

1 **Dispersal of larval and juvenile seabream: Implications for**

2 **Mediterranean marine protected areas**

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21recruitment, two banded seabream, Marine Protected Areas

22**Abstract**

23

24In the marine context, information about dispersal is essential for
25the design of networks of marine protected areas (MPAs). Generally,
26most of the dispersal of demersal fishes is thought to be driven by
27the transport of eggs and larvae in currents, with the potential
28contribution of dispersal in later life stages relatively minimal.

29Using otolith chemistry analyses, we estimate dispersal patterns
30across a spatial scale of approximately 180 km at both propagule
31(i.e. eggs and larvae) and juvenile/sub-adult (i.e. between
32settlement and recruitment to the fishery) stages of a
33Mediterranean coastal fishery species, the two-banded seabream
34*Diplodus vulgaris*.

35We detected three major natal sources of propagules replenishing
36local populations in the entire study area, suggesting that propagule
37dispersal distance extends to at least 90 km. For the juvenile stage,
38we detected dispersal of up to 165 km.

39Our work highlights the surprising and significant role of dispersal
40during the juvenile life stages as an important mechanism
41connecting populations. Such new insights are crucial for creating
42effective management strategies (e.g. MPAs and MPA networks) and
43to gain support from policymakers and stakeholders, highlighting
44that MPA benefits can extend well beyond MPA borders, and not only
45via dispersal of eggs and larvae, but also through movement by
46juveniles.

48 **1. Introduction**

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

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

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

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

73the occurrence of spawning aggregations and, therefore, to
74generate greater propagule production compared to fished areas
75(Evans et al. 2008, Di Franco et al. 2012a). In a network of MPAs,
76each individual MPA should be adequately connected to the others
77via dispersal to support the persistence and/or the recovery of local
78populations from disturbance (Planes et al. 2009, Gaines et al.
792010). If MPAs are isolated from one another and not connected by
80dispersal between them, MPAs are more vulnerable to local
81extinctions because of local perturbations, since they cannot be
82replenished by immigration from elsewhere (Gaines et al. 2010).

83The management-oriented need for information on dispersal was
84recently recognized even at policy level, as highlighted by the
85implementation of the California Marine Life Protection Act in the
86USA (Anadon et al. 2013) and by the 'Marine Strategy Framework
87Directive' (MSFD; 2008/56/EC) in the EU, where the creation of
88coherent and effective networks of MPAs is considered a key tool to
89reach conservation targets in the marine environment (Anadon et al.
902013).

91Despite the variety of approaches currently used to tackle this issue,
92tracking the movements of marine fauna and quantifying dispersal
93patterns is, however, a complex task due to the difficulty in
94following individuals throughout their entire life cycles (Calò et al.
952013). Many larval dispersal patterns are estimated using models
96(e.g. Lagrangian models) parameterized with information about
97species life history traits (e.g. pelagic larval duration (PLD) and

98 spawning date (SpD)) and oceanographic data (Pujolar et al. 2013,
99 Andrello et al. 2013, 2015). Other approaches that have proved
100 highly valuable in estimating fish movements and dispersal use
101 genetics (Planes et al. 2009, Weersing and Toonen 2009) and
102 tagging (both natural and artificial, Thorrold et al. 2002, Di Lorenzo
103 et al. 2014). Among natural tags, otolith chemical signatures have
104 proven to be a valuable approach to both tracking fish movements
105 and modelling dispersal patterns (Elsdon et al. 2008, Gillanders
106 2009, Di Franco et al. 2012b). Focusing on natural tags, otoliths (ear
107 bones) are carbonate structures usually in the form of aragonite
108 (even if they can be found also in form of vaterite) located in inner
109 ear of fishes and grow by the daily accretion of calcium carbonate
110 increments throughout the fish's entire lifetime (Campana 1999).
111 Otoliths, starting from their formation during the embryonic stage,
112 incorporate chemical signatures of the water mass the fish is in
113 during each life history stage (Green et al. 2009). Though under
114 physiological constraints otolith chemistry reflects the water
115 chemistry of the surrounding environment, and once laid down,
116 increments (that can be referenced to specific ages) remain
117 unaltered (Campana 1999, Elsdon et al. 2008). The chemical
118 information acquired locally within the otoliths can be used to derive
119 profiles of the movement history of an individual (Campana 1999,
120 Green et al. 2009). Despite some limitations (see Elsdon et al. 2008
121 for detailed description of the method), otolith chemistry is
122 nowadays largely accepted as a useful method for unravelling fish

123dispersal and connectivity patterns (Calò et al. 2013, Starrs et al.
1242014, but see Berumen et al. 2010).

125In order to provide crucial information for the design of a network of
126effective MPAs, in this study we estimate dispersal patterns at both
127propagule (i.e. eggs and larval stages) and juvenile stages of an
128ecologically and economically important Mediterranean coastal fish,
129the two-banded seabream *Diplodus vulgaris* (Geoffroy Saint-Hilaire,
1301817), using analysis of otolith chemistry. Specifically we aim to
131estimate, for the two-banded seabream *Diplodus vulgaris*, the scale
132of dispersal at propagule stage (i.e. eggs and larvae) and to build a
133dispersal kernel for juvenile (i.e. post-settlement) dispersal. This can
134allow us to assess the paradigm that dispersal at juvenile stage is
135negligible and that dispersal and connectivity for coastal fish equate
136with propagule dispersal.

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139 **2. Material and methods**

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141 **2.1. Study species**

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143The common two-banded seabream (*Diplodus vulgaris*) is a
144demersal reef fish distributed throughout the Mediterranean and the
145eastern Atlantic. It usually grows to a length of about 30 cm,

146although it can reach a maximum length of 45 cm (Fisher et al.
1471987) and exceed 30 years in age (Guidetti et al. unpublished data).
148*Diplodus vulgaris*, with the congeneric *D. sargus sargus*, is an
149economically important fish exploited both by professional and
150recreational fisheries (Lloret et al. 2008) and plays an ecologically
151relevant role in Mediterranean coastal ecosystems. Preying on sea-
152urchins (grazers), the two *Diplodus* species indirectly control the
153transition from macroalgal forests to coralline barrens (i.e. bare
154rocks with encrusting algae), and may therefore have strong effects
155on rocky-reef community structure and ecosystem function (Guidetti
156et al. 2006).

157Seabream eggs, released in the water column, hatch two days after
158fertilization and then larvae develop in pelagic waters for more than
1591 month (Di Franco et al. 2013). Larvae metamorphose and settle (a
160stage called 'settlement') in shallow coastal habitats (mainly small
161bays characterised by mixed sandy and rocky bottoms) at
162approximately 10 mm TL (Planes et al. 1999, Vigliola and Harmelin-
163Vivien 2001). About six months later, the juveniles (i.e. small-sized
164subadults, approximately 8 cm TL) join the adults (at a phase that is
165operatively defined recruitment) and at about 2 years of age (i.e.
166approximately 18 cm TL) they reach sexual maturity. Adults are
167relatively sedentary, with evidence of high site fidelity and
168movement at the scale of few kilometers (La Mesa et al. 2013).
169Much less is known about dispersal during the propagule and
170juvenile stages, with the only information concerning the Atlantic

171coasts of Portugal and showing dispersal at the scale of 1 km for
172juveniles (Abecasis et al. 2009) and inconclusive evidence for larvae
173(Correia et al. 2011).

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175 **2.2. Sampling scheme**

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177We used otolith chemistry to obtain information on: 1) natal origin
178and larval dispersal by analysis of the core (laid down during
179embryogenesis, Green et al. 2009), of post-settler otoliths; 2) “site
180fidelity” and/or juvenile dispersal (i.e. the movement between
181settlement and recruitment) by analysis of the post-settlement rings
182of otoliths (i.e. about 10 daily increments after the settlement mark,
183which marks the transition from pelagic larva to demersal settler, Di
184Franco et al. 2013) of both post-settlers and juveniles. The second
185issue has been very scarcely studied despite its potential relevance.
186Assaying otoliths of post-settlers (i.e. transitional juveniles *sensu*
187Vigliola and Harmelin-Vivien 2001) collected along a stretch of coast
188and identifying groups of similar origins based on elemental
189signatures in otolith cores provided information about the spatial
190extent of larval dispersal. Larval dispersal distance was estimated
191on the basis of the distance among different sampling sites that
192were replenished by a single source. Evaluating “site fidelity” of
193juvenile fish between settlement and recruitment, and/or the
194distance travelled between settlement and recruitment sites,
195provided information about juvenile movement after settlement. A

196prerequisite for this kind of investigation is to assess the spatial
197patterns of elemental signatures in otoliths among sampling sites.
198The elemental composition of the portion of the otolith formed just
199after settlement (the portion chemically characterized by the site
200where the fish settled) of post-settlers was assessed for 14 sites
201(see 2.3) and used to generate a reference set of site-specific
202chemical fingerprints representing potential settlement sites in the
203study area. Post-settlement movement (i.e. the distance travelled
204by juveniles) between settlement and recruitment stages was
205inferred by comparing chemical fingerprints of the same portion of
206the otolith (i.e. corresponding to about 10 days after settlement)
207between juveniles (collected 8-10 months after settlement) and
208post-settlers (collected shortly after settlement) from multiple sites.
209The analysis of the same portion of the otolith in both post-settlers
210and juveniles prevented us from any bias related to potential
211temporal variability in water chemistry between settlement and
212recruitment. In addition the choice of analysing the portion of the
213otolith corresponding to 10 days after settlement (based on visual
214identification of otolith microstructure) could reduce the risk related
215to temporal mismatch between microstructural and microchemical
216processes (see Freshwater et al. 2015). No evidences of this
217mismatch exists for Mediterranean species and findings from
218sockeye salmon *Oncorhynchus nerka* highlight, in 50% of individuals
219examined, a lag of about 9 days with microchemical process
220occurring before microstructural ones. If this would be the case also

221in our model species, the portion of otolith that we chemically
222analysed would still correspond to a moment when settlers
223inhabited settlement sites and therefore would allow us to properly
224characterize settlement sites.

225

226 **2.3. Sample collection and study area**

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228Both propagule and juvenile (i.e. post-settlement to recruitment)
229dispersal was investigated at the scale of approximately 180 km.
230Post-settlers and juveniles of *Diplodus vulgaris* were collected at 14
231sites along ~180 km of the Apulian Adriatic coast of Italy (Fig. 1).

232Post-settlers of *D. vulgaris* were collected in May 2010. At each site,
23310-12 individuals were collected (total n= 157) with a hand-net.
234Post-settlers were euthanized in an ice water slurry in accordance
235with authorisation protocols by the Italian Ministry of Agriculture,
236Foods and forestry politics (permit number 0011267-2010). By
237spearfishing juveniles (i.e. small size subadults, 8-10 cm TL) were
238collected 8-10 months later, after recruitment, from the same 14
239sites where post-settlers were previously collected. Therefore, post-
240settlers and juveniles collected in the present study belonged to the
241same annual cohort. At each site, 10-14 juveniles were collected
242(total n= 164). Fish were frozen until otolith removal was
243undertaken.

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246 **2.4. Sample preparation and analysis**

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

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

258 Otoliths of juveniles were only analysed for the chemical
259 composition of the post-settlement portion. Ten elements were
260 analyzed (^{24}Mg , ^{44}Ca , ^{55}Mn , ^{66}Zn , ^{88}Sr , ^{138}Ba , ^{208}Pb , ^7Li , ^{57}Fe , ^{59}Co).

261 Despite some evidences suggest that Mg uptake can be
262 physiologically regulated, and may not represent ambient conditions
263 (see Woodcock et al. 2012) we included this element because it has
264 been found useful for distinguishing fish from different locations
265 when used in combination with other elements (Swan et al., 2003;
266 Sarimin et al., 2009). Details about chemical analyses procedures
267 can be found in Appendix A.

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269 **2.5. Data analyses**

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

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276 **2.5.1. Natal sources and propagule dispersal**

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

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

295chosen based on recommendations regarding 'cost'-optimal
296allocation of sampling effort from Di Franco et al. 2014.

297Once different natal origins were identified (see results), we tested
298for possible differences in settlement site replenishment for each
299identified natal source with a univariate one-way PERMANOVA on
300core multivariate composition using site number as a variable (i.e.
301from 1 to 14, from Northern to Southern sampling site). Natal source
302was treated as a single factor with different levels corresponding to
303the major natal sources identified. The same experimental design
304was used to test for potential differences in SpD and PLD of
305individuals from each natal source.

306Statistical analyses were run using Primer 6 PERMANOVA + software
307package (Clarke and Gorley 2006).

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309 **2.5.2. Juvenile dispersal**

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311To account for possible uncharacterized settlement sites, which
312represents an inevitable bias despite our extensive sampling effort,
313we compared otolith elemental signatures of juveniles with those of
314settlers using principal component analysis (PCA). Juveniles that fell
315outside a 95% confidence ellipse around the settlers baseline data
316(elemental signatures of settlement sites) were assumed to have
317originated from uncharacterized settlement site(s) and were
318excluded from further analyses (see Appendix B for details).

319 Canonical analysis of principal coordinates (CAP, Anderson and Willis
320 2003) and jackknife cross validation (% of correct classification)
321 were performed on the edge portion of the elemental data of post-
322 settlers to assess how accurately post-settlers were classified to
323 sites where they were collected in each region. A specific
324 randomization test (White and Ruttenberg 2007) was used to
325 estimate the probability that reclassification success (% of correct
326 classification) was better than random. Juveniles were assigned to
327 settlement sites (i.e. the sites where the post-settlers were
328 collected) through linear discriminant functions previously
329 parameterized with data from post-settler otoliths. Centroids per
330 specimen for both post-settler and juvenile data (i.e. centroid of the
331 three replicate sample pits for each specimen) were calculated and
332 used for CAP analysis.

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

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

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

355

356 **3. Results**

357

358 ***3.1. Natal sources and propagule dispersal***

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

363 Three of the seven groups (A, B, and D) consisted of a single
364 individual, while group G consisted of three individuals (~2% of all
365 settlers sampled). Groups C, E and F consisted of ~26%, ~45% and
366 ~24%, respectively, of settlers. These three major groups
367 significantly differed in terms of their multivariate core elemental
368 fingerprints (PERMANOVA $p < 0.01$). The Mg:Ca and Sr:Ca ratios

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

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

380 Considering spawning date (SpD), the three major natal origins
381 differed significantly by a few days (Permanova pseudo-f: 4.4664,
382 $p = 0.014$). Pairwise tests revealed that group C significantly differed
383 from E ($p < 0.01$) and F ($p < 0.05$), while no difference was detected
384 between E and F. SpD of group C took place about 10 days after that
385 of groups E and F (2010 December 21st vs 2010 December 10th).
386 Post-settlers size (SL) ranged from 15 to 30 mm (mean \pm SE = $25 \pm$
387 0.2 mm). Considering PLD, no significant difference was detected
388 among the three groups, with 47.6 ± 1.2 (mean \pm s.e.), 44.5 ± 1.1 and
389 44.9 ± 1.4 days respectively for C, E and F. Within each natal source,
390 a large range in PLD was detected: 29-61 days in C, 29-58 in E, and
391 25-56 in F.

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

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

399

400 **3.2. Juvenile dispersal**

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

407 Approximately 10% of juveniles were assigned to a settlement site
408 corresponding to the site where they were collected, indicating that
409 they recruited to the same site where they settled (i.e. 0 km
410 dispersal). Approximately 51% of juveniles moved between 5 and 55
411 km, 22% between 55 and 100 km, and 15% between 100 and 135
412 km. A single fish (0.75%) moved approximately 165 km. Overall,
413 median dispersal was 40 km and average dispersal was 51 km (\pm
414 3.2, s.e.).

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

419($p < 0.0001$, Fig. 4b), suggesting that this trend is due to the spatial
420arrangement of sampling sites and could be due to chance.

421Comparing the two dispersal kernels (measured and randomised), a
422significant difference was detected (Wilcoxon-Mann-Whitney test,
423 $p = 0.020$), with the measured kernel more skewed towards shorter
424dispersal (Fig. A4), indicating that fish disperse long distances less
425often than predicted by chance.

426The adjusted dispersal kernel had a median dispersal distance of 50
427km and average of 63.42 km (± 3.74 , s.e.), and did not follow an
428exponential decay model ($p > 0.05$). Compared to the measured
429dispersal kernel, the adjusted dispersal kernel had a fatter tail (Fig.
4304c), corresponding to a greater frequency of long distance dispersal
431events.

432

433

434 **4. Discussion**

435

436Here we highlight the existence of three major natal sources of
437propagules for the two banded seabream (*Diplodus vulgaris*) that
438replenish the study area (i.e. about 180 km of coastline), suggesting
439that propagule dispersal extends to at least 90 km.

440In addition, we observed extensive dispersal – up to 165 km – at the
441juvenile stage and built a juvenile dispersal kernel. This evidence, as
442far as we know, is novel and has important implications for the
443ecology and management of MPAs.

444

445 **4.1. Natal sources and propagule dispersal**

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

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

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

469relative frequency of short- and long-distance propagule dispersal
470events. This would be possible by focusing on nesting fishes where
471the exact location of the propagule source (i.e. the nest) is known
472(e.g. Buston et al. 2012) or by using marking methods based on
473maternal transmission of stable isotopes to offspring (Almany et al.
4742007, Munro et al. 2009).

475Despite we cannot identify where the natal sources are located we
476can speculate that a relevant percentage of propagules could
477originate from the Torre Guaceto Marine Protected Area (TGMPA)
478that is located within our study area and that has been shown to
479host high density and biomass of fishes (Sala et al. 2012, Di Franco
480et al. 2012a). Evidences on the congeneric *Diplodus sargus* suggest
481that TGMPA host high density of spawners and contribute through
482propagule export to the replenishment of populations inhabiting
483unprotected areas (Di Franco et al. 2012a, Pujolar et al. 2013). A
484similar pattern could be attended also for *D. vulgaris*, with one (or
485more) of the three major natal origins located within TGMPA and
486part of the propagules exported toward unprotected areas following
487sea currents dominating western Adriatic (Artegiani et al. 1997)
488during *D. vulgaris* spawning period (i.e. mainly winter).

489The replenishment of multiple sites by each natal source suggests
490high variability in propagule dispersal, because propagules from a
491single source reach settlement sites located at different distances.

492This evidence could result from the flexibility of the PLD as
493expressed by the wide range highlighted among the individuals from

494each natal origin. Two of the three major spawning events
495(corresponding to the three major natal sources) occurred
496simultaneously while the third spawning event began approximately
49710 days later. We detected spawning events that occurred over a
498long time period, suggesting an extended spawning season for this
499species. This evidence agrees with findings on *D. vulgaris* from other
500Mediterranean (Mouine et al. 2012, Di Franco et al. 2013) and non-
501Mediterranean areas (Gonçalves & Erzini 2000, Pajuelo et al. 2006)
502indicating spawning season lasting 3-7 months.

503

504 **4.2. Juvenile dispersal**

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506Here we provide evidence of extensive dispersal during the juvenile
507stage of up to 165 km. This finding agrees with recent findings for
508other temperate coastal fishes, which have suggested dispersal up
509to 600 km (Tobin et al. 2010, Hamer et al. 2011, McMahon et al.
5102012, Di Franco et al. 2012b, Reis Santos et al. 2013, Bouchard et al.
5112015). In the present study, our dispersal estimates are from otolith
512chemistry analyses, but other evidence from a study adopting tag-
513recapture techniques on the congeneric species *Diplodus sargus*
514*sargus* reported a dispersal distance of ~17 km for juveniles (~11
515cm TL) within one month (D'Anna et al. 2004), confirming the
516potential for coastal fishes to disperse significant distances as
517juveniles.

518 There was much variability in juvenile dispersal distances among
519 individuals, as demonstrated by the measured dispersal kernel. Few
520 individuals dispersed large distances after settlement (the tail of the
521 kernel), and about 10% of individuals did not disperse at all. Overall,
522 we observed lower site fidelity in *Diplodus vulgaris* compared to its
523 congener *D. sargus sargus* in the same study area (Di Franco et al.
524 2012b). Interspecific differences in dispersal are common, and can
525 be related to a number of species-specific factors (e.g. aspect ratio
526 of the caudal fin, Radinger and Wolter 2013) or environmental
527 factors (e.g. habitat heterogeneity, Fraser et al. 2001).

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

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

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

567et al. 2009). In that study, small *D. vulgaris* (<12 cm TL) were
568reported to usually remain in the same area for up to one month, or
569if they did disperse, they only moved a few kilometres. In that study,
570however, the time period study was much shorter than in the
571present study, and their findings were from a coastal lagoon, a
572different environment than the open, rocky coast we investigated.
573Moreover, conclusions drawn from conventional tag-recapture
574studies (as in Abecasis et al. 2009) are highly dependent on
575recapture effort. The otolith chemistry approach implemented in this
576study provided a quantitative dispersal estimate unaffected by any
577recapture bias.

578Another study using microchemical analyses of *D. vulgaris* otoliths
579indicates that 2+ years individuals disperse across tens of square
580kilometres (Correia et al. 2011). However, these analyses by Correia
581et al. (2011) were based on examination of the whole otolith using
582solution based analyses, which provide less useful information than
583our analyses for detecting dispersal; analysing the whole otolith
584loses information related to the location of the individual during
585particular times and thus life stages.

586

587 **5. Conclusion**

588

589Our estimate of propagule dispersal falls within the range identified
590for other temperate fishes (50-500 km, Anadon et al. 2013 and
591references therein) and, therefore our evidence supports the

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

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

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

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

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

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

641pointed out for a system of small customary tenure areas in Papua
642New Guinea (Almany et al. 2013), understanding whether and at
643what spatial scale human communities can benefit from
644management actions is key to designing effective strategies,
645obtaining support for management, and providing greater incentives
646for compliance.

647

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668

669

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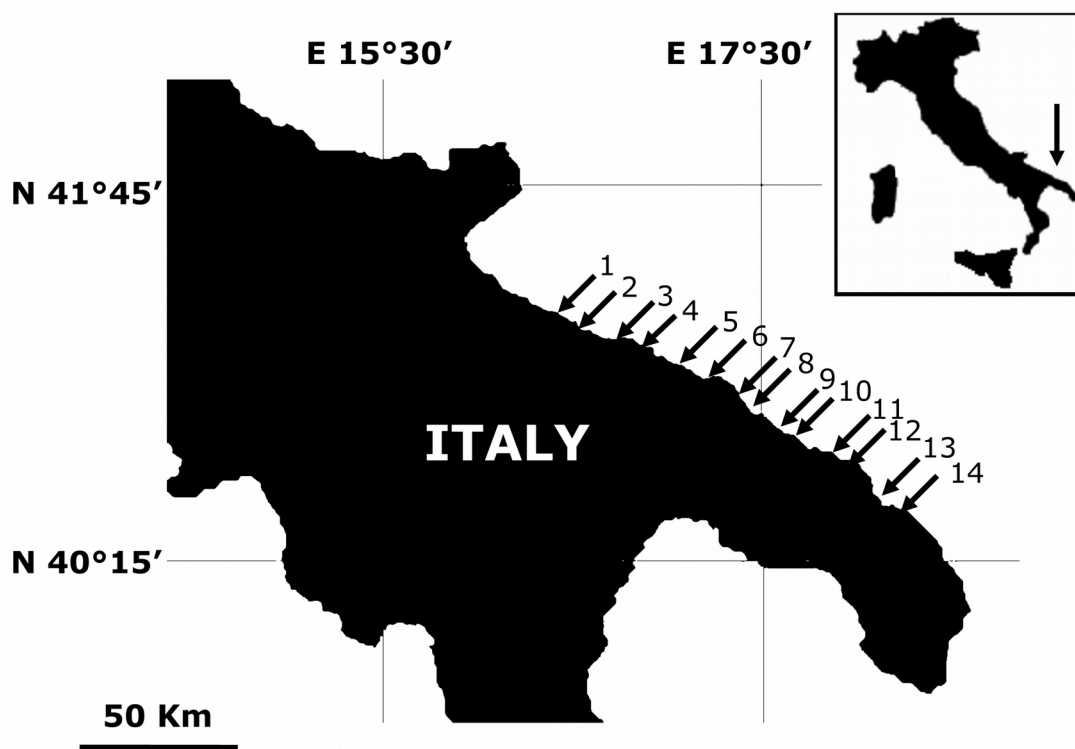
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888 **Figure legends**

889

890



891

892 **Figure 1.** Study area. Sampling sites are indicated with arrows.

893 Sites are numbered progressively from 1 (most northern site) to 14

894 (most southern site).

895

896

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

907

908

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

914 **Supporting Information**

915

916 **Appendix A.**

917

918 **Otolith preparation and chemical analyses**

919

920 **Otolith preparation**

921

922 In the laboratory, one sagittal otolith was removed from each
923 specimen, cleaned of soft tissue using plastic dissecting pins, and
924 mounted sulcus side up on a glass slide using crystal bond (Aremco
925 Products, Inc.). Otoliths were polished with 3 μm and 1 μm Imperial
926 3M lapping film to expose inner growth layers for analysis. We chose
927 not to polish the otolith to the core and to leave material above it in
928 order to ensure the core was not removed during pre-ablation
929 procedures, which potentially allowed us to sample all the material
930 associated with the core. After polishing with lapping film, otoliths
931 were rinsed and sonicated for 10 minutes in ultra-pure water.
932 Otoliths were dried and arranged onto new glass slides (6 otoliths
933 per slide). All otoliths were randomly ordered to prevent sample
934 batch bias.

935

936 **Otolith chemical analyses**

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

87

44

88

939(identified previously as approximate core size of the cores) from
940the surface of the otolith through the visible core. The spike in
941Mn:Ca was used as an indicator of the core location, as previous
942studies have reported elevated Mn concentrations in the core
943(Brophy et al. 2004, Ruttenberg et al. 2005), and therefore just one
944out of the three pits sampled in the core (the one showing at least 3-
945fold higher Mn:Ca concentration than surrounding material, Brophy
946et al. 2004) was considered in subsequent analysis. A Mn:Ca spike
947could not be detected in 13% (21 otoliths) of the core samples of
948post-settlers; these samples were not used in the analysis of natal
949origins.

950In the post-settlement portion of otoliths of both post-settlers and
951juveniles, we analysed the same otolith portion (i.e. corresponding
952to about 10 days after settlement). We ablated three horizontal pits
953and all three were considered in the subsequent analysis in order to
954account for within-otolith variability and to optimize sampling design
955(Di Franco et al. 2011, see Di Franco et al. 2014 for an in-depth
956discussion about this issue).

957Once otoliths were inside the laser ablation chamber, they were
958viewed remotely on a computer screen where the area for ablation
959was selected. The laser was focused on the sample surface and fired
960through the microscope objective lens using a spot size of 30 μm .
961Each run generally consisted of 40 s acquisition, 10 s blank to
962correct for background which was subtracted from each sample, 10
963s ablation (laser at 65% power, about 6 J/cm^2) resulting in a pit

964 about 10 μm deep, and 20 s for washout. Prior to analysis, samples
965 were pre-ablated to remove any surface contamination (laser at
966 50% power). Helium gas was flushed into the ablation cell to reduce
967 the deposition of ablated aerosols and to improve signal intensity.
968 The ablated aerosol was then mixed with argon before entering the
969 inductively coupled plasma (ICP) torch. All otoliths were analysed
970 using a Thermo Elemental inductively coupled plasma mass
971 spectrometer (ICP-MS) connected to a NewWave Research UP213
972 with aperture imaging laser ablation (LA) system (see table S1 for a
973 summary of operating conditions and data acquisition parameters).
974 External calibration was performed with two Standard References
975 Materials (SRM) from National Institute of Standards and Technology,
976 NIST 610 and NIST 612. Calcium was used as an internal standard to
977 account for variation in ablation and aerosol efficiency (Yoshinaga et
978 al. 2000).

979 All 9 elements analyzed (^{24}Mg , ^{55}Mn , ^{66}Zn , ^{88}Sr , ^{138}Ba , ^{208}Pb , ^7Li , ^{57}Fe ,
980 ^{59}Co) were expressed as ratios relative to ^{44}Ca . Detection limits were
981 calculated from the concentration of analyte yielding a signal
982 equivalent to $3\times$ the standard deviation of the blank signal for each
983 of the elements (see Table A2).

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

989

990

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992

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1009 **Appendix B**

1010

1011 **Accounting for uncharacterized settlement site(s)**

1012

1013 Accurate assessment of site fidelity and juvenile dispersal (i.e.
1014 assignment of juveniles to settlement sites) relies on the
1015 assumption that all possible settlement sites contributing to the
1016 juvenile pool investigated have been sampled and included in the
1017 data set (Campana 1999, Reis Santos et al. 2013). However, despite
1018 our intensive sampling of a number of settlement sites identified as
1019 important for the study area based on a preliminary survey carried
1020 out by authors, it is in practice impossible to include all possible
1021 settlement sites across the study area (180 km of coastline). From
1022 this perspective, other non-sampled settlement sites may have
1023 contributed to juveniles analysed in the present study, and indeed in
1024 some cases, the juvenile otolith signature did not match those of
1025 any settlers used as the baseline data set. In order to reduce the
1026 potential bias related to uncharacterized settlement sites we
1027 adopted a statistical approach used in similar studies (Hamer et al.
1028 2005, Chittaro et al. 2009, Reis-Santos et al. 2013): we compared
1029 otolith elemental signatures of juveniles with those of settlers using
1030 principal component analysis (PCA). Juveniles that fell outside a 95%
1031 confidence ellipse around the settler baseline data (elemental
1032 signatures of settlement sites) were assumed to have originated

1033from uncharacterized settlement site(s) and were excluded from
1034further analyses.

1035The elemental fingerprints from the juvenile portion of otoliths were
1036mostly distributed within the 95% confidence ellipses of the post-
1037settler baseline data (Fig. A3). However, there were 31 juveniles
1038(~19%) that fell outside the confidence ellipses of the post-settler
1039data (i.e. putatively originating from uncharacterized settlement
1040sites) and were excluded from further analysis, and thus the
1041analysis consisted of a total of 133 individuals.

1042

1043

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1059

1060 **Appendix C**

1061

1062 **Juvenile dispersal kernels**

1063

1064 The probability of detecting dispersal declines with distance from
1065 the source and it depends on the spatial arrangement of sampling
1066 sites and on number of sampled specimens. Specifically, in our case,
1067 we would be able to record a displacement corresponding to the
1068 maximum distance between sites (i.e. approx. 180 km) only for the
1069 specimens collected at the northernmost and southernmost
1070 sampling sites, while we would be able to record zero dispersal (0
1071 km, i.e. juvenile collected at the same site where it settled) for all
1072 individuals from all the sampling sites. As highlighted by Matthysen
1073 et al. 1995, several reported dispersal patterns are in fact due to the
1074 limitations of the set of all potential observations. From this
1075 perspective, a comparison of the observations that are actually
1076 made with the set of observations that could have been made must
1077 be carried out (Matthysen et al. 1995).

1078 We would expect a decline in the frequency of observations as
1079 dispersal distance increases simply as a result of the spatial
1080 arrangement of sampling sites. To account for this inevitable bias,
1081 we used the approach of Matthysen et al. 1995, and constructed a
1082 null dispersal kernel (*sensu* Caley 1991) describing the null
1083 hypothesis of random dispersal. The null hypothesis is that each
1084 individual has the same chance to disperse all possible distances

101

102

1085among sampling sites (e.g. to not disperse and to disperse over the
1086maximum distance allowed within the study area). To construct the
1087null dispersal kernel, we accounted for the effect of sample size (i.e.
1088number of juveniles collected from each site), using real sampling
1089numbers. This dispersal kernel provides information about our
1090“ability” to detect dispersal given the spatial arrangement of our
1091sampling sites.

1092We then compared a randomised dispersal kernel with the measured
1093dispersal kernel (based on our observed data) using a Wilcoxon-
1094Mann-Whitney test. Any differences between the two dispersal
1095kernels would indicate higher or lower real dispersal compared to
1096the dispersal pattern predicted by the null kernel.

1097Based on Matthysen et al. 1995, we corrected our dispersal
1098estimates for the inverse probability to detect dispersal at a given
1099distance. This probability was taken from the randomized dispersal
1100kernel. In other words, we used the inverse probability to observe
1101dispersal at a given distance (i.e. probability described in the
1102random dispersal kernel) as a distance-weight correction: dispersal
1103distances that were less likely to be observed (e.g. high-distance
1104dispersal) were overweighted compared to dispersal distances with
1105a high probability of observation (e.g. no dispersal).

1106The use of more sophisticated correction techniques (e.g. Baker et
1107al. 1995, Cooper et al. 2008) would require greater knowledge about
1108the distribution of available sites for settlement and recruitment

1109 across our study area. This task is, in a field situation, impossible for
1110 the studied species in such a large study area.

1111 Statistical analyses were run using the open source software 'R' (see
1112 www.r-project.org).

1113

1114 **References**

1115

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1125

1126 **Appendix D**

1127

1128 **Settlement sites replenishment by natal origins**

1129

1130 Among the seven groups of post-settlers identified, four groups of
1131 post-settlers consisted of 1-3 individuals. Group G consisted of a
1132 total of three individuals, and single fish was collected at each of
1133 three sites located in the south of the study area. Group A consisted
1134 of one individual from a site located approximately in the middle of
1135 study area, Group B consisted of one individual from a site in the
1136 north of the study area, Group D consisted of one individual from
1137 the southernmost sampling site (Fig. A2). Note that in Figure A2
1138 these Groups - A, B, D and G - are omitted to improve clarity.

1139

1140 **Table A1. Operating conditions and data acquisition**
 1141 **parameters for LA-ICP-MS analysis**

<i>ICP-MS</i>	
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
<i>Laser</i>	
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
<i>Analysis protocol</i>	

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

1142

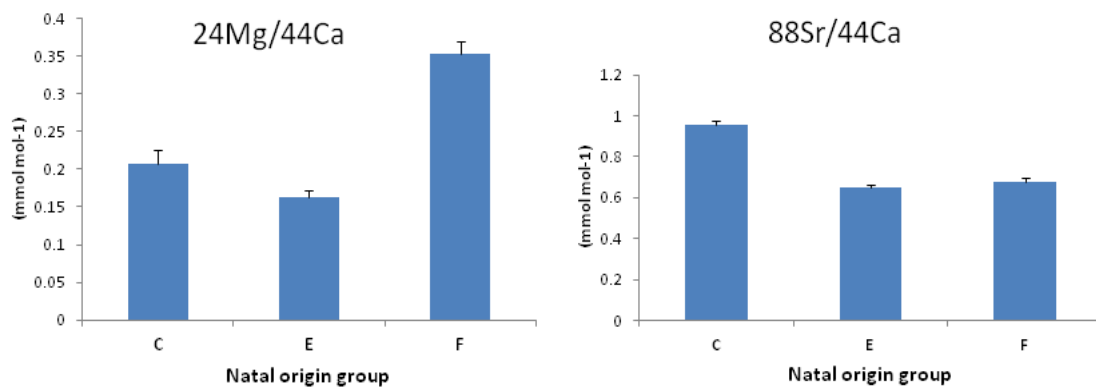
1143

1144 **Table A2. Estimates of precision, accuracy and limits of**
 1145 **detection (LOD).** Values for %RSD (% relative standard deviation)
 1146 and % accuracy are dimensionless. LOD are given in mmol mol⁻¹.

1147

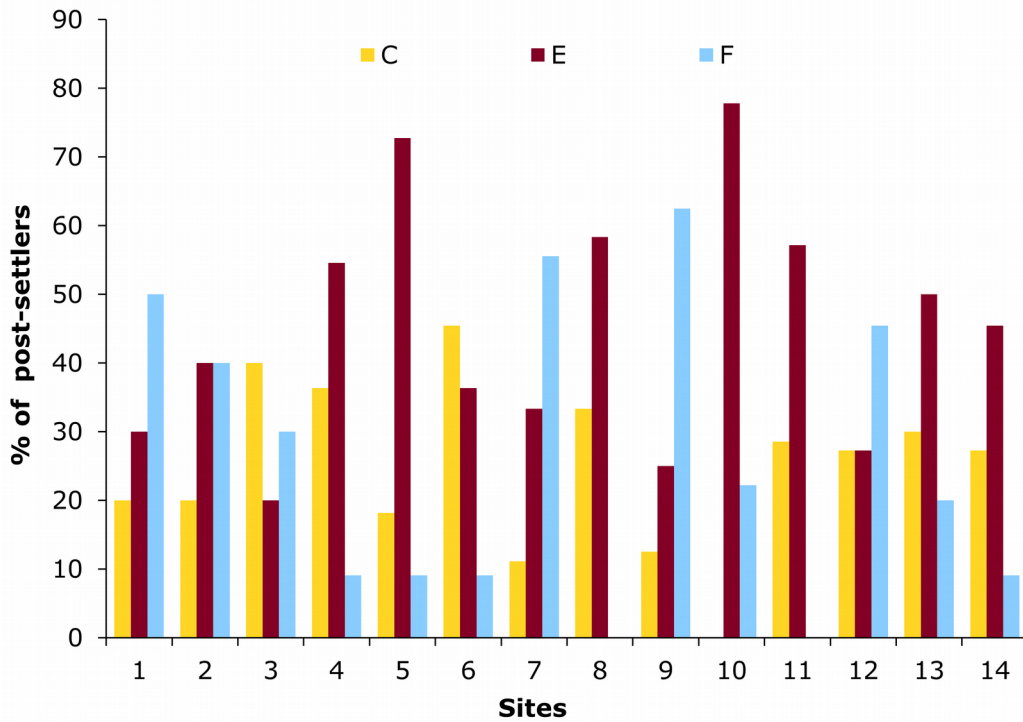
Element Ratio	NIST 610 % RSD	NIST 612 % RSD	% Accuracy NIST 610	% Accuracy NIST 612	LOD
Mg:Ca	8.95	15.44	103	110.2	0.056
Mn:Ca	6.40	10.95	101.55	113.73	0.077
Sr:Ca	4.60	10.51	100.90	93.62	0.027
Ba:Ca	9.30	9.52	102.23	89.78	0.0031

1148



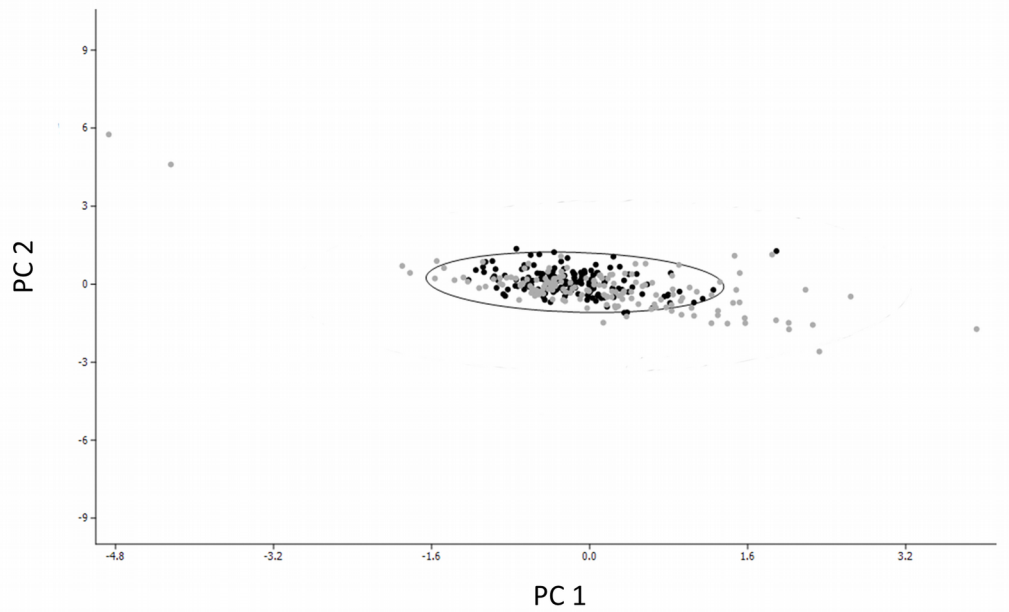
1149

1150 **Figure A1.** Average Mg:Ca and Sr:Ca calcium ratios (\pm standard
 1151 error) in the otolith core region for the three major natal source
 1152 groups identified by SIMPROF analysis. Group C was characterized
 1153 by intermediate concentrations of Mg:Ca and high concentrations of
 1154 Sr:Ca compared to groups E and F. Group E was characterized by low
 1155 Mg:Ca concentrations and intermediate Sr:Ca concentrations.
 1156 Group F was characterized by high Mg:Ca concentrations and
 1157 intermediate Sr:Ca concentrations.



1158

1159 **Figure A2.** Percentage of post-settlers originating from the three
 1160 major putative natal source groups based on otolith core signatures
 1161 and their contributions to replenishment at the 14 sampling sites.
 1162 Different colors represent the three groups identified by SIMPROF
 1163 analysis. Sites are numbered progressively on the x-axis from 1
 1164 (most northern sampling site) to 14 (most southern sampling site).
 1165 Note that the four marginal groups each contributing only 1-3
 1166 individuals - A, B, D and G - are omitted to improve graph clarity.



1167

1168 **Figure A3.** Ordination plot of principal component analysis (PCA)
1169 comparing multi-element otolith signatures of juveniles (grey
1170 circles) and post-settlers of known origin (black circles) forming the
1171 baseline group. Ellipsis represents the 95% confidence ellipse
1172 around the baseline group data.

1173

1174**Figure A4.** Juvenile dispersal kernel from observed (red) and
1175randomised (blue) data (see Appendix C for further details).

1176