Dispersal of larval and juvenile seabream: Implications for Mediterranean marine protected areas

Antonio Di Franco [1,2*], Antonio Calò [2,3], Antonio Pennetta [4], Giuseppe De Benedetto [4], Serge Planes [5], Paolo Guidetti [1,2]

[1] Université Nice Sophia Antipolis, Faculté des Sciences, EA 4228 ECOMERS, Nice, France; [2] CoNISMa (Consorzio Nazionale Interuniversitario per le Scienze del Mare), Rome, Italy; [3] Departamento de Ecologìa e Hidrologìa, Universidad de Murcia, Murcia, Spain; [4] Laboratorio di Spettrometria di massa analitica ed isotopica, Dipartimento di Beni Culturali, University of Salento, Lecce, Italy; [5] USR 3278 CNRS-EPHE, Laboratoire d'excellence 'CORAIL', Centre de Recherches Insulaires et Observatoire de l'Environnement, Université de Perpignan, 66860 Perpignan Cedex, France.

*corresponding author e-mail: difry@libero.it. Tel. 0033492076848

Keywords: dispersal, juvenile, otolith, propagule, settlement, recruitment, two banded seabream, Marine Protected Areas

Abstract

In the marine context, information about dispersal is essential for the design of networks of marine protected areas (MPAs). Generally, most of the dispersal of demersal fishes is thought to be driven by the transport of eggs and larvae in currents, with the potential contribution of dispersal in later life stages relatively minimal.

Using otolith chemistry analyses, we estimate dispersal patterns across a spatial scale of approximately 180 km at both propagule (i.e. eggs and larvae) and juvenile/sub-adult (i.e. between settlement and recruitment to the fishery) stages of a Mediterranean coastal fishery species, the two-banded seabream *Diplodus vulgaris*.

We detected three major natal sources of propagules replenishing local populations in the entire study area, suggesting that propagule dispersal distance extends to at least 90 km. For the juvenile stage, we detected dispersal of up to 165 km.

Our work highlights the surprising and significant role of dispersal during the juvenile life stages as an important mechanism connecting populations. Such new insights are crucial for creating effective management strategies (e.g. MPAs and MPA networks) and to gain support from policymakers and stakeholders, highlighting that MPA benefits can extend well beyond MPA borders, and not only via dispersal of eggs and larvae, but also through movement by juveniles.

47

7 4

8

1. Introduction

Dispersal, defined as the movement of individuals away from their "source" (Nathan et al. 2003), determines the spatial scale at which local populations are ecologically connected to each other. Dispersal is widely considered a major determinant of the: 1) distribution and local abundance of species; 2) dynamics of spatially structured metapopulations (and of community structure) and 3) extent to which populations and assemblages of species are able to respond to perturbations (Clobert et al. 2001).

In the marine context, the development of spatial management using marine protected areas (MPAs) in the 90s, and later the concept of MPA networks, has identified dispersal and connectivity as key factors in designing effective networks (Planes et al. 2009, Gaines et al. 2010, Almany et al. 2013).

The overall framework driving MPA design is that the size of MPAs should be set to allow for 1) effective protection of populations of target species inside MPA borders, 2) both self-replenishment and export of propagules (i.e. pelagic eggs and larvae) and 3) spillover of some juveniles, subadults and adults beyond boundaries (Harrison et al. 2012, Di Lorenzo et al. 2014). Knowledge about dispersal/movement patterns is, therefore, of paramount importance in designing effective MPAs and MPA networks (Green et al. 2014).

Effective MPAs generally have a high density of spawners (large-sized, sexually mature individuals), thus the potential to increase

the occurrence of spawning aggregations and, therefore, to generate greater propagule production compared to fished areas (Evans et al. 2008, Di Franco et al. 2012a). In a network of MPAs, each individual MPA should be adequately connected to the others via dispersal to support the persistence and/or the recovery of local populations from disturbance (Planes et al. 2009, Gaines et al. 2010). If MPAs are isolated from one another and not connected by dispersal between them, MPAs are more vulnerable to local extinctions because of local perturbations, since they cannot be replenished by immigration from elsewhere (Gaines et al. 2010).

The management-oriented need for information on dispersal was recently recognized even at policy level, as highlighted by the implementation of the California Marine Life Protection Act in the USA (Anadon et al. 2013) and by the 'Marine Strategy Framework Directive' (MSFD; 2008/56/EC) in the EU, where the creation of coherent and effective networks of MPAs is considered a key tool to reach conservation targets in the marine environment (Anadon et al. 2013).

Despite the variety of approaches currently used to tackle this issue, tracking the movements of marine fauna and quantifying dispersal patterns is, however, a complex task due to the difficulty in following individuals throughout their entire life cycles (Calò et al. 2013). Many larval dispersal patterns are estimated using models (e.g. Lagrangian models) parameterized with information about species life history traits (e.g. pelagic larval duration (PLD) and

spawning date (SpD)) and oceanographic data (Pujolar et al. 2013, Andrello et al. 2013, 2015). Other approaches that have proved highly valuable in estimating fish movements and dispersal use genetics (Planes et al. 2009, Weersing and Toonen 2009) and tagging (both natural and artificial, Thorrold et al. 2002, Di Lorenzo et al. 2014). Among natural tags, otolith chemical signatures have proven to be a valuable approach to both tracking fish movements and modelling dispersal patterns (Elsdon et al. 2008, Gillanders 2009, Di Franco et al. 2012b). Focusing on natural tags, otoliths (ear bones) are carbonate structures usually in the form of aragonite (even if they can be found also in form of vaterite) located in inner ear of fishes and grow by the daily accretion of calcium carbonate increments throughout the fish's entire lifetime (Campana 1999). Otoliths, starting from their formation during the embryonic stage, incorporate chemical signatures of the water mass the fish is in during each life history stage (Green et al. 2009). Though under physiological constraints otolith chemistry reflects the water chemistry of the surrounding environment, and once laid down, increments (that can be referenced to specific ages) remain unaltered (Campana 1999, Elsdon et al. 2008). The chemical information acquired locally within the otoliths can be used to derive profiles of the movement history of an individual (Campana 1999, Green et al. 2009). Despite some limitations (see Elsdon et al. 2008 for detailed description of the method), otolith chemistry is nowadays largely accepted as a useful method for unravelling fish

dispersal and connectivity patterns (Calò et al. 2013, Starrs et al. 2014, but see Berumen et al. 2010).

In order to provide crucial information for the design of a network of effective MPAs, in this study we estimate dispersal patterns at both propagule (i.e. eggs and larval stages) and juvenile stages of an ecologically and economically important Mediterranean coastal fish, the two-banded seabream *Diplodus vulgaris (*Geoffroy Saint-Hilaire, 1817), using analysis of otolith chemistry. Specifically we aim to estimate, for the two-banded seabream *Diplodus vulgaris*, the scale of dispersal at propagule stage (i.e. eggs and larvae) and to build a dispersal kernel for juvenile (i.e. post-settlement) dispersal. This can allow us to assess the paradigm that dispersal at juvenile stage is negligible and that dispersal and connectivity for coastal fish equate with propagule dispersal.

2. Material and methods

2.1. Study species

The common two-banded seabream (*Diplodus vulgaris*) is a demersal reef fish distributed throughout the Mediterranean and the eastern Atlantic. It usually grows to a length of about 30 cm,

although it can reach a maximum length of 45 cm (Fisher et al. 1987) and exceed 30 years in age (Guidetti et al. unpublished data). *Diplodus vulgaris*, with the congeneric *D. sargus sargus*, is an economically important fish exploited both by professional and recreational fisheries (Lloret et al. 2008) and plays an ecologically relevant role in Mediterranean coastal ecosystems. Preying on sea-urchins (grazers), the two *Diplodus* species indirectly control the transition from macroalgal forests to coralline barrens (i.e. bare rocks with encrusting algae), and may therefore have strong effects on rocky-reef community structure and ecosystem function (Guidetti et al. 2006).

Seabream eggs, released in the water column, hatch two days after fertilization and then larvae develop in pelagic waters for more than 1 month (Di Franco et al. 2013). Larvae metamorphose and settle (a stage called ‘settlement’) in shallow coastal habitats (mainly small bays characterised by mixed sandy and rocky bottoms) at approximately 10 mm TL (Planes et al. 1999, Vigliola and Harmelin-Vivien 2001). About six months later, the juveniles (i.e. small-sized subadults, approximately 8 cm TL) join the adults (at a phase that is operatively defined recruitment) and at about 2 years of age (i.e. approximately 18 cm TL) they reach sexual maturity. Adults are relatively sedentary, with evidence of high site fidelity and movement at the scale of few kilometers (La Mesa et al. 2013). Much less is known about dispersal during the propagule and juvenile stages, with the only information concerning the Atlantic

coasts of Portugal and showing dispersal at the scale of 1 km for juveniles (Abecasis et al. 2009) and inconclusive evidence for larvae (Correia et al. 2011).

2.2. Sampling scheme

We used otolith chemistry to obtain information on: 1) natal origin and larval dispersal by analysis of the core (laid down during embryogenesis, Green et al. 2009), of post-settler otoliths; 2) “site fidelity” and/or juvenile dispersal (i.e. the movement between settlement and recruitment) by analysis of the post-settlement rings of otoliths (i.e. about 10 daily increments after the settlement mark, which marks the transition from pelagic larva to demersal settler, Di Franco et al. 2013) of both post-settlers and juveniles. The second issue has been very scarcely studied despite its potential relevance. Assaying otoliths of post-settlers (i.e. transitional juveniles *sensu* Vigliola and Harmelin-Vivien 2001) collected along a stretch of coast and identifying groups of similar origins based on elemental signatures in otolith cores provided information about the spatial extent of larval dispersal. Larval dispersal distance was estimated on the basis of the distance among different sampling sites that were replenished by a single source. Evaluating “site fidelity” of juvenile fish between settlement and recruitment, and/or the distance travelled between settlement and recruitment sites, provided information about juvenile movement after settlement. A

prerequisite for this kind of investigation is to assess the spatial patterns of elemental signatures in otoliths among sampling sites. The elemental composition of the portion of the otolith formed just after settlement (the portion chemically characterized by the site where the fish settled) of post-settlers was assessed for 14 sites (see 2.3) and used to generate a reference set of site-specific chemical fingerprints representing potential settlement sites in the study area. Post-settlement movement (i.e. the distance travelled by juveniles) between settlement and recruitment stages was inferred by comparing chemical fingerprints of the same portion of the otolith (i.e. corresponding to about 10 days after settlement) between juveniles (collected 8-10 months after settlement) and post-settlers (collected shortly after settlement) from multiple sites. The analysis of the same portion of the otolith in both post-settlers and juveniles prevented us from any bias related to potential temporal variability in water chemistry between settlement and recruitment. In addition the choice of analysing the portion of the otolith corresponding to 10 days after settlement (based on visual identification of otolith microstructure) could reduce the risk related to temporal mismatch between microstructural and microchemical processes (see Freshwater et al. 2015). No evidences of this mismatch exists for Mediterranean species and findings from sockeye salmon *Oncorhynchus nerka* highlight, in 50% of individuals examined, a lag of about 9 days with microchemical process occurring before microstructural ones. If this would be the case also

in our model species, the portion of otolith that we chemically analysed would still correspond to a moment when settlers inhabited settlement sites and therefore would allow us to properly characterize settlement sites.

2.3. Sample collection and study area

Both propagule and juvenile (i.e. post-settlement to recruitment) dispersal was investigated at the scale of approximately 180 km. Post-settlers and juveniles of *Diplodus vulgaris* were collected at 14 sites along ~180 km of the Apulian Adriatic coast of Italy (Fig. 1). Post-settlers of *D. vulgaris* were collected in May 2010. At each site, 10-12 individuals were collected (total n= 157) with a hand-net. Post-settlers were euthanized in an ice water slurry in accordance with authorisation protocols by the Italian Ministry of Agriculture, Foods and forestry politics (permit number 0011267-2010). By spearfishing juveniles (i.e. small size subadults, 8-10 cm TL) were collected 8-10 months later, after recruitment, from the same 14 sites where post-settlers were previously collected. Therefore, post-settlers and juveniles collected in the present study belonged to the same annual cohort. At each site, 10-14 juveniles were collected (total n= 164). Fish were frozen until otolith removal was undertaken.

2.4. Sample preparation and analysis

In the laboratory before removing the otoliths, standard lengths (SL) of the post-settlers were measured to the nearest 1 mm. Then one sagittal otolith was prepared for chemical analyses 'as outlined in supplementary material Appendix A.. Otoliths of post-settlers were analysed for the chemical composition of both the core (in order to acquire information about natal origin) and the post-settlement portion (i.e. ten increments after the settlement mark).

For post-settlers we obtained SpD and PLD data through otolith microstructure analyses. Otolith daily rings were read using a high-powered microscope (see Di Franco et al. 2013 for details).

Otoliths of juveniles were only analysed for the chemical composition of the post-settlement portion. Ten elements were analyzed (^{24}Mg, ^{44}Ca, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb, ^{7}Li, ^{57}Fe, ^{59}Co). Despite some evidences suggest that Mg uptake can be physiologically regulated, and may not represent ambient conditions (see Woodcock et al. 2012) we included this element because it has been found useful for distinguishing fish from different locations when used in combination with other elements (Swan et al., 2003; Sarimin et al., 2009). Details about chemical analyses procedures can be found in Appendix A.

2.5. Data analyses

Otolith elemental concentrations were converted to molar concentrations and standardised to calcium. All further data analyses were carried out on log (x+1) transformed element:^{44}Ca data.

2.5.1. *Natal sources and propagule dispersal*

To determine the number of potential natal (i.e. propagule) sources, the multivariate elemental concentrations of otolith cores from post-settlers (as a proxy for identifying the existence of single or multiple areas of origin, Papetti et al. 2013) were analysed using agglomerative hierarchical clustering based on group average on the Euclidean resemblance matrix. The SIMPROF permutation procedure was used to determine which clusters were significantly different at the 5% level (Clarke et al. 2008).

Because homogeneity may simply reflect environmental similarity, we used permutational multivariate analysis of variance (PERMANOVA) to test for differences between the 14 sampling sites by analysing the otolith edge of post-settlers (i.e. post-settlement portion laid down just before capture). ‘Site’ (Si) was treated as a random factor (fourteen levels), ‘Otolith’ (Ot) as a random factor nested within (Si) (10-12 levels). Three replicate samples from each otolith were analyzed (total n=471). This analytical design, encompassing within-otolith replication for the otolith edge, was

chosen based on recommendations regarding 'cost'-optimal allocation of sampling effort from Di Franco et al. 2014.

Once different natal origins were identified (see results), we tested for possible differences in settlement site replenishment for each identified natal source with a univariate one-way PERMANOVA on core multivariate composition using site number as a variable (i.e. from 1 to 14, from Northern to Southern sampling site). Natal source was treated as a single factor with different levels corresponding to the major natal sources identified. The same experimental design was used to test for potential differences in SpD and PLD of individuals from each natal source.

Statistical analyses were run using Primer 6 PERMANOVA + software package (Clarke and Gorley 2006).

2.5.2. *Juvenile dispersal*

To account for possible uncharacterized settlement sites, which represents an inevitable bias despite our extensive sampling effort, we compared otolith elemental signatures of juveniles with those of settlers using principal component analysis (PCA). Juveniles that fell outside a 95% confidence ellipse around the settlers baseline data (elemental signatures of settlement sites) were assumed to have originated from uncharacterized settlement site(s) and were excluded from further analyses (see Appendix B for details).

Canonical analysis of principal coordinates (CAP, Anderson and Willis 2003) and jackknife cross validation (% of correct classification) were performed on the edge portion of the elemental data of post-settlers to assess how accurately post-settlers were classified to sites where they were collected in each region. A specific randomization test (White and Ruttenberg 2007) was used to estimate the probability that reclassification success (% of correct classification) was better than random. Juveniles were assigned to settlement sites (i.e. the sites where the post-settlers were collected) through linear discriminant functions previously parameterized with data from post-settler otoliths. Centroids per specimen for both post-settler and juvenile data (i.e. centroid of the three replicate sample pits for each specimen) were calculated and used for CAP analysis.

Statistical analyses were run using Primer 6 PERMANOVA + software package (Clarke and Gorley 2006).

Based on assignment outputs, we calculated juvenile dispersal (i.e. distance travelled between settlement and recruitment sites) for each individual, and from this we constructed a dispersal kernel (i.e. dispersal frequency distribution), which we here called the "measured dispersal kernel". We tested the kernel fit using an exponential decay model, commonly used as an approximation for the decline in frequency of observations as dispersal distance increases (Nathan et al. 2003). However, the measured dispersal frequency distribution is necessarily restricted to the spatial

arrangement of sampling sites and to the number of specimens collected at each site (Cooper et al. 2008), with only a limited number of specimens able to disperse over the maximum distance among sites considered in the study (in our case, we would have been able to record a maximum displacement that corresponds to the maximum distance between sites only for the specimens collected at the northernmost and southernmost sampling sites). To account for this limitation, we calculated both a “randomized” and a “adjusted” dispersal kernel (i.e. adjusted for the inverse probability to observe dispersal at a given distance) following Matthysen et al. 1995 as detailed in Appendix C.

3. Results

3.1. Natal sources and propagule dispersal

Based on elemental fingerprints from otolith cores of post-settlers, SIMPROF detected seven statistically different groups (Fig. 3), corresponding to seven natal sources.

Three of the seven groups (A, B, and D) consisted of a single individual, while group G consisted of three individuals (~2% of all settlers sampled). Groups C, E and F consisted of ~26%, ~45% and ~24%, respectively, of settlers. These three major groups significantly differed in terms of their multivariate core elemental fingerprints (PERMANOVA $p<0.01$). The Mg:Ca and Sr:Ca ratios

contributed most to the differentiation of these three major groups (about 99% of the total dissimilarity in pairwise comparisons, SIMPER analysis), and, individually, both the Mg:Ca and Sr:Ca ratios differed significantly among the three groups (PERMANOVA $p<0.01$ for both elemental ratios) (Fig. A1).

Each of the three major groups was composed by specimens sampled in almost all settlement sites, with group E that included specimens from all the 14 sampling sites. There was no difference among the three major groups in terms of number of settlers that replenished the 14 sites (Fig. A2, Permanova pseudo-f: 0.66, $p=0.51$; Appendix D).

Considering spawning date (SpD), the three major natal origins differed significantly by a few days (Permanova pseudo-f: 4.4664, $p=0.014$). Pairwise tests revealed that group C significantly differed from E ($p<0.01$) and F ($p<0.05$), while no difference was detected between E and F. SpD of group C took place about 10 days after that of groups E and F (2010 December 21st vs 2010 December 10th). Post-settlers size (SL) ranged from 15 to 30 mm (mean ± SE= 25 ± 0.2 mm). Considering PLD, no significant difference was detected among the three groups, with 47.6±1.2 (mean±s.e.), 44.5±1.1 and 44.9±1.4 days respectively for C, E and F. Within each natal source, a large range in PLD was detected: 29-61 days in C, 29-58 in E, and 25-56 in F.

Significant differences for the factor ‘Site’ (pseudo-f: 5.51, $p< 0.001$) were detected in elemental concentrations of the otolith edge in

post-settlers. Significant differences ‘among otoliths’ were also detected (pseudo-f: 3.82, p< 0.001), suggesting within-site variation among individuals. Mg:Ca contributed the most to the observed differences among sites (ranging from ~48% to ~91% of total dissimilarity in pairwise comparisons, SIMPER analysis).

3.2. Juvenile dispersal

For post-settlers, a significant jackknife reclassification success was found (randomization test p=0.0002) with 22.9% of samples correctly classified to collection site in cross-validation of CAP analysis (i.e. 7.1% correct classification to one of 14 sites due to chance alone).

Approximately 10% of juveniles were assigned to a settlement site corresponding to the site where they were collected, indicating that they recruited to the same site where they settled (i.e. 0 km dispersal). Approximately 51% of juveniles moved between 5 and 55 km, 22% between 55 and 100 km, and 15% between 100 and 135 km. A single fish (0.75%) moved approximately 165 km. Overall, median dispersal was 40 km and average dispersal was 51 km (± 3.2, s.e.).

The measured dispersal kernel for juveniles did not follow an exponential decay distribution with p value at threshold of significance (p=0.054, Fig 4a). Considering a randomised dispersal kernel, a significant exponential decay trend was detected

(p<0.0001, Fig. 4b), suggesting that this trend is due to the spatial arrangement of sampling sites and could be due to chance.

Comparing the two dispersal kernels (measured and randomised), a significant difference was detected (Wilcoxon-Mann-Whitney test, p= 0.020), with the measured kernel more skewed towards shorter dispersal (Fig. A4), indicating that fish disperse long distances less often than predicted by chance.

The adjusted dispersal kernel had a median dispersal distance of 50 km and average of 63.42 km (± 3.74, s.e.), and did not follow an exponential decay model (p>0.05). Compared to the measured dispersal kernel, the adjusted dispersal kernel had a fatter tail (Fig. 4c), corresponding to a greater frequency of long distance dispersal events.

4. Discussion

Here we highlight the existence of three major natal sources of propagules for the two banded seabream (*Diplodus vulgaris*) that replenish the study area (i.e. about 180 km of coastline), suggesting that propagule dispersal extends to at least 90 km.

In addition, we observed extensive dispersal – up to 165 km – at the juvenile stage and built a juvenile dispersal kernel. This evidence, as far as we know, is novel and has important implications for the ecology and management of MPAs.

4.1. Natal sources and propagule dispersal

We detected multiple natal sources replenishing the study area, with three sources providing major contributions. The number of natal sources detected, however, is likely function of the sampling effort (in terms of number of post-settlers collected per site), therefore an higher number of natal sources could be detected by increasing sampling effort. Putative additional natal sources are however likely minor ones (i.e. providing relatively low contribution to settlement sites) that could be difficult to be identified at present sampling effort.

Each major natal source appears to replenish multiple (almost all) settlement sites spread along the 180 km of coastline in the study area, suggesting that propagule dispersal may take place at least over 90 km (in the case of natal sources located near the middle of the study area). We can only provide this conservative estimate of dispersal because it is impossible to spatially locate the natal sources that could be even located outside the study area. Thus, our estimate of maximum propagule dispersal of 90 km is conservative, and could in fact be much farther (e.g. ≥180km in the case of natal sources located near the edge of the study area or outside it).

Due to the approach adopted here, we cannot spatially locate the natal source, track propagule dispersal and build a propagule dispersal kernel, so we cannot provide any hypotheses about the

relative frequency of short- and long-distance propagule dispersal events. This would be possible by focusing on nesting fishes where the exact location of the propagule source (i.e. the nest) is known (e.g. Buston et al. 2012) or by using marking methods based on maternal transmission of stable isotopes to offspring (Almany et al. 2007, Munro et al. 2009).

Despite we cannot identify where the natal sources are located we can speculate that a relevant percentage of propagules could originate from the Torre Guaceto Marine Protected Area (TGMPA) that is located within our study area and that has been shown to host high density and biomass of fishes (Sala et al. 2012, Di Franco et al. 2012a). Evidences on the congeneric *Diplodus sargus* suggest that TGMPA host high density of spawners and contribute through propagule export to the replenishment of populations inhabiting unprotected areas (Di Franco et al. 2012a, Pujolar et al. 2013). A similar pattern could be attended also for *D. vulgaris*, with one (or more) of the three major natal origins located within TGMPA and part of the propagules exported toward unprotected areas following sea currents dominating western Adriatic (Artegiani et al. 1997) during *D. vulgaris* spawning period (i.e. mainly winter).

The replenishment of multiple sites by each natal source suggests high variability in propagule dispersal, because propagules from a single source reach settlement sites located at different distances.

This evidence could result from the flexibility of the PLD as expressed by the wide range highlighted among the individuals from

each natal origin. Two of the three major spawning events (corresponding to the three major natal sources) occurred simultaneously while the third spawning event began approximately 10 days later. We detected spawning events that occurred over a long time period, suggesting an extended spawning season for this species. This evidence agrees with findings on *D. vulgaris* from other Mediterranean (Mouine et al. 2012, Di Franco et al. 2013) and non-Mediterranean areas (Gonçalves & Erzini 2000, Pajuelo et al. 2006) indicating spawning season lasting 3-7 months.

4.2. Juvenile dispersal

Here we provide evidence of extensive dispersal during the juvenile stage of up to 165 km. This finding agrees with recent findings for other temperate coastal fishes, which have suggested dispersal up to 600 km (Tobin et al. 2010, Hamer et al. 2011, McMahon et al. 2012, Di Franco et al. 2012b, Reis Santos et al. 2013, Bouchard et al. 2015). In the present study, our dispersal estimates are from otolith chemistry analyses, but other evidence from a study adopting tag-recapture techniques on the congeneric species *Diplodus sargus sargus* reported a dispersal distance of ~17 km for juveniles (~11 cm TL) within one month (D'Anna et al. 2004), confirming the potential for coastal fishes to disperse significant distances as juveniles.

There was much variability in juvenile dispersal distances among individuals, as demonstrated by the measured dispersal kernel. Few individuals dispersed large distances after settlement (the tail of the kernel), and about 10% of individuals did not disperse at all. Overall, we observed lower site fidelity in *Diplodus vulgaris* compared to its congener *D. sargus sargus* in the same study area (Di Franco et al. 2012b). Interspecific differences in dispersal are common, and can be related to a number of species-specific factors (e.g. aspect ratio of the caudal fin, Radinger and Wolter 2013) or environmental factors (e.g. habitat heterogeneity, Fraser et al. 2001).

The measured dispersal kernel for juveniles consists of a declining function with distance, similar to the larval dispersal kernel reported for a tropical fish (Almany et al. 2013). We observed a maximum dispersal of juveniles of 165 km, more than three times greater than the maximum dispersal of larvae (~50 km) predicted for a coral grouper using genetic parentage analyses (Almany et al. 2013). However, it is important to note that all dispersal studies to date are limited by the spatial scale over which they sample individuals, and a “complete” dispersal kernel – one with relatively narrow confidence intervals around the mean prediction across a large distance – has never been reported. The adjusted dispersal kernel for juveniles consists of a higher probability of long distance dispersal compared to the measured kernel, and suggests greater role for juvenile movements in connecting local populations.

We detected a single instance of long-distance dispersal (LDD, Nathan et al. 2003) in *D. vulgaris*, identified as dispersal greater than the 99th percentile of the dispersal kernel: an individual travelled farther (about 30 km more) than the next farthest dispersing individual recorded (165 vs 135 km approx.). LDD could have effects on a species' ecology (resource use, species co-existence, and large-scale meta-population dynamics) and evolutionary trajectory (gene flow, genetic structure and species diversity) (Nathan et al. 2003). However, accurate estimates of the frequency of LDD are difficult to obtain, because LDD processes are, by their nature, highly stochastic (Nathan et al. 2003). In addition, methodological constraints are associated with the quantification of LDD. A key problem is the under-sampling of LDD events using sampling designs that involve an array of sites (Koenig et al. 1996). To properly estimate LDD, the spatial scale of the study area should correspond to the scale of LDD events (Koenig et al. 1996). Unfortunately, maintaining equal probability of disperser collection constant across large spatial scales requires an unfeasible sampling effort at more distant locations (Nathan et al. 2003). This problem may still hold even if sampling effort is intense and spatially extensive, but can be addressed by using a distance-weighted correction (Baker et al. 1995), as we have done in this study through the construction of the adjusted dispersal kernel.

Our findings regarding juvenile dispersal disagree with those of another study using conventional tag-recapture methods (Abecasis

et al. 2009). In that study, small *D. vulgaris* (<12 cm TL) were reported to usually remain in the same area for up to one month, or if they did disperse, they only moved a few kilometres. In that study, however, the time period study was much shorter than in the present study, and their findings were from a coastal lagoon, a different environment than the open, rocky coast we investigated. Moreover, conclusions drawn from conventional tag-recapture studies (as in Abecasis et al. 2009) are highly dependent on recapture effort. The otolith chemistry approach implemented in this study provided a quantitative dispersal estimate unaffected by any recapture bias.

Another study using microchemical analyses of *D. vulgaris* otoliths indicates that 2+ years individuals disperse across tens of square kilometres (Correia et al. 2011). However, these analyses by Correia et al. (2011) were based on examination of the whole otolith using solution based analyses, which provide less useful information than our analyses for detecting dispersal; analysing the whole otolith loses information related to the location of the individual during particular times and thus life stages.

5. Conclusion

Our estimate of propagule dispersal falls within the range identified for other temperate fishes (50-500 km, Anadon et al. 2013 and references therein) and, therefore our evidence supports the

conclusion that a distance of 100 km between MPAs within a network would be appropriate for this species (Di Franco et al. 2012a,b, Anadon et al. 2013). This conclusion is further strengthened by our estimate of dispersal at juvenile stage, which demonstrates that some *D. vulgaris* disperse tens of kilometres, and a few travel more than 100 km.

Generally, dispersal and connectivity in demersal fishes (particularly for coastal species) are equated with dispersal just at propagule stages, and the contribution of movement during later life stages is usually considered negligible. This view resembles what in freshwater fish ecology is termed the "restricted-movement paradigm" (RMP, Rodriguez 2002). This propagule-centred view is frequent in the literature on MPA network design. In contrast, our findings stress the importance of dispersal during other life stages in connecting sites and potentially driving export/import of biomass from/to MPAs. This dispersal of individuals at different stages can have important consequences for population dynamics and genetics (Gaines and Bertness 1993), and thus a more complete understanding of dispersal processes across multiple life stages is required. In this perspective only few studies assessed dispersal and movement patterns over multiple life history stages and evidences suggest that juveniles can play a relevant role in contributing to species dispersal (Tobin et al. 2010, McMahaon et al. 2012, Bouchard et al. 2015, see Green et al. 2014 for a review about

Larval dispersal and movement patterns of coral reef fishes). Our findings further contribute to strength these evidences.

Despite the critical importance of understanding dispersal (Jones et al. 2007, Planes et al. 2009), there is still relatively little information about the scale of dispersal and connectivity, especially for temperate fishes. Here we provide information about dispersal at both the propagule and juvenile stages for a temperate coastal fish that highlights the important role of dispersal during the juvenile life stages in connecting populations. This represents a new and surprising piece of information, one with direct implications for management and the design of effective MPAs and MPA networks.

By highlighting extensive dispersal during two life stages, our findings further contribute to the conclusion that MPAs can provide fisheries benefits across large distances and to communities relying on fishing resources, and that they can contribute to ecosystem-wide recovery from disturbance. In fact, in addition to the well-known propagule export from MPAs, which typically have higher density and biomass of spawners than surrounding fished areas, and have the potential to replenish unprotected areas 100s km from the MPAs (Pelc et al. 2010, Di Franco et al. 2012a), our work identifies the possible role of juvenile dispersal in replenishing fishing grounds and connecting MPAs within a network.

Such information can play a powerful role in strengthening stakeholder support by demonstrating that benefits of MPAs extend across a larger spatial scale than previously recognized. In fact, as

pointed out for a system of small customary tenure areas in Papua New Guinea (Almany et al. 2013), understanding whether and at what spatial scale human communities can benefit from management actions is key to designing effective strategies, obtaining support for management, and providing greater incentives for compliance.

Acknowledgements

This research was funded by Total Foundation (http://foundation.total.com/foundation/total-foundation-200090.html). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors wish to thank Christian Vaglio, Manfredi Di Lorenzo, Giorgio Aglieri, Giacomo Milisenda, (University of Salento), Pasquale Baiata (Coop Coris, Palermo), Commander Ugo Adorante (Nucleo Subacqueo Carabinieri, Bari, Italy) and his team (Pietro Di Pinto, Gianfranco Simonini, Carlo Del Console, Fabrizio Pichierri, Gianni Sgariglia, Roberto Ciccacci, Mauro Bellini) for invaluable assistance during fieldwork.

The authors wish to thank the editor and three anonymous reviewers for their useful comments which have helped us to improve the manuscript.

The paper is dedicated to the memory of Glenn Almany, who tragically passed away, that provided constructive comments and revisions that improved the manuscript.

References

Abecasis, D., Bentes, L., Erzini, K., 2009. Home range, residency and movements of *Diplodus sargus* and *Diplodus vulgaris* in a coastal lagoon: connectivity between nursery and adult habitats. Estuarine Coastal and Shelf Science 85, 525–529.

Almany, G., Hamilton, R., Matawal, M., Bode, M., Potuko, T., Saenz-Agudelo, P., Planes, S., Berumen, M.L., Rhodes, K., Thorrold, S.R., Jones, J.P., Russ, G.R. 2013. Dispersal of grouper larvae drives local resource sharing in a coral reef fishery. – Curr. Biol. 23: 626–630. doi:10.1016/j.cub.2013.03.006.

Anadón, J. D., Mancha-Cisneros, M.M., Best, B.D., Gerber, L.R. 2013. Habitat-specific larval dispersal and marine connectivity: implications for spatial conservation planning. - Ecosphere 4(7): 82. http://dx.doi.org/10.1890/ ES13-00119.1

Anderson, M. J., Willis, T. J. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. - Ecology 84: 511–525.

Andrello, M., Mouillot, D., Beuvier, J., Albouy, C., Thuiller, W., Manel, S. 2013. Low Connectivity between Mediterranean Marine Protected Areas: A Biophysical Modeling Approach for the Dusky Grouper Epinephelus marginatus. PLoS ONE 8(7): e68564. doi:10.1371/journal.pone.0068564

Andrello, M., Mouillot, D., Somot, S., Thuiller, W., Manel, S. 2015. Additive effects of climate change on connectivity between

694 marine protected areas and larval supply to fished areas. -
695 Divers. Distrib. 21: 139–150. doi: 10.1111/ddi.12250
696Baker, M., Nur, N., Geupel, G. R. 1995. Correcting biased estimates
697 of dispersal and survival due to limited study area: theory and
698 an application using wrentits. - Condor 97: 663–674.
699Berumen, M. L. et al. 2010. Otolith geochemistry does not reflect
700 dispersal history of clownfish larvae. - Coral Reefs 29: 883–
701 891.
702Buston, P. M., Jones, G.P., Planes, S., Thorrold, S.R. 2012. Probability
703 of successful larval dispersal declines fivefold over 1 km in a
704 coral reef fish. - Proc. R. Soc. B 279: 1883–1888.
705Calò, A., Félix-Hackradt, F.C., Garcia, J., Hackradt, C.W., Rocklin, D.,
706 Otón, J.T., García Charton, J.A. 2013. A review of methods to
707 assess connectivity and dispersal between fish populations in
708 the Mediterranean Sea. - Adv. Oceanogr. Limnol. 4:2: 150-175.
709 DOI: 10.1080/19475721.2013.840680.
710Campana, S. E. 1999. Chemistry and composition of fish otoliths:
711 pathways, mechanisms and applications. – Mar. Ecol. Prog.
712 Ser. 188: 263−297.
713Clarke, K. R., Gorley, R. N. 2006. PRIMER v6: User Manual/Tutorial.
714 PRIMER-E, Plymouth.
715Clarke KR, Somerfield PJ, Gorley RN (2008) Testing of Null
716 Hypotheses in Exploratory Community Analyses: Similarity
717 Profiles and Biota-environment Linkage. J Exp Mar Biol Ecol.
718 366(1-2):56–69.

Clobert, J., Danchin, E., Dhondt, A., Nichols, J. 2001. Dispersal. Oxford University Press, Oxford.

Cooper, C. B., Daniels, S.J., Walters, Jr. 2008. Can we improve estimates of juvenile dispersal and survival? - Ecology 89: 3349–3361.

Correia, A.T., Pipa, T., Gonçalves, J.M.S., Erzini, K., Hamer, P.A. 2011. Insights into population structure of *Diplodus vulgaris* along the SW Portuguese coast from otolith elemental signatures. - Fish. Res. 111: 82–91.

D'Anna, G., Giacalone, V.M., Badalamenti, F., Pipitone, C. 2004. Releasing of hatchery-reared juveniles of the white seabream *Diplodus sargus* (L., 1758) in the Gulf of Castellammare artificial reef area (NW Sicily). - Aquaculture 233: 251–268.

Di Franco, A., Coppini, G., Pujolar, J.M., De Leo, G.A., Gatto, M., Lyubartsev, V., Melià, P., Zane, L., Guidetti, P. 2012a. Assessing Dispersal Patterns of Fish Propagules from an Effective Mediterranean Marine Protected Area. - PLOS ONE 7(12): e52108. doi:10.1371/journal.pone.0052108

Di Franco, A., Gillanders, B.M., De Benedetto, G., Pennetta, A., De Leo, G.A., Guidetti, P. 2012b. Dispersal Patterns of Coastal Fish: Implications for Designing Networks of Marine Protected Areas. PLOS ONE 7(2): e31681. doi:10.1371/journal.pone.0031681

Di Franco, A., Qian, K., Calò, A., Di Lorenzo, M., Planes, S., Guidetti, P. 2013. Patterns of variability in early life traits of a

744 Mediterranean coastal fish. - Mar. Ecol. Prog. Ser. 476: 227-
745 235.
746Di Franco, A., Bulleri, F., Pennetta, A., De Benedetto, G., Clarke, K.R.,
747 Guidetti, P. 2014. Within-Otolith Variability in Chemical
748 Fingerprints: Implications for Sampling Designs and Possible
749 Environmental Interpretation. PLOS ONE 9(7): e101701.
750 doi:10.1371/journal.pone.0101701
751Di Lorenzo, M., D'Anna, G., Badalamenti, F., Giacalone, V.M., Starr,
752 R., Guidetti, P. 2014. Fitting the size of marine reserves to
753 movement patterns of protected species: a case study on the
754 white seabream (*Diplodus sargus sargus*) in the Mediterranean
755 Sea. - Mar. Ecol. Prog. Ser. 502: 245-255.
756Elsdon, T. S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones,
757 C.M., Limburg, K.E., Secor, D.H., Thorold, S.R., Walther, B.D.
758 2008. Otolith chemistry to describe movements and life-
759 history parameters of fishes: hypotheses, assumptions,
760 limitations and inferences. - Oceanogr. Mar. Biol. 46: 297−330.
761Evans, R. D., Russ, G.R., Kritzer, J.P. 2008. Batch fecundity of
762 *Lutjanus carponotatus* (Lutjanidae) and implications of no-take
763 marine reserves on the Great Barrier Reef, Australia. - Coral
764 Reefs 27: 179–189.
765Fisher, W., Bauchot, M.L., Schneider, M. (eds) 1987. Fiches FAO
766 d'identification des espèces pour les besoins de la pèche. In
767 Méditerranée et mer Noire. Zone de pêche 37. Volume II.
768 Vertebres. Rome: FAO, pp. 761–1530.

Fraser, D. F., Gilliam, J., Daley, M.J., Le, A.N., Skalski, G.T. (2001) Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. - Am. Nat. 158: 124–135.

Gaines, S. D., Bertness, M. 1993. The dynamics of juvenile dispersal: why field ecologists must integrate. – Ecology 74: 2430-2435.

Gaines, S. D., White, C., Carr, M.H., Palumbi, S.R. 2010. Designing marine reserve networks for both conservation and fisheries management. - P. Natl. Acad. Sci. USA 107: 18286–18293.

Gillanders, B. M. 2009. Tools for studying biological marine ecosystem interactions—natural and artificial tags. In: Nagelkerken I (ed) Ecological connectivity among tropical coastal systems. Springer, New York, NY, p 457−492.

Gonçalves, J.M.S., Erzini, K. 2000. The reproductive biology of the two banded sea bream *Diplodus vulgaris* from the southwest coast of Portugal. – J. Appl. Ichthyology 16: 110−116

Green, B. S., Mapstone, B., Carlos, G., Begg, G.A. 2009. Tropical fish otoliths: Information for assessment, management and ecology. Springer, New York, NY. 314 pp.

Green A. L., Maypa, A.P., Almany, G.R., Rhodes, K.L., Weeks, R., Abesamis, R.A., Gleason, M.G., Mumby, P.J., White, A.T. 2014. Larval dispersal and movement patterns of coral reef fishes, and implications for marine reserve network design. - Biol. Rev. doi: 10.1111/brv.12155.

Guidetti, P. 2006. Marine reserves re-establish lost predatory interactions and cause community-wide changes in rocky reefs. – Ecol. Appl. 16: 963–976.

Hamer, P.A., Acevedo, S., Jenkins, G.P., Newman, A. 2011. Connectivity of a large embayment and coastal fishery: spawning aggregations in one bay source local and broad-scale fishery replenishment. - J. Fish Biol. 78: 1090−1109.

Harrison, H.B., Williamson, D.H., Evans, R.D., Almany, G.R., Thorrold, S.R., Russ, G.R., Fedheim, K.A., van Herwerden, L., Planes, S., Srinivasan, M., Berumen, M.L., Jones, G.P. 2012. Larval export from marine reserves and the recruitment benefit for fish and fisheries. - Curr Biol 22:1023–1028. doi:10.1016/j.cub.2012.04.008

Jones, G.P., Srinivasan, M., Almany, G.R. 2007. Population connectivity and conservation of marine biodiversity. - Oceanography 20: 100–111.

Koenig, W.D., Van Vuren, D., Hooge, P. N. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. - Trends Ecol. Evol. 11: 514–517.

La Mesa, G., Consalvo, I., Annunziatellis, A., Canese, S. 2013. Spatio-temporal movement patterns of *Diplodus vulgaris* (Actinopterygii, Sparidae) in a temperate marine reserve (Lampedusa, Mediterranean Sea). - Hydrobiologia 20: 129-144.

Lloret, J., Zaragoza, N., Caballero, D., Font, T., Casadevall, M., Riera, V. 2008. Spearfishing pressure on fish communities in rocky coastal habitats in a Mediterranean marine protected area. – Fish Res. 94: 84–91.

Matthysen, E., Adriaensen, F., Dhondt, A. A. 1995. Dispersal distances of nuthatches, *Sitta europaea*, in a highly fragmented habitat. - Oikos 72: 375–381.

Mouine, N., Francour, P., Ktari, M.H., Chakroun-Marzouk, N. 2012. Reproductive biology of four *Diplodus* species *Diplodus vulgaris*, *D. annularis*, *D. sargus sargus* and *D. puntazzo* (Sparidae) in the Gulf of Tunis (central Mediterranean). J. Mar. Biol. Assoc. UK 92: 623–631.

Nathan, R., Perry, G., Cronin, J.T., Strand, A.E., Cain, M.L. 2003. Methods for estimating long-distance dispersal. - Oikos 103: 261–273.

Papetti, C., Di Franco, A., Zane, L., Guidetti, P., De Simone, V., Spizzottin, M., Zorica, B., Čikeš Keč, V., Mazzoldi, C. 2013. Single population and common natal origin for Adriatic *Scomber scombrus* stocks: evidence from an integrated approach. - ICES J. Mar. Sci. 70: 387–398. doi: 10.1093/icesjms/fss201

Pajuelo, J.G., Lorenzo, J.M., Bilbao, A., Ayza, O., Ramos, A.G. 2006. Reproductive characteristics of the benthic coastal fish *Diplodus vulgaris* (Teleostei: Sparidae) in the Canarian

archipelago, northwest Africa. – J. Appl. Ichthyology 22: 414−418

Pelc, R. A., Warner, R.R., Gaines, S., Paris, C.B. 2010. Detecting larval export from marine reserves. - P. Natl. Acad. Sci. USA 107(43): 18266–18271.

Planes, S., Macpherson, E., Biagi, F., Garcia-Rubies, A., Harmelin, J., Harmelin-Vivien, M., Jouvenel, J.-Y., Tunesi, L., Vigliola, L., Galzin, R. 1999. Spatio-temporal variability in growth of juvenile sparid fishes from the Mediterranean littoral zone. - J. Mar. Biol. Assoc. UK 79: 137-143.

Planes, S., Jones, G.P., Thorrold S. 2009. Larval dispersal connects fish populations in a network of marine protected areas. - P. Natl. Acad. Sci. USA 106: 5693–5697.

Pujolar J. M., Schiavina, M., Di Franco, A., Melià, P., Guidetti, P., Gatto, M., De Leo, G. A., Zane, L. 2013. Understanding the effectiveness of marine protected areas using genetic connectivity patterns and Lagrangian simulations. - Divers. Distrib. 19: 1531–1542. DOI: 10.1111/ddi.12114.

Radinger, J., Wolter, C. 2013. Patterns and predictors of fish dispersal in rivers. - Fish Fish. 13: 456–473. doi:10.1111/faf.12028.

Reis-Santos, P., Tanner, S.E., Vasconcelos, R.P., Elsdon, T.S., Cabral, H.N., Gillanders, B.M. 2013. Connectivity between estuarine and coastal fish populations: contributions of estuaries are not consistent over time. – Mar. Ecol. Prog. Ser. 491: 177-186.

Rodriguez, M. A. 2002. Restricted movement in stream fish: the paradigm is incomplete, not lost. - Ecology 83: 1–13.

Starrs, D., Ebner, B.C., Fulton, C.J. 2014. All in the ears: Unlocking the early life history biology and spatial ecology of fishes. - Biol. Rev. doi: 10.1111/brv.12162

Thorrold, S. R., Jones, G. P., Hellberg, M. E., Burton, R. S., Swearer, S. E., Neigel, J. E., Morgan, S. G., Warner, R. R. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. – Bull. Mar. Sci. 70: 291-308.

Tobin, D., Wright, P.J., Gibb, F.M., Gibb, I.M. 2010. The importance of life stage to population connectivity in whiting (*Merlangius merlangus*) from the northern European shelf. – Mar. Biol. 157: 1063−1073.

Vigliola, L., Harmelin-Vivien, M.L. 2001. Post-settlement ontogeny in three Mediterranean reef fish species of the genus *Diplodus*. - Bull. Mar. Sci. 68: 271-286.

Weersing, K., Toonen, R. J. 2009. Population genetics, larval dispersal, and connectivity in marine systems. – Mar. Ecol. Prog. Ser. 393: 1–12.

White, J. W., Ruttenberg, B. I. 2007. Discriminant function analysis in marine ecology: some oversights and their solutions. – Mar. Ecol. Prog. Ser. 329: 301–305.

Figure legends

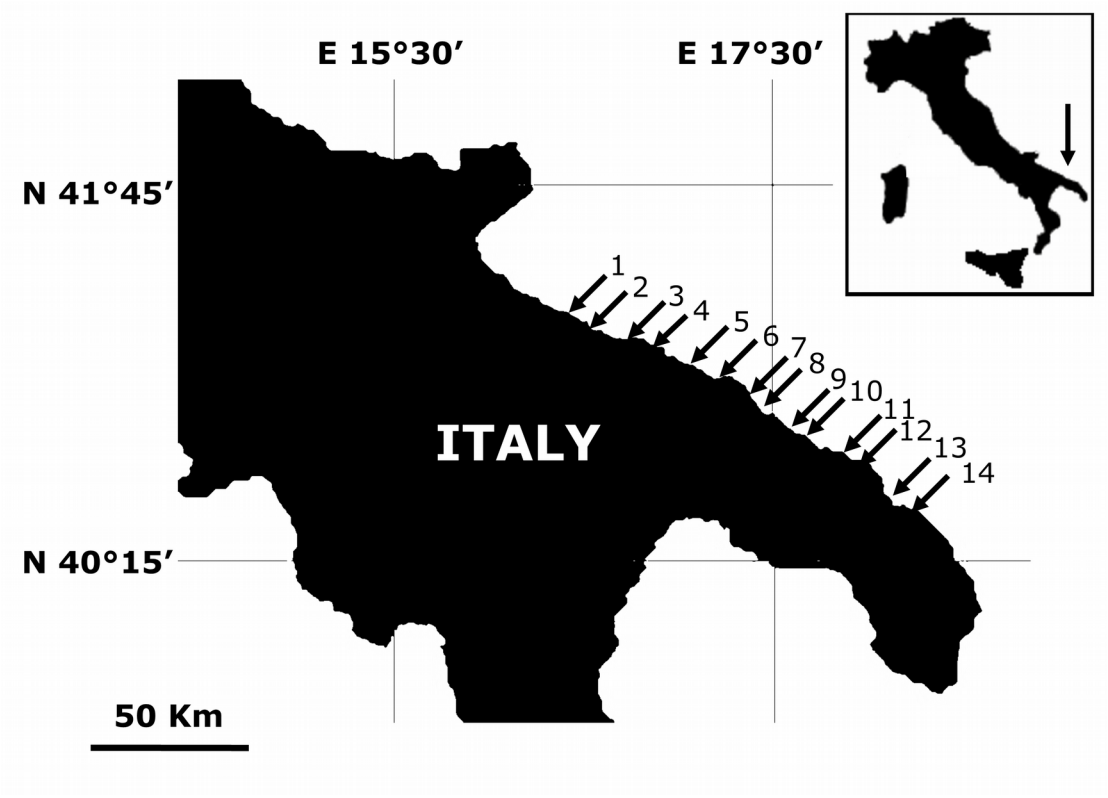

Figure 1. Study area. Sampling sites are indicated with arrows. Sites are numbered progressively from 1 (most northern site) to 14 (most southern site).

Figure 2. Classification of post-settlers otolith cores into groups based on differences in elemental composition. Letters indicate the seven statistically different groups (arbitrarily named from left to right) identified by SIMPROF analysis. Thick black lines indicate significant differences among groups. Red lines indicate non-significant differences among samples. Individual samples are labelled on the x-axis with a symbol corresponding to the sampling site from which they were collected (see legend on the right of the figure). Sites are numbered progressively from 1 (most northern site) to 14 (most southern site).

907

908

909**Figure 3**. Exponential decay fitting for juvenile dispersal kernels
910estimated from a) otolith chemistry data, b) randomised data, and
911c) adjusted data. Dotted red lines are 95% confidence intervals
912calculated using simultaneous Working-Hotelling procedure.

913

85 43

86

Supporting Information

Appendix A.

Otolith preparation and chemical analyses

Otolith preparation

In the laboratory, one sagittal otolith was removed from each specimen, cleaned of soft tissue using plastic dissecting pins, and mounted sulcus side up on a glass slide using crystal bond (Aremco Products, Inc.). Otoliths were polished with 3 µm and 1 µm Imperial 3M lapping film to expose inner growth layers for analysis. We chose not to polish the otolith to the core and to leave material above it in order to ensure the core was not removed during pre-ablation procedures, which potentially allowed us to sample all the material associated with the core. After polishing with lapping film, otoliths were rinsed and sonicated for 10 minutes in ultra-pure water. Otoliths were dried and arranged onto new glass slides (6 otoliths per slide). All otoliths were randomly ordered to prevent sample batch bias.

Otolith chemical analyses

In post-settlers we used laser ablation to sample material associated with the core using three discrete vertical pits 30 µm deep

(identified previously as approximate core size of the cores) from the surface of the otolith through the visible core. The spike in Mn:Ca was used as an indicator of the core location, as previous studies have reported elevated Mn concentrations in the core (Brophy et al. 2004, Ruttenberg et al. 2005), and therefore just one out of the three pits sampled in the core (the one showing at least 3-fold higher Mn:Ca concentration than surrounding material, Brophy et al. 2004) was considered in subsequent analysis. A Mn:Ca spike could not be detected in 13% (21 otoliths) of the core samples of post-settlers; these samples were not used in the analysis of natal origins.

In the post-settlement portion of otoliths of both post-settlers and juveniles, we analysed the same otolith portion (i.e. corresponding to about 10 days after settlement). We ablated three horizontal pits and all three were considered in the subsequent analysis in order to account for within-otolith variability and to optimize sampling design (Di Franco et al. 2011, see Di Franco et al. 2014 for an in-depth discussion about this issue).

Once otoliths were inside the laser ablation chamber, they 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 using a spot size of 30 µm. Each run generally consisted of 40 s acquisition, 10 s blank to correct for background which was subtracted from each sample, 10 s ablation (laser at 65% power, about 6 J/cm^2) resulting in a pit

about 10 µm deep, and 20 s for washout. Prior to analysis, samples were pre-ablated to remove any surface contamination (laser at 50% power). Helium gas was flushed into the ablation cell to reduce the deposition of ablated aerosols and to improve signal intensity. The ablated aerosol was then mixed with argon before entering the inductively coupled plasma (ICP) torch. All otoliths were analysed using a Thermo Elemental inductively coupled plasma mass spectrometer (ICP-MS) connected to a NewWave Research UP213 with aperture imaging laser ablation (LA) system (see table S1 for a summary of operating conditions and data acquisition parameters). External calibration was performed with two Standard References Materials (SRM) from National Institute of Standards and Technology, NIST 610 and NIST 612. Calcium was used as an internal standard to account for variation in ablation and aerosol efficiency (Yoshinaga et al. 2000).

All 9 elements analyzed (^{24}Mg, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb, ^{7}Li, ^{57}Fe, ^{59}Co) 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 (see Table A2).

Mean estimates of precision (%RSD, relative standard deviation) and accuracy for NIST 610 and NIST 612 were calculated based on 109 replicate measurements (Table A1). Recorded values of Li, Fe, Zn, Pb and Co were consistently below detection limits and therefore excluded from the analyses.

References

Brophy, D. et al. 2004. Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. – Mar. Biol. 144: 779–786.

Di Franco, A. et al. 2011. Large scale variability in otolith microstructure and microchemistry: the case study of *Diplodus sargus sargus* (Pisces: Sparidae) in the Mediterranean Sea. – Ital. J. Zool. 78(2): 182–192.

Di Franco, A. et al. 2014. Within-Otolith Variability in Chemical Fingerprints: Implications for Sampling Designs and Possible Environmental Interpretation. – PLOS ONE 9(7): e101701. doi:10.1371/journal.pone.0101701

Ruttenberg, B. I. et al. 2005. Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. - Mar. Ecol. Progr. Ser. 297: 273–281.

Yoshinaga, J. et al. 2000. Fish otolith reference material for quality assurance of chemical analyses. – Mar. Chem. 69: 91−97.

Appendix B

Accounting for uncharacterized settlement site(s)

Accurate assessment of site fidelity and juvenile dispersal (i.e. assignment of juveniles to settlement sites) relies on the assumption that all possible settlement sites contributing to the juvenile pool investigated have been sampled and included in the data set (Campana 1999, Reis Santos et al. 2013). However, despite our intensive sampling of a number of settlement sites identified as important for the study area based on a preliminary survey carried out by authors, it is in practice impossible to include all possible settlement sites across the study area (180 km of coastline). From this perspective, other non-sampled settlement sites may have contributed to juveniles analysed in the present study, and indeed in some cases, the juvenile otolith signature did not match those of any settlers used as the baseline data set. In order to reduce the potential bias related to uncharacterized settlement sites we adopted a statistical approach used in similar studies (Hamer et al. 2005, Chittaro et al. 2009, Reis-Santos et al. 2013): we compared otolith elemental signatures of juveniles with those of settlers using principal component analysis (PCA). Juveniles that fell outside a 95% confidence ellipse around the settler baseline data (elemental signatures of settlement sites) were assumed to have originated

from uncharacterized settlement site(s) and were excluded from further analyses.

The elemental fingerprints from the juvenile portion of otoliths were mostly distributed within the 95% confidence ellipses of the post-settler baseline data (Fig. A3). However, there were 31 juveniles (~19%) that fell outside the confidence ellipses of the post-settler data (i.e. putatively originating from uncharacterized settlement sites) and were excluded from further analysis, and thus the analysis consisted of a total of 133 individuals.

References

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. – Mar. Ecol. Progr. Ser. 188: 263−297.

Chittaro, P.M. et al. 2009. Spatial and temporal patterns in the contribution of fish from their nursery habitats. - Oecologia 160: 49−61.

Hamer, P.A. et al. 2005. Chemical tags in otoliths indicate the importance of local and distant settlement areas to populations of a temperate sparid, *Pagrus auratus*. – Can. J. Fish. Aquat. Sci. 62: 623−630.

Reis-Santos, P. et al. 2013. Connectivity between estuarine and coastal fish populations: contributions of estuaries are not consistent over time. – Mar. Ecol. Progr. Ser. 491: 177-186.

Appendix C

Juvenile dispersal kernels

The probability of detecting dispersal declines with distance from the source and it depends on the spatial arrangement of sampling sites and on number of sampled specimens. Specifically, in our case, we would be able to record a displacement corresponding to the maximum distance between sites (i.e. approx. 180 km) only for the specimens collected at the northernmost and southernmost sampling sites, while we would be able to record zero dispersal (0 km, i.e. juvenile collected at the same site where it settled) for all individuals from all the sampling sites. As highlighted by Matthysen et al. 1995, several reported dispersal patterns are in fact due to the limitations of the set of all potential observations. From this perspective, a comparison of the observations that are actually made with the set of observations that could have been made must be carried out (Matthysen et al. 1995).

We would expect a decline in the frequency of observations as dispersal distance increases simply as a result of the spatial arrangement of sampling sites. To account for this inevitable bias, we used the approach of Matthysen et al. 1995, and constructed a null dispersal kernel (*sensu* Caley 1991) describing the null hypothesis of random dispersal. The null hypothesis is that each individual has the same chance to disperse all possible distances

among sampling sites (e.g. to not disperse and to disperse over the maximum distance allowed within the study area). To construct the null dispersal kernel, we accounted for the effect of sample size (i.e. number of juveniles collected from each site), using real sampling numbers. This dispersal kernel provides information about our "ability" to detect dispersal given the spatial arrangement of our sampling sites.

We then compared a randomised dispersal kernel with the measured dispersal kernel (based on our observed data) using a Wilcoxon-Mann-Whitney test. Any differences between the two dispersal kernels would indicate higher or lower real dispersal compared to the dispersal pattern predicted by the null kernel.

Based on Matthysen et al. 1995, we corrected our dispersal estimates for the inverse probability to detect dispersal at a given distance. This probability was taken from the randomized dispersal kernel. In other words, we used the inverse probability to observe dispersal at a given distance (i.e. probability described in the random dispersal kernel) as a distance-weight correction: dispersal distances that were less likely to be observed (e.g. high-distance dispersal) were overweighted compared to dispersal distances with a high probability of observation (e.g. no dispersal).

The use of more sophisticated correction techniques (e.g. Baker et al. 1995, Cooper et al. 2008) would require greater knowledge about the distribution of available sites for settlement and recruitment

across our study area. This task is, in a field situation, impossible for the studied species in such a large study area.

Statistical analyses were run using the open source software ‘R’ (see www.r-project.org).

References

Baker, M. et al. 1995. Correcting biased estimates of dispersal and survival due to limited study area: theory and an application using wrentits. - Condor 97: 663–674.

Caley, M. J. 1991. A null model for testing distributions of dispersal distances. – Am. Nat. 138: 524–532.

Cooper, C. B. et al. 2008. Can we improve estimates of juvenile dispersal and survival? - Ecology 89: 3349–3361.

Matthysen, E. et al. 1995. Dispersal distances of nuthatches, *Sitta europaea*, in a highly fragmented habitat. - Oikos 72: 375–381.

Appendix D

Settlement sites replenishment by natal origins

Among the seven groups of post-settlers identified, four groups of post-settlers consisted of 1-3 individuals. Group G consisted of a total of three individuals, and single fish was collected at each of three sites located in the south of the study area. Group A consisted of one individual from a site located approximately in the middle of study area, Group B consisted of one individual from a site in the north of the study area, Group D consisted of one individual from the southernmost sampling site (Fig. A2). Note that in Figure A2 these Groups – A, B, D and G – are omitted to improve clarity.

Table A1. Operating conditions and data acquisition parameters for LA-ICP-MS analysis

ICP-MS	
Model	Thermo Elemental XSeriesII
Forward power	1200 W
Gas flows	
Coolant (plasma)	Ar: 13 l min^{-1}
Auxiliary	Ar: 0.7 l min^{-1}
Sample transport	He: ca 0.5 l min^{-1} (in the ablation cell), Ar: ca 0.9 l min^{-1}
Laser	
Model	NewWave Research UP213 with aperture imaging
Wavelength	213 nm (Nd:YAG)
Pulse width (FWHM)	3 ns
Energy distribution	Homogenized, flat beam, aperture imaged
Energy density (fluence)	6.0 J cm^{-2}
Repetition rate	2 Hz
Crater diameter	30 µm
Analysis protocol	

Scanning mode	Peak jumping, 1 point per peak, 10 ms dwel time
Acquisition mode	Time resolved analysis
Analysis duration	40 s (10 s background, 10 s signal, 20 s washout)
Isotopes monitored	^{7}Li, ^{24}Mg, ^{44}Ca ^{55}Mn, ^{57}Fe, ^{59}Co, ^{66}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb

1142

1143

Table A2. Estimates of precision, accuracy and limits of detection (LOD). Values for %RSD (% relative standard deviation) and % accuracy are dimensionless. LOD are given in mmol mol^{-1}.

Element Ratio	NIST 610 % RSD	NIST 612 % RSD	% Accuracy NIST 610	% Accuracy NIST 612	LOD
Mg:Ca	8.95	15.44	103	110.2	0.056
Mn:Ca	6.40	10.95	101.55	113.73	0.077
Sr:Ca	4.60	10.51	100.90	93.62	0.027
Ba:Ca	9.30	9.52	102.23	89.78	0.0031

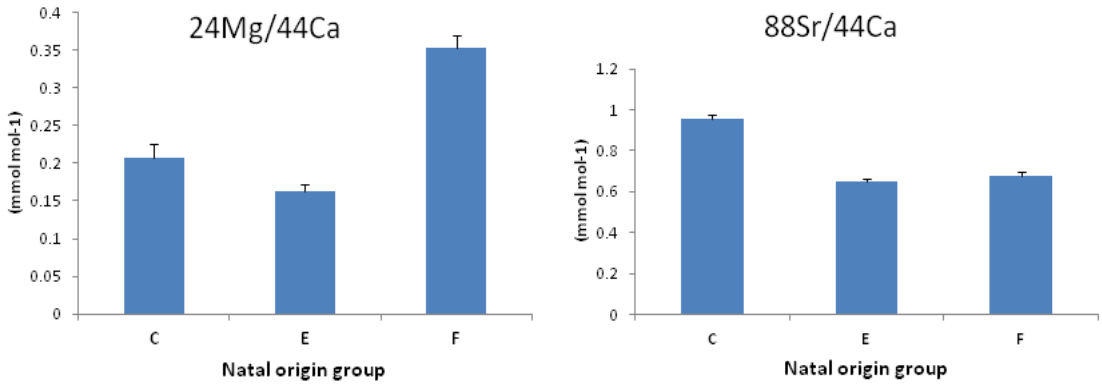

Figure A1. Average Mg:Ca and Sr:Ca calcium ratios (± standard error) in the otolith core region for the three major natal source groups identified by SIMPROF analysis. Group C was characterized by intermediate concentrations of Mg:Ca and high concentrations of Sr:Ca compared to groups E and F. Group E was characterized by low Mg:Ca concentrations and intermediate Sr:Ca concentrations. Group F was characterized by high Mg:Ca concentrations and intermediate Sr:Ca concentrations.

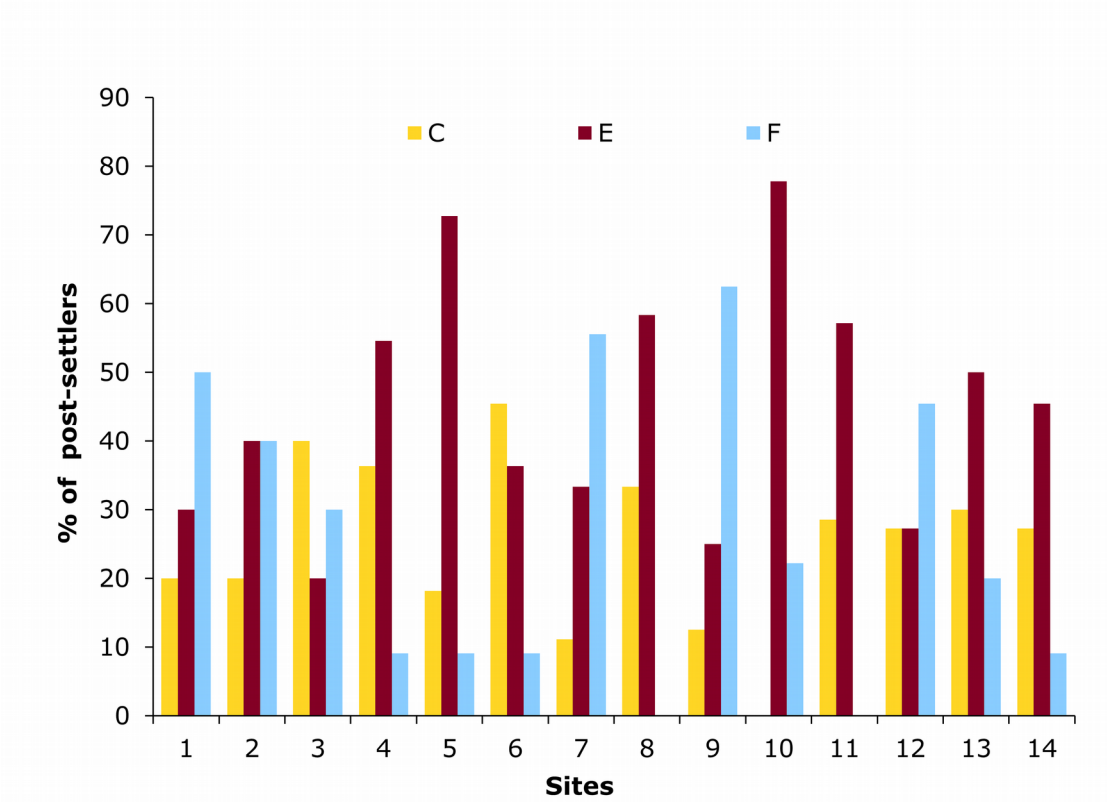

Figure A2. Percentage of post-settlers originating from the three major putative natal source groups based on otolith core signatures and their contributions to replenishment at the 14 sampling sites. Different colors represent the three groups identified by SIMPROF analysis. Sites are numbered progressively on the x-axis from 1 (most northern sampling site) to 14 (most southern sampling site). Note that the four marginal groups each contributing only 1-3 individuals – A, B, D and G – are omitted to improve graph clarity.

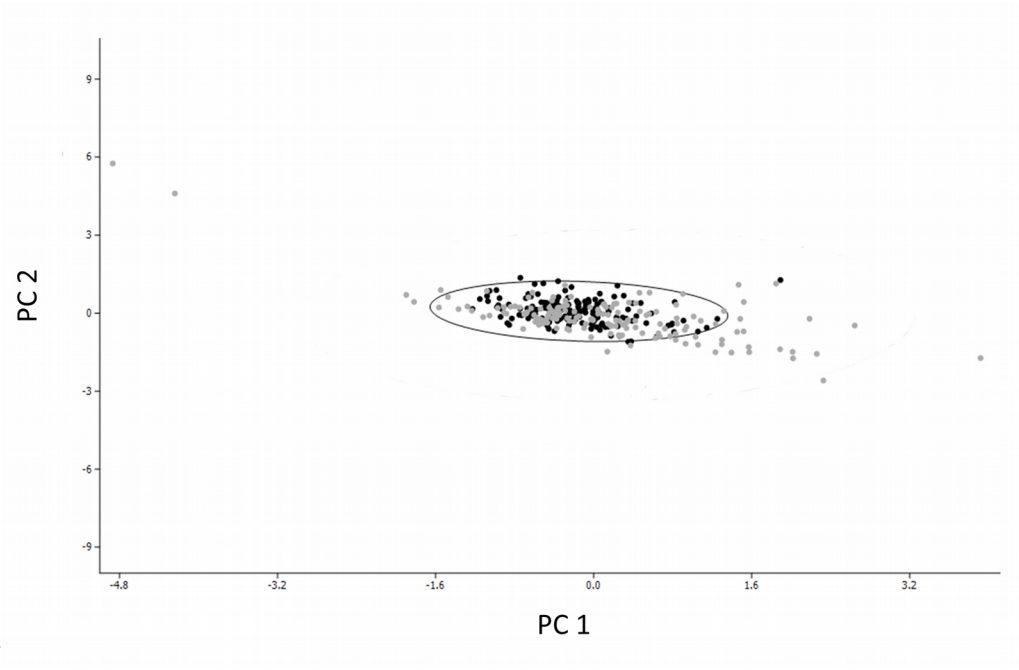

Figure A3. Ordination plot of principal component analysis (PCA) comparing multi-element otolith signatures of juveniles (grey circles) and post-settlers of known origin (black circles) forming the baseline group. Ellipsis represents the 95% confidence ellipse around the baseline group data.

Figure A4. Juvenile dispersal kernel from observed (red) and randomised (blue) data (see Appendix C for further details).