlers replenishment was recorded a the scale of zone suggesting that post-settler demographic composition can change at the scale of 50-100 km. A similar result was found by (Fontes et al. 2009) analysing otolith core composition of the temperate wrasse *Coris julis* in the Azores. The spatial difference in natal source composition between the northernmost and the southernmost zones of the study is also in accordance with the difference in pelagic larval duration of *O. melanura* between the same zones (Calò et al. submitted). This concordant results could indicate that the geographic origin of propagules and early life history traits (e.g. the duration of the larval phase) could have a common

source of variability or be non-independent. Patterns of post-settlers distribution along the coast are likely to be a consequence of both pre- and/or post-settlement processes as well as both physical (i.e. current dynamics during the larval dispersal phase) or biological ones (e.g. behaviour, inter- and intra-specific competition for food and space, mortality). From this perspective the different natal origin composition found between the southernmost zone (WMR) and the other two zones of the study area (SAP and CMR) could be a consequence of the mesoscale oceanographic pattern that characterizes the region. In a recent work, Rossi et al (2014) pointed out that the separation between the Alboran sea and the rest of the Mediterranean sea, generally thought to have place along the Almeria-Oran front, could actually have its Spanish coastal extreme around Cartagena, that in our study separate the CMR zone from the WMR zone. The presence of this oceanographic boundary, already suggested to be a biogeographic barrier in the Western Mediterranean sea (Pérez-Ruzafa 2010), could reduce the dispersal of propagules from the WMR zone toward the northernmost zones and vice versa. However, it is very hard to draw conclusions on the forces that determined the spatially dependent outcomes recorded in this study, as no further information are available to elucidate them, especially considering the lack of information on fish spawning grounds. From this perspective, further investigation are to be conducted using other methodologies that would permit to directly track the movements of propagules from their source to the settlement site. These methods (e.g. otolith artificial tagging or genetic parentage analysis), although undoubtedly powerful, have been rarely or never applied so far in the Mediterranean context given the lack of data about spawning ground locations and the huge sampling effort needed for obtaining representative results (Calò et al. 2013).

The analysis of larval patch cohesiveness suggests that groups of larvae can merge in the pelagic environment after having travelled separately for some days. In recent works a similar larval patch formation was evidenced in a small triplefin fish of New Zeland reef, using otolith chemical analysis (Shima & Swearer 2009, Shima et al. 2015). However, cases of protracted larval patch

cohesiveness were also documented, with larvae experiencing the same dispersal pathway during the entire pelagic phase (Ben-Tzvi et al. 2012, Bernardi et al. 2012). In our study, in two light-traps, an increasing otolith chemical variability was recorded between the sampling day and the day immediately before, suggesting that, during the sampling night two or more discrete groups of larvae were attracted toward the same trap, after having travelled different dispersal pathways. In other two light-traps, a stable chemical composition between the sampling day and the previous ones was observed, suggesting that the aggregation of distinct patches can take place in different moments of the larval phase. It is difficult to establish if this aggregation tendency is a result of larval behaviour or particular oceanic features (e.g. gyres) that can mix together patches previously separated. Kiparissis et al (2008) found evidences of aggregation behaviour in post-settlement individual of *O. melanura*, differently from other Mediterranean sparid fishes (Harmelin-Vivien et al. 1995). In particular, post-settlers arrive in small shoals and then actively aggregate in larger shoals with increasing fish size after various aggregation steps (Kiparissis et al. 2008). From this perspective, the same aggregation behaviour could characterize the larval phase, determining a mix of distinct larval patches, potentially coming from different natal sources, during the pelagic phase. This patch mixing tendency could be an adaptive strategy that would guarantee a higher genetic diversity, and thus higher survival probability, when facing the environmental uncertainty of the settlement habitat and the high mortality rates associated to it (Nanninga & Berumen 2014, Shima et al. 2015). In the light of these considerations, it resulted evident that post-settler natal sources diversity recorded here in each sampling site could be a consequence of multiple aggregation steps that can occur both during the propagule dispersal phase and early after the settlement process and would contribute together to demographic heterogeneity, fundamental in driving meta-population dynamics and the persistence of species (Kritzer & Sale 2004).

To conclude, with the present study we provided insights on the propagule dispersal traits of a temperate coastal fish. This is the first study aimed to estimate dispersal distances in the south-

western Mediterranean Sea and, to our knowledge, the first to provide information on larval patch dispersal cohesiveness during the pelagic phase of a Mediterranean coastal fish. Information on propagule dispersal patterns are fundamental for the correct planning of MPAs and networks (Planes et al. 2009, Gaines et al. 2010). From this perspective, our results are particularly relevant considering the environmental value of the region and the strong effort that is being accomplished to build an effective network of MPAs in the sector of Mediterranean sea considered. Certainly, the study of one species is not sufficient for drawing conclusions on the effective MPAs spatial arrangement, but it represents an important piece of knowledge, that together with information on other species and/or using different approaches, can provide the fundamental background for correct marine spatial conservation actions.

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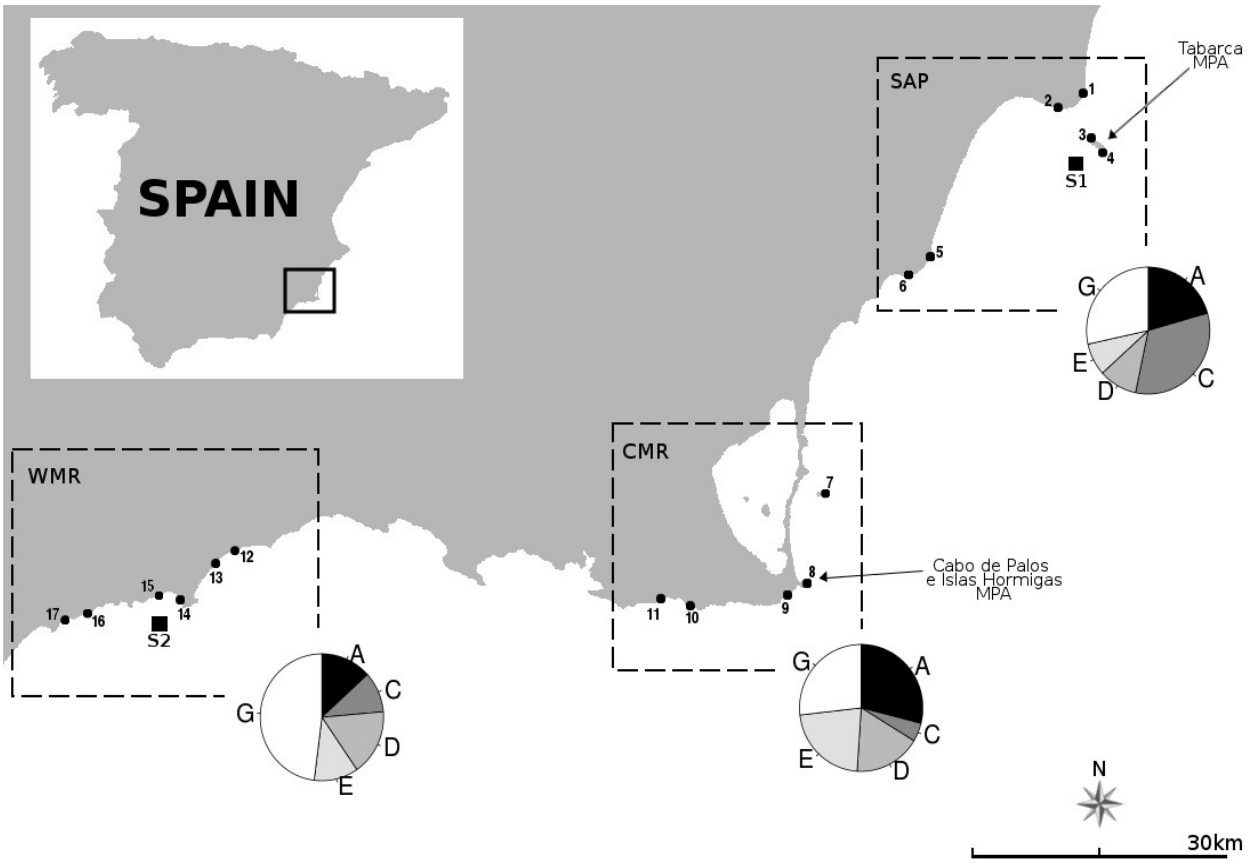
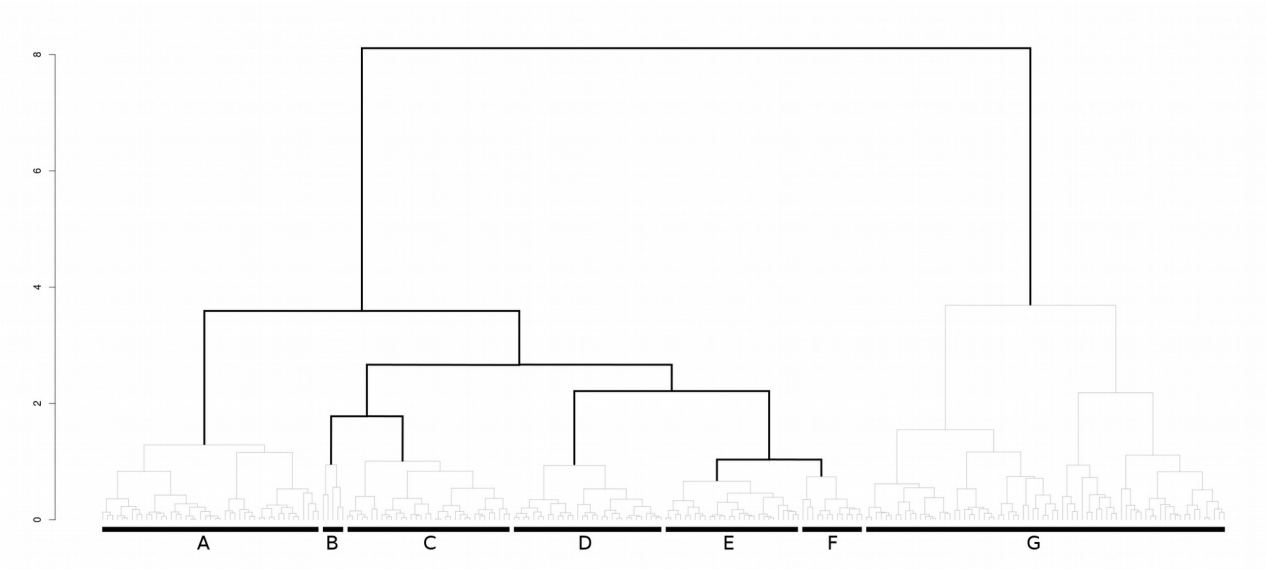


517 Figure 1. Map of the study area. Black dots indicate post-settlers sampling sites (numbers represent
518 site names); dashed line rectangles represents zones: south Alicante province (SAP), central Murcia
519 region (CMR) and west Murcia region (WMR). Black squares represent pre-settlers sampling sites.
520 Pie charts indicate, for each zone, the proportion of post-settlers belonging to the five major natal
521 sources clustered (see results).

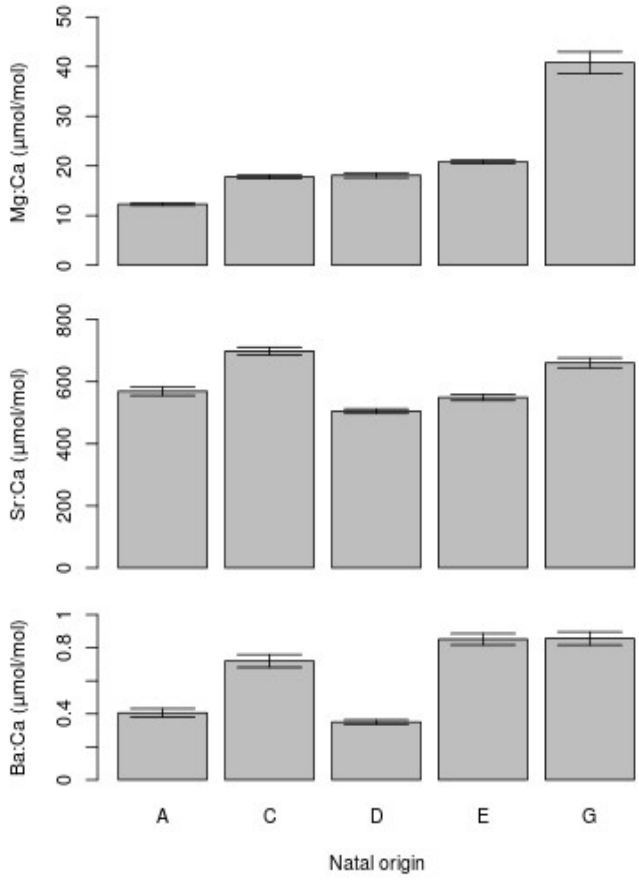
Element ratio	NIST 610 %RSD	NIST 612 %RSD	NIST 610 %Accuracy	NIST 612 %Accuracy	LOD mmol/mol
Mg:Ca	9.8	11.1	84	92	0.03852
Sr:Ca	7.8	8.3	115	98	0.00148
Ba:Ca	7.1	9.4	91	89	0.00066

Element ratio	NIST 610 %RSD	NIST 612 %RSD	NIST 610 %Accuracy	NIST 612 %Accuracy	LOD mmol/mol
Mg:Ca	6.6	14.4	98	106	0.04568
Sr:Ca	7	9.3	98	93	0.01441
Ba:Ca	6.1	7	98	82	0.00076

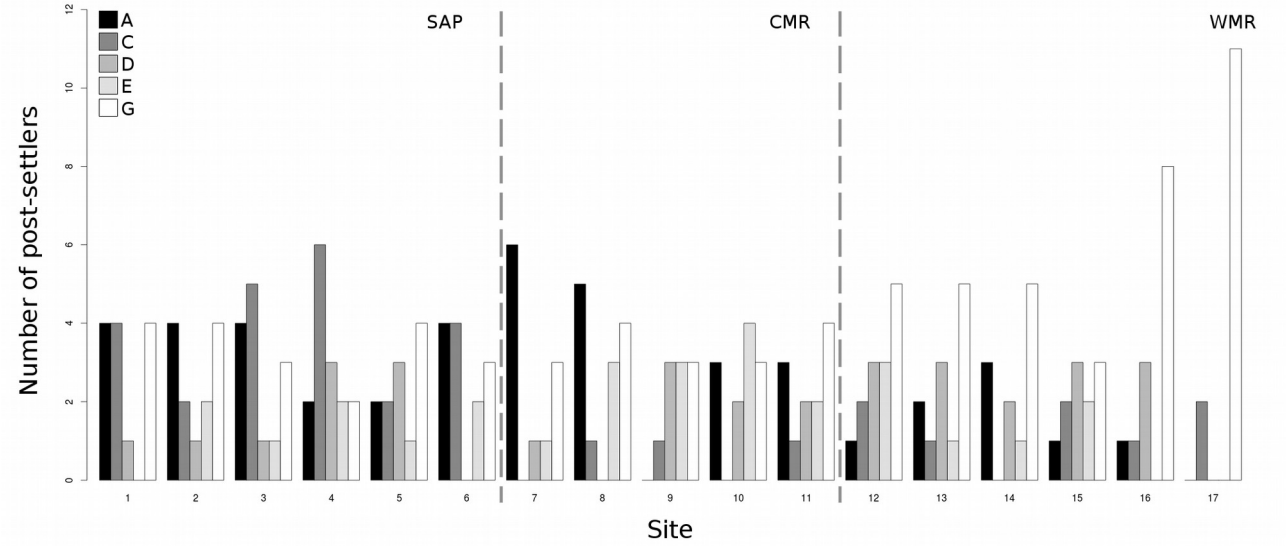
523 Table 1. Estimates of precision, accuracy and limits of detection (LOD) for post settlers' otoliths
524 (upper table) and pre settlers' otoliths (lower table). Values for %RSD (% relative standard
525 deviation) and % accuracy are dimensionless.



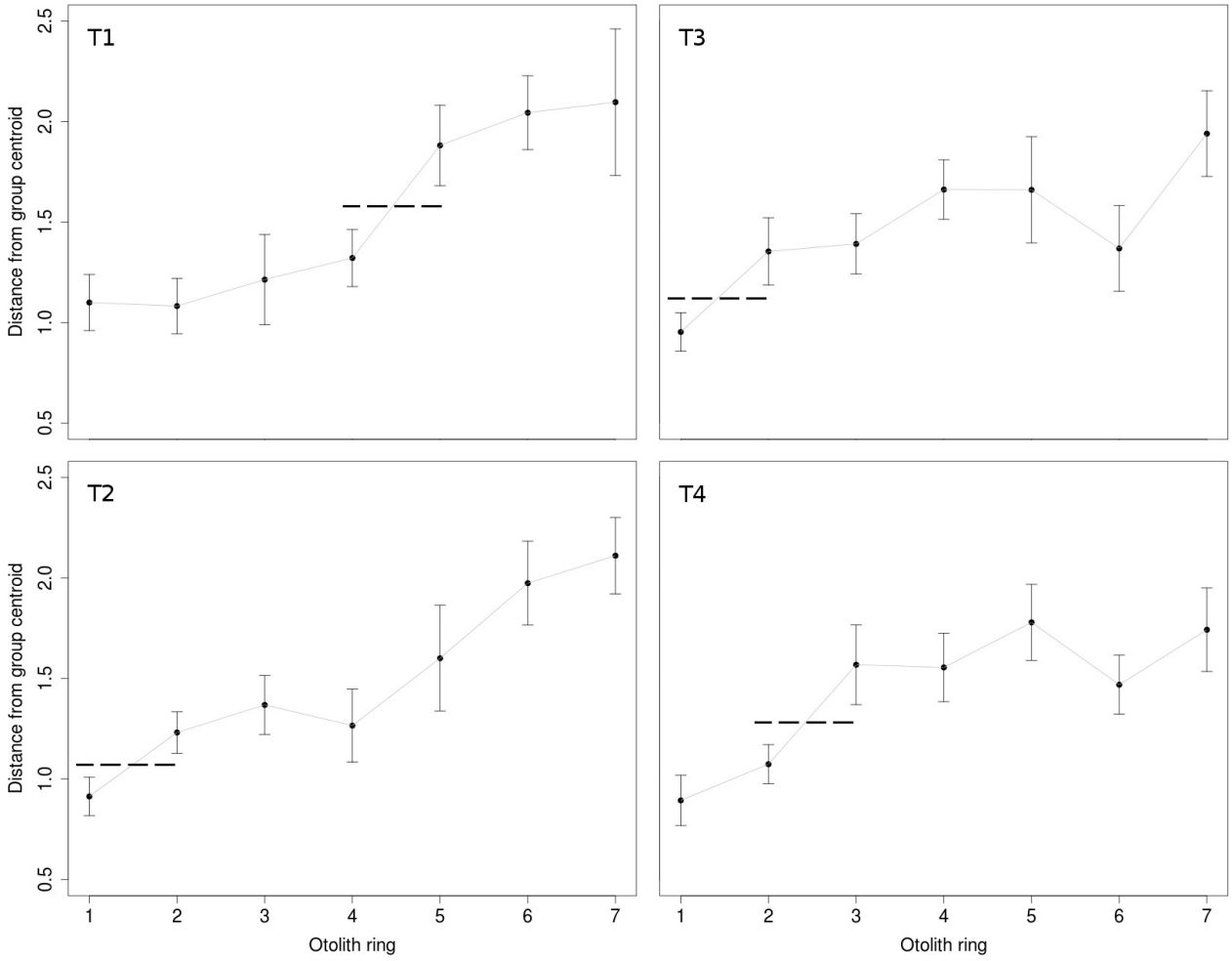
527 Figure 2. Cluster (based on Ward's approach) of post-settlers otolith cores into groups considering
528 Mg:Ca, Sr:Ca and Ba:Ca elemental concentrations. Black branches indicate statistically significant
529 groups resulted from SIMPROF (arbitrarily named from left to right). Grey branches indicate non-
530 significant differences.



531 Figure 3. Average (± standard error) element/calcium ratios in the core region of the five major
532 clusters identified by SIMPROF (see Figure 2).



534 Figure 4. Total number of post-settlers, for each sampling site, coming from the five major natal
535 sources. Different colors represent the five major groups identified by SIMPROF. Sites are
536 numbered progressively from the northernmost to the southernmost (See Fig. 1). Vertical dashed
537 lines separate the three zones considered. Source B and F (minor sources) were omitted for enhance
538 readability.



539 Figure 5. Plots of average distance (± standard error) from group centroid per each otolith ring (day
540 of life) analysed, as calculated by PERMDISP. Each plot represent a light-trap sample: T1 (10
541 specimens), T2 (8 specimens), T3 (11 specimens) and T4 (11 specimens). Dashed segments
542 represent statistically significant increments in chemical fingerprint variability between pairs of
543 consecutive rings.