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Marine Ecology: progress series

DOI:
10.3354/meps11664

Published: 21/03/2016

Discriminating nursery grounds of juvenile plaice (*Pleuronectes platessa*) in the south eastern Irish Sea using otolith microchemistry

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ABSTRACT: Nursery grounds are valuable habitats providing sources of food and refuge during early life stages for many commercially caught marine fish. Distinguishing between different nursery grounds and identifying habitat origin using trace elemental concentrations in aragonite structures of teleost fish have proved valuable in fish ecology and fisheries. This study aimed to: (1) compare chemical signatures (elemental fingerprints) within sagittal otoliths of juvenile plaice (*Pleuronectes platessa*) sampled from known nursery habitats in the SE Irish Sea; and (2) assess their potential and robustness as natural tags for identifying nursery grounds for the putative SE Irish Sea plaice stock. Otoliths from 1-group juvenile plaice (6-15 cm total length) were obtained from 8 nursery grounds in coastal areas off North West England and North Wales (including Anglesey) between June and August 2008. Solution-based inductively-coupled plasma mass spectrometry determined the concentrations of 10 elements (Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn, Ba), with significant differences in otolith element composition observed between all nursery grounds. Cross-validation linear discriminant function analysis (CV-LDFA) classified fish to their nursery ground of capture (46.2% to 93.3%), with a total group CV-LDFA accuracy of 71.0%. CV-LDFA between regions (North West England and North Wales) classified fish with 82% accuracy. The discrimination of juvenile plaice from all 8 nursery grounds within the southeast Irish Sea using otolith microchemistry offers significant opportunities in the development of future effective fisheries management strategies through understanding the supply of juveniles from specific nursery grounds and adult plaice in the Southeast Irish Sea.

KEY WORDS: Nursery grounds· Otolith microchemistry· Natural tag· Juvenile plaice· *Pleuronectes platessa*

INTRODUCTION

For many coastal fish species, the adult and juvenile life stages exhibit spatial segregation in habitat (Gillanders et al. 2003), where juveniles are often recruited into near shore nursery habitats through entrainment into surface water currents and gyres (Collas et al. 1997, Hamilton et al. 2008) and where, depending on the species, residency can vary from months to years (Vasconcelos et al. 2007, 2008) before migrating offshore to join adult populations (Brown 2006a, Fodrie & Herzka 2008). The ability to understand and track movement patterns of fish with complex life cycles is necessary if we are to estimate habitat 'value' in the context of new recruits to sustain the adult population (Beck et al. 2001). Furthermore, the importance of identifying which nursery areas are the most productive and their connectivity through larval and juvenile exchange should be considered if effective management protocols are to be implemented (Cowen et al. 2000, Vasconcelos et al. 2008, Cuveliers et al. 2010). However, mark and recapture studies on juvenile fish have provided some insight (e.g. Burrows et al. 2004, Pickett et al. 2004, Tupper 2007) but these methods can be labour intensive, logistically difficult to implement, with constraints including the small size of juveniles in comparison to the tags, high rates of juvenile mortality, low recapture rates and the requirement for large numbers of individuals tagged to yield meaningful results (Gillanders 2005, Brown 2006b, Herzka et al. 2009). However, techniques used to study natural tags such as trace-element chemistry in calcified structures in fishes are providing a wealth of information on population dynamics, movement patterns and early life history strategies (See reviews in Elsdon et al. 2008, Sturrock et al. 2012).

The use of otolith microchemistry can be a valuable alternative to manual tagging in distinguishing between the habitats of origin in juvenile marine fishes (Thorrold et al. 2001, Gillanders 2005, Brown 2006b). Due to the nature and composition of otoliths,

material deposited within the aragonite matrix is metabolically inert, not susceptible to resorption and remains unaltered after deposition (Thorrold et al. 1998, Campana 1999). Therefore, otoliths of juvenile fish that have long residency times within a particular habitat or nursery ground should reflect those physico-chemical characteristics of their surrounding environment and record a chronological record within the otolith matrix (de Pontual & Geffen 2002, Fodrie & Herzka 2008). Otolith microchemistry is proving to be a valuable natural tag in the study of fish ecology in general (Elsdon et al. 2008, Sturrock et al. 2012) and in particular, it has been successfully applied in identifying distinct otolith chemical signatures between different nursery grounds and in studying connectivity and movement patterns for a range of flatfish species (Geffen et al. 2003, Brown 2006a, b, Chittaro et al. 2009, Cuveliers et al. 2010, Nims & Walther 2014, Bailey et al. 2015).

The plaice *Pleuronectes platessa* is one of the most commercially important flatfish species landed by demersal fisheries in England and Wales, with populations along the west coast of the UK currently managed as either single or multiple International Council for the Exploration of the Sea divisions (ICES area VIIa and ICES areas VIIf and g, Dunn & Pawson 2002, Ellis et al. 2012). However, there is strong evidence to suggest that separate stocks exist within these divisions. Evidence of possible sub-stocks based on tagging studies identified different migratory patterns, differences in reproductive biology (fecundity, age at first maturity) and differences in growth patterns for the north eastern and western Irish Sea and within the south eastern Irish Sea (including Cardigan Bay and a small migratory contingent to the Bristol Channel and Celtic Sea) (Dunn & Pawson 2002, Fox et al. 2007, ICES 2014).

Within the southeast Irish Sea, the main nursery grounds for juvenile plaice have been identified along the coastal waters of northwest England and North Wales (Dunn & Pawson 2002, Ellis et al. 2012), where the newly benthic-orientated juveniles spend

between 1 to 3 years before migrating offshore into deeper water (Nash et al. 1994, Dunn & Pawson 2002, Fox et al. 2007). In light of the commercial importance of this species, it is therefore the aim of this paper to identify whether the main plaice nursery grounds in the south-eastern Irish Sea exhibit distinct otolith microchemical signals and whether these naturally occurring chemical tags can be used to classify individual juvenile back to their nursery ground of origin.

MATERIALS AND METHODS

Sample Collection. Juvenile plaice (1-group) with a total length (TL) between 6 and 15 cm were collected from 8 sites identified as main nursery grounds around the north coast of Wales and North West England (Dunn & Pawson 2002) during June and August 2008 (Figure 1). 1-group plaice were chosen (as opposed to 0-group) to represent an integrated signal over 12 months and to account for any possible seasonal fluctuations or movements made during the first year within their chosen nursery ground. Sampling sites were selected due to their recognised importance as major nursery grounds for juvenile plaice within the putative South-East Irish Sea stock (Dunn & Pawson 2002, Fox et al. 2007). Fish were collected using two techniques: a push-net was used in water depths of < 1m; and, a nylon beach-seine net (Dimensions: depth 2.2 m cod end mesh 5 mm), used in water > 1 m in depth. On capture, juvenile plaice were immediately euthanized using the Home Office Schedule 1 method and stored on ice within a portable refrigeration unit for transportation back to the laboratory where fish were frozen at -20 ^{o}C until otolith extraction.

Otolith Preparation. All equipment used in extraction, cleaning, and storage of the sagittal otoliths were non-metallic and pre-acid-washed in analytical grade 10% HNO_3 (>69% HNO_3, Sigma Aldrich), triple-rinsed in ultra-pure 18 MΩ Milli-Q water (hereafter

109 referred to as Milli-Q) and dried under a laminar flow hood for 24 hours prior to use.
110 Similarly, analytical tubes were prepared as outlined above with one minor alteration
111 where they were acid-cleaned using a solution of 1% HNO_3 / 0.5% HCl (both analytical
112 grade). To prevent the possible risk of zinc contamination, powder-free vinyl gloves
113 (Shermond) were used during all sample procedures (Batley 1989, Friel et al. 1996,
114 Dugan et al. 2008).

115 A maximum of 15 fish, were collected from each of the 8 nursery grounds for otolith
116 extraction and analysis. However, due to poor weather conditions at the time of
117 collection, only 6 1-group plaice were caught at Hoylake. Both left and right sagittal
118 otoliths were extracted using fine-tipped plastic forceps and cleaned of any adhering
119 tissue using a fine-bristled nylon brush. Left and right sagittal otoliths were stored
120 separately in 1.5 mL polypropylene micro-centrifuge tubes and dried under a laminar
121 flow hood for 24 hours. Otoliths were immersed in a 3% hydrogen peroxide solution
122 (30% H_2O_2 analytical grade) and sonicated for 5 minutes to remove organics (Brophy et
123 al. 2003), triple-rinsed in Milli-Q and dried under a laminar flow hood for 24 hours.
124 Individual otoliths were weighed to the nearest 0.001 mg (Mettler Toledo MX/UMX series
125 5) and stored in micro-centrifuge tubes prior to analysis.

126 Right sagittal otoliths were used for the chemical analysis and were dissolved in 0.1
127 mL of a 50% HNO_3 / 25% HCl solution and diluted to a volume of 5 mL with Milli-Q.
128 Repeat samples (n = 12) using the remaining left sagittal otolith were analysed to
129 determine if the elemental composition between otolith pairs was similar i.e. either
130 otolith could have been used.

131 Calibration solutions were prepared using a commercial multi-element standard
132 (SPEX-CertiPrep) diluted with Milli-Q to give concentrations of 100, 10, 1 ng ml^{-1} for the
133 multi-element assessments. Elements observed at a higher concentration in otolith

material, such as Ca, Na and K, were measured using multi-element standards consisting of Ca levels measured at 200, 100 and 50 μg ml^{-1}, with additional measurement of Sr, Na, K at 2000 and 200 ng ml^{-1} to extend the calibration range for these more abundant elements. The use of procedural blanks enabled limits of detection (LOD) tests to correct for instrument instability and/or signal drift and any non-spectral interference caused by the matrix (Vanhaecke et al. 1992, Wells et al. 2003). Measurements of samples, repeat samples and blanks were randomised to remove the possibility of systematic bias.

Sample Analysis. Juvenile plaice otolith solutions were analyzed using an Agilent Technologies 7500 series inductively-coupled plasma mass spectrometer (ICP-MS) equipped with a quadrupole reaction cell combined with an ASX 500 series auto-sampler. LOD for each element were defined as the mean blank value plus 3 x standard deviations (Gray, 1989; Wells et al 2003). Twenty elements were determined: Li, Na, Mg, $Al^{\#}$, K, Ca, Mn, Fe^{*}, $Cu^{\#}$, Zn, As^{*}, Rb, Sr, $Cd^{\#}$, Sn, $Cs^{\#}$, Ba, $La^{\#}$, $Pb^{\#}$, $U^{\#}$. Elements affected by polyatomic interferences (*) and those falling below the LOD (#) were subsequently removed from any further analysis (Gray 1989, Evans & Ebdon 1990). Additionally, four samples were excluded due to their concentrations (μg g^{-1}) being observed at higher levels than expected for all elements measured and thus believed to be contaminated. From the initial 20 elements measured, 11 were quantifiable and were found to be above theoretical limits of detection (LOD) at the 8 nursery grounds (Li, Na, Mg, K, Ca, Mn, Zn, Rb, Sr, Sn, and Ba).

Statistical Analysis. Elemental concentrations were expressed as μg g^{-1} otolith and were transformed to an element: Ca ratio (Forrester & Swearer, 2002, Swearer et al. 2003, Brown 2006a b). Data for each element were analysed for univariate normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test) (Minitab v.14.0), with the assumptions being met following Log_{10} transformation of all 10

159 elements. Prior to the analysis of elemental concentrations observed in juvenile plaice
160 otoliths between nursery grounds an assessment of both left and right sagittal otoliths
161 was performed. Results showed no significant differences in the elemental
162 concentrations of the 10 elements between otolith pairs (Paired t-test; all $P > 0.05$). A
163 combination of both univariate and multivariate statistical techniques were used to
164 investigate single and multi-elemental fingerprints of the otoliths from each of the 8
165 nursery grounds. To analyse and quantify the variation in elemental composition of
166 juvenile plaice otoliths within and between the 8 nursery grounds a multivariate analysis
167 of variance (MANOVA) using Wilks' criterion was performed followed by pairwise
168 comparisons between nursery sites. Examination of the differences in otolith chemical
169 composition for each element between the 8 nursery grounds was conducted using a
170 One-Way analysis of variance (ANOVA). Where the ANOVA indicated significant
171 differences, pairwise comparisons (Bonferroni test) were used to identify which
172 sampling locations differed from the other. Cross-validation linear discriminant function
173 analysis (CV LDFA. SPSS v.16.0) was used to determine the accuracy with which juvenile
174 plaice could be classified back to their nursery ground of capture and through
175 geographical separation by region i.e. North West of England (NWE) and North West
176 Wales (NWW) based on the element concentrations within their otoliths (Clarke et al.
177 2007, Ramsay et al. 2011). Canonical score plots were used to provide a visual
178 representation of the classification of individual fish back to their nursery ground. To
179 evaluate the chance-corrected agreement between the actual and predicted site of
180 capture, Cohen's kappa statistic was calculated. Scores range between 0 and 1, with 0
181 indicating no improvement to that achieved by pure chance and 1 indicating perfect
182 agreement in classification to site (Titus et al. 1984, Ramsay et al. 2011).
183

184 **RESULTS**

185 Observations of the elemental box plots (Figure. 2) indicated apparent differences
186 between nursery grounds. Some elements indicated elevated concentrations at some
187 sites, most notably Zn, Rb and Sn at Hoylake and Zn at Benllech Beach. Similarly, elevated
188 peaks of Mn and Ba were observed at Ainsdale on Sea. Conversely, decreased Zn
189 concentrations were detected at Penmaenmawr and Llandulas and decreased
190 concentrations of Mg, K and Rb were observed at the three most westerly sites, Llandulas,
191 Penmaenmawr and Benllech Beach.

192 Multi-elemental fingerprints of otolith chemistry were found to differ significantly
193 between the 8 nursery grounds (MANOVA: $F_{10, 96} = 6.64$, $P < 0.001$), with significant
194 differences observed for the pairwise comparisons between the 8 nursery grounds
195 sampled (Table 1). In addition, an ANOVA test on the otolith concentrations for each of
196 the 10 elements measured indicated significant differences between the 8 nursery
197 grounds (Table 2). For each element, *post hoc* Bonferroni pairwise comparisons between
198 sites revealed significant differences between sites, most notably the elements Mn, Zn, Rb
199 and Sn (Table 2). Sn exhibited the most variability between the 8 sampling locations (16
200 out of 28 pairwise comparisons). Similarly, Rb showed significant differences in
201 elemental concentrations between sites in 12 out of 28 pairwise comparisons (Table 2).

202 Using CV LDFA, 71.0% of juvenile plaice were correctly classified back to their nursery
203 ground of origin based on their elemental composition, with classification results ranging
204 from 46.2% for Seascale to 93.3% for Penmaenmawr (Table 3). The first two canonical
205 discriminant functions of the CV LDFA explained 73.2% of the total variance and were
206 based on the differences in Li, K, Mn, Sr and Sn amongst the nursery grounds. Cohen's
207 kappa statistic indicated the chance corrected CV LDFA, classification was 0.66 (±0.1
208 confidence intervals, CI's) for all elements between sites. Classification results showed

209 that where incorrectly classified, many of the fish were assigned to an adjacent nursery
210 ground (Table 3). For example, for fish collected from Heysham, 2 juvenile plaice were
211 assigned to Seascale and 2 to Cleveleys, both adjacent sites to Heysham. Similarly, 2
212 juvenile plaice from Cleveleys were assigned to the adjacent site at Heysham. Two sites
213 along the North Wales coast, Llandulas and Benllech Beach both had 2 juvenile plaice
214 assigned to Penmaenmawr (Table 3). Differences in between the 8 nursery grounds can
215 be seen when the first two discriminant functions are plotted (Figure 3).
216 Graphical separation using the 8 nursery grounds within the first two discriminant
217 functions is more apparent in Figure 3 when the multielement fingerprints of the 107
218 juveniles sampled were separated by region, with sites sampled from North West Wales
219 (NWW) becoming distinguishable from those juvenile fish sampled from the North West
220 of England (NWE). Cross-validation LDFA results indicated high classification accuracy of
221 juvenile *P. platessa* with 82.2% (NWE: 53/63; NWW: 35/44) of cases correctly assigned
222 to their regional location of capture for the NWE and NWW (Figure 3). Cohen's kappa
223 statistic indicated the CV-LDFA, classification was 0.64 (±0.1CI) for all elements between
224 regional boundaries.
225
226 **DISCUSSION**
227 The use of otolith microchemistry in the present study allowed for the accurate
228 classification of an inshore population of juvenile plaice (*Pleuronectes platessa*) collected
229 from 8 nursery grounds along the North Western coast of England and Wales. Using a
230 multi-element approach (Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn, and Ba), significant differences
231 were found between all sites indicating the potential use of these natural tags in
232 distinguishing between individual nursery grounds for a coastal marine species (Rooker
233 et al. 2001b, Forrester & Swearer 2002, Brown 2006b). Similarly, using a multi-element

234 approach (11 elements; Table 4), Geffen et al. (2003) reported high classification success
235 for post-juvenile plaice collected from 5 sites in the eastern Irish Sea with their results
236 revealing separation between groups of plaice that related to previously identified
237 spawning grounds within the Irish Sea (Dunn & Pawson 2002). In general, otolith
238 microchemistry in flatfishes has been very successful at identifying both individual fish
239 back to site and between sites over differing geographical ranges i.e. 10s to 100s km (see
240 Table 4). Furthermore, the results attained during this study are comparable with
241 classification rates observed in similar otolith microchemistry studies in flatfish (range
242 70 – 92%, see Table 4) over a similar spatial scale (100's of km, see Table 4).
243 A multi-element approach in discriminating between populations in different
244 geographical locations has been regularly used in fishes (see Table 4). However, otolith
245 microchemistry studies in fishes have adopted two approaches, where the discriminant
246 function analysis used to classify fish back to source has used all measured elements or
247 has selected a reduced set of elements which were found to be statistically significant in
248 discriminating between areas. A comparison between the two analytical approaches was
249 conducted by Vasconcelos et al. (2007) who obtained high classification accuracies using
250 a multi-element approach (Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba and Pb) that allowed
251 discrimination between populations (Table 4). However, reducing the set of elements in
252 their discriminant analysis failed to improve classification success and Vasconcelos et al.
253 (2007) concluded that the best outcome was to use the larger dataset in the
254 discrimination model. Adopting a similar analytical approach, the data from the present
255 study were re-analysed to determine if classification success could be improved by
256 analyzing a reduced set of statistically significant elements (in our case; Li, K, Mn, Sr, Sn).
257 However, we also found no improvement in our classification success (CV-LDFA: 65.4%)

from our initial analysis using all the 10 elements which provided the most accurate discrimination between the 8 marine nursery grounds.

Some studies using biogeochemical tags to discriminate between geographical locations have tended to focus on a small suite of elements that have similar ionic radii and ionic charge to calcium, e.g. Mn, Sr and Ba (Swearer et al. 2003, Hedges et al. 2004, Clarke et al. 2007) and which substitute for Ca in the otolith matrix e.g. Mg (Rooker et al. 2001a, Swan et al. 2006). However, focusing solely on the use of those elements which are the primary drivers determining classification in microchemistry studies of freshwater and diadromous fishes (e.g. Sr and Ba, Table 4) may not be as robust for microchemistry analysis for fish sampled from marine waters (e.g. Mg, Mn, Sr, Ba: CV-LDFA: 31.8% this study) (Brown & Severin 2009).

To determine which elements are the primary drivers of spatial discrimination using otolith microchemistry in differing waterbodies is beyond the scope of this paper. However, a review of the elements used in such studies (Table 4) suggests that certain metals may contribute more to spatial discrimination within fresh, estuarine and marine waters. For instance, in estuarine environments, Mg, Mn, Sr and Cd are significant in discrimination between sites (Table 4) whilst studies identifying the movement between estuarine and coastal waters have identified Li, Mn, Rb and Sc as significant in discriminant analyses (Table 4). In the marine environment, Mn, Mg, Sr, Ba, Li, K and Pb have been identified as significant in discrimination (Table 4). Using elements such as lithium (due to its fluvial inputs from continents) and Rb (due to higher dissolved concentrations in marine waters) may be advantageous in discriminating fish from coastal/marine habitats from fish collected from freshwater/estuarine habitats (Brown 2006a,b, Leakey et al. 2009). Similarly, Mn (due to its elevated particulate phase within the marine environment) may be beneficial in future studies in distinguishing fish from

283 other non-marine environments (Leaky et al. 2009). Additionally, Mn may be particularly
284 useful in discriminating flatfish habitats due to the nature of their benthic lifestyle and
285 their close proximity to the sediment. The resuspension of those sediments via
286 bioturbation (Geffen et al. 2003) and the heavy metals associated with them may allow
287 benthic fluxes of Mn to be reflected in their otolith chemistry (Leaky et al. 2009).
288 One of the main obstacles found to limit the use of otolith microchemistry to identify
289 movement patterns in marine fish appears to be the homogeneous distribution of the
290 more reliably identified elements (Sturrock et al., 2012). However, the use of a larger
291 suite of elements such as Na, Mg, K, Zn, Rb, Sr and Sn and those elements deemed likely
292 to prove reliable geographical markers such as Li, Mn and Ba (Sturrock et al., 2012) may
293 increase the complexity of the otolith elemental signature and extend the scope of those
294 spatially explicit low level elements to allow for better classification results for fish
295 sampled from marine environments (Geffen et al. 2003, Vasconcelos et al. 2007, Leakey
296 et al. 2009, Sturrock et al., 2012, this study). This was apparent when looking at marine
297 studies conducted within close proximity of each other (≤ 500Km Table 4), where a larger
298 set of elements (between 5-11) were necessary to discriminate between sampling
299 locations compared to studies conducted over larger geographical ranges (> 500Km)
300 where 4-6 elements were used. However, caution must be taken in using the elements
301 just described in future studies as primary drivers and should only be used in the context
302 of the results for individual sites where all elements measured from natural and
303 anthropogenic inputs have been taken into account.
304 As analytical costs decrease the application of a multi-tag approach, using a
305 combination of trace elements and stable isotopes to observe movement patterns and
306 assign origin of fish over geologically diverse environments are becoming increasingly
307 used in migration studies. Studies of this nature have tended to look at population

connectivity to reconstruct migratory movements using elements such as Sr and Ba in conjunction with stable isotopes of $\delta^{13}C$ and $\delta^{18}O$ in freshwater environments (Walther & Thorrold 2008, Walther et al. 2008, Whitledge 2009). However, more recent studies on marine fish (including flatfishes) are also adopting a dual isotope ($\delta^{13}C$ and $\delta^{18}O$) and multi-element approach to investigate otolith chemistry (e.g. Dierking et al. 2012, Kajajian et al. 2014, Wells et al. 2015).

Site fidelity of *Pleuronectes platessa*

One explanation for the high classification observed for the present study may be due to the life history patterns observed for juvenile plaice with their prolonged residency times on defined nursery grounds (Dunn & Pawson 2002) during their first years of growth. Juvenile (0-group) plaice have been found to exhibit both site fidelity and homing behavior for their chosen nursery ground (Burrows et al. 2004, Gibson et al. 2011), with tag and release studies indicating when displaced juvenile plaice will return to their site of capture (Riley 1973, Burrows et al. 2004). Although it is known that both 0-group and 1-group plaice enter relatively deeper water to avoid colder temperatures during October-November, they return to shallower depths the following spring (Wennhage et al. 2001). In addition, Riou et al. (2001) has shown that 1-group plaice individuals are more numerous close to shore during spring and autumn. Total residency times on nursery grounds for juvenile plaice can range between 1 and 3 years before juveniles migrate into deeper water as they enter the sub-adult phase and begin the process of sexual maturity (Nash et al. 1994, Dunn & Pawson 2002, Fox et al. 2007).

Thus, the spatial distribution patterns of juvenile plaice, combined with their site fidelity make them a perfect species to show spatial signals using otolith microchemistry. The utilization of integrated chemical signals from the various trace metals within the juvenile plaice otoliths along the North West coast of England and North Wales (including

333 Anglesey) suggest that both 1-group (the present study) and 2/3-group plaice (Geffen et
334 al. 2003) move little from their chosen sites. If however juvenile place were found to
335 move, evidence would suggest they move to sites which are in close proximity of each
336 other e.g. within a chosen region, have similar geologies and therefore similar chemical
337 signals. A factor which seems evident when we take into account the high classification
338 accuracy observed within the regional areas for this study.
339 Thorrold et al. (1988) have stated that in order to identify fish back to source, all
340 source locations need to be sampled. By way of explanation, within the context of the
341 present study, to assess which nursery areas contribute the greatest proportions of
342 juvenile fish to the adult stock requires the sampling of all possible sources of recruits.
343 For the present study, it was not possible to sample all sources of juvenile plaice in the
344 southeast Irish Sea as it is likely that these are not known. In addition, licensing
345 conditions restricted how many sites could be sampled, and accessibility to some sites
346 was difficult (e.g. within Morecambe Bay). However, fish were sampled from the major
347 nursery grounds identified by previous studies (Dunn and Pawson 2002, Fox et al., 2007;
348 Ellis et al. 2012) which are likely to produce the majority of recruits for the putative
349 southeast Irish Sea stock. It is possible that plaice larvae derived from spawning grounds
350 in the western Irish Sea may be transported onto nursery grounds in the eastern Irish Sea
351 (Fox et al. 2009). However, we targeted 1-group plaice in our study to ensure that the
352 dominant chemical signal measured in the otolith would be derived from the residency
353 period on the nursery ground itself and any signal derived from the mother or the pelagic
354 larval phase would be significantly diluted.
355 Determining the connectivity between juvenile nursery grounds is critical if we are to
356 understand recruitment patterns and the relative importance of different nursery
357 grounds to the adult stocks (see review by Gillanders et al. 2003). The use of a multi-

358 elemental otolith tag in the present study suggests that it may be possible identify adults
359 to nursery ground, or region of origin by looking at the juvenile portion of the adult
360 otoliths (Forrester and Swearer 2002, Cuveliers et al. 2010). Given the relative sizes of
361 the otoliths derived from juvenile and adult plaice, it is likely that solution-based ICP-MS
362 would be used on juvenile otoliths whilst laser ablation ICP-MS would be used to assess
363 the otolith core of adults. The former approach would be used to obtain an integrated
364 'signature' for the juvenile whilst the latter would be used to derive the juvenile
365 'signature' for that fish. However, one must be cautious when using two different
366 analytical techniques to determine otolith elemental concentrations as both methods will
367 vary in their sensitivity and detection limits (see Campana 1999, de Pontual et al. 2000,
368 Ludsin et al. 2006) which may affect which elements are available for inclusion in the
369 discriminant analysis.
370 The understanding of a stock's structure, ecology and, more importantly, the exchange
371 rates between spatially separated sub-populations of both juvenile fish and adults is
372 essential for future management programmes if we are to continue sustainable fishing
373 (Tanner et al. 2012). For one to effectively manage a species, a clear understanding of
374 habitat importance and therefore its productivity in maintaining the population has to be
375 identified (Chittaro et al 2009). The use of otolith microchemistry has helped in
376 classifying juvenile plaice to individual nursery grounds for this study and the possible
377 identification of a regional split hitherto unknown. Although the role of dispersal in
378 marine population dynamics is still incomplete (Cook 2011), the use of natural chemical
379 tags has enabled researchers to quantify these movements. Furthermore, the use of
380 established baselines based on the elemental chemistry of these otoliths would further
381 the understanding of movement and connectivity between nursery grounds. In doing so
382 future assessments of those nursery grounds combined with changes over temporal

383 scales may assist in the understanding of their relative importance to adult stocks and
384 assist in the prioritization of management and conservation of the more productive
385 nursery grounds.

386 The site fidelity observed in juvenile plaice suggests that they are likely to experience
387 the same physical and biological conditions since settlement and this, combined with
388 their natural homing trait (Burrows et al. 2004), makes them an ideal model to study
389 inter-annual variability (i.e. temporal stability) of the elemental "tag" for local nursery
390 grounds using otolith microchemistry. A recent study using otoliths extracted from
391 juvenile plaice collected from two sites in North Wales found that the elemental
392 concentration of Mg, Na, K, Sr and Ba varied little over an inter-annual (3-4 year) period
393 (Marriott 2014), further strengthening the use of plaice as a study species to assess
394 elemental changes over temporal scales.

395 The identification of natal origin of South Eastern Irish Sea plaice will allow future
396 management and conservation efforts to be directed towards prioritizing the more
397 important nursery and juvenile habitats within this area (in the form of recruitment rates
398 of juveniles to the adult population) and assist in future fisheries and integrated coastal
399 management (Vasconcelos et al. 2007, Cuveliers et al. 2010).

400

401 *Acknowledgements*. The authors would like to thank Berwyn Roberts and Craig
402 Robertson for their invaluable assistance during sample collection during this study and
403 the North Wales and North West Sea Fisheries Commission for issuing the licenses to
404 catch undersized fish in this study. We thank the referees for their helpful comments on
405 the manuscript. The project was funded by the European Social Fund.

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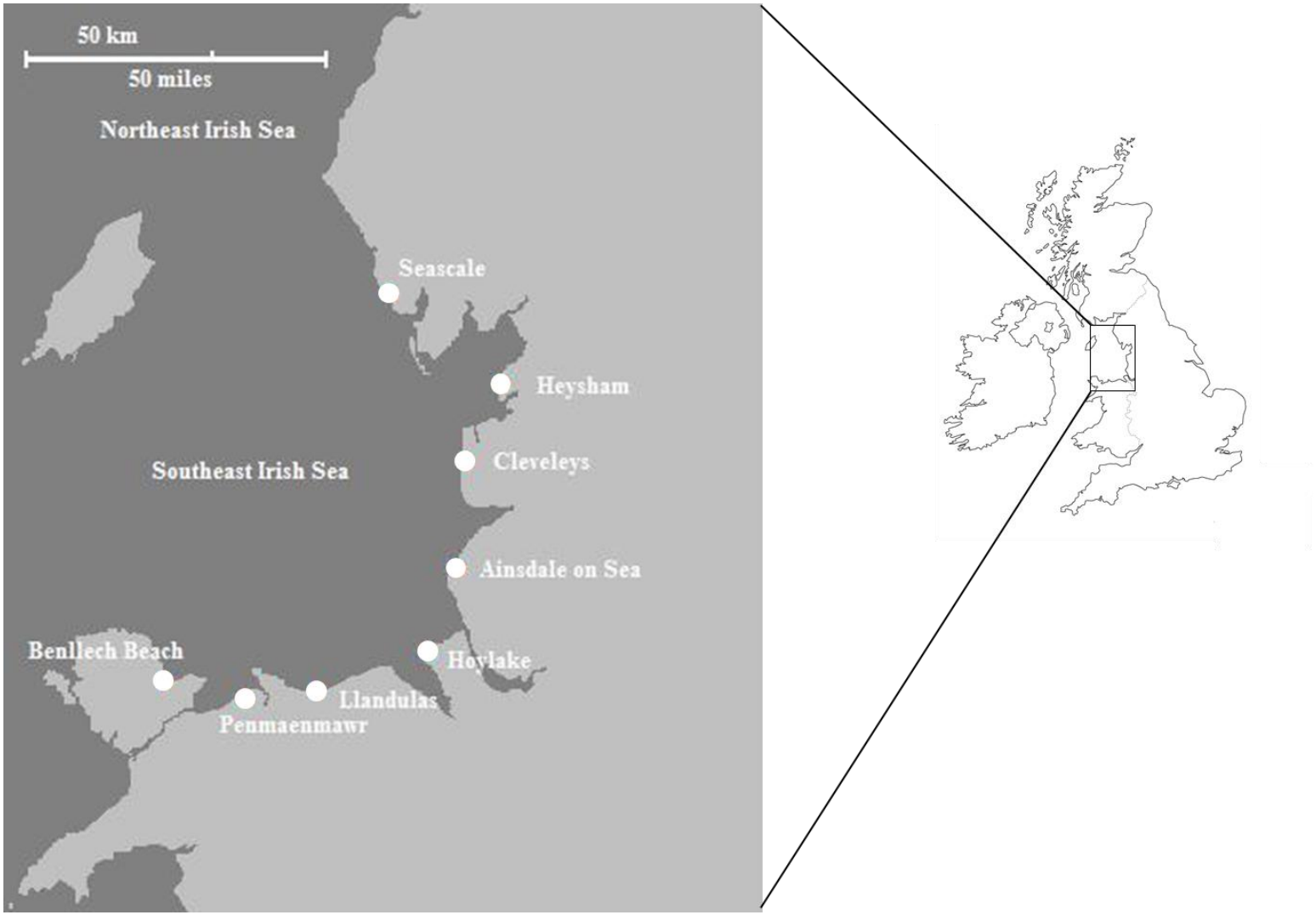

Figure 1. Geographical locations of the 8 juvenile plaice *Pleuronectes platessa* nursery grounds (recognised by Dunn and Pawson 2002) along the North West coasts of England and North Wales sampled during the present study.

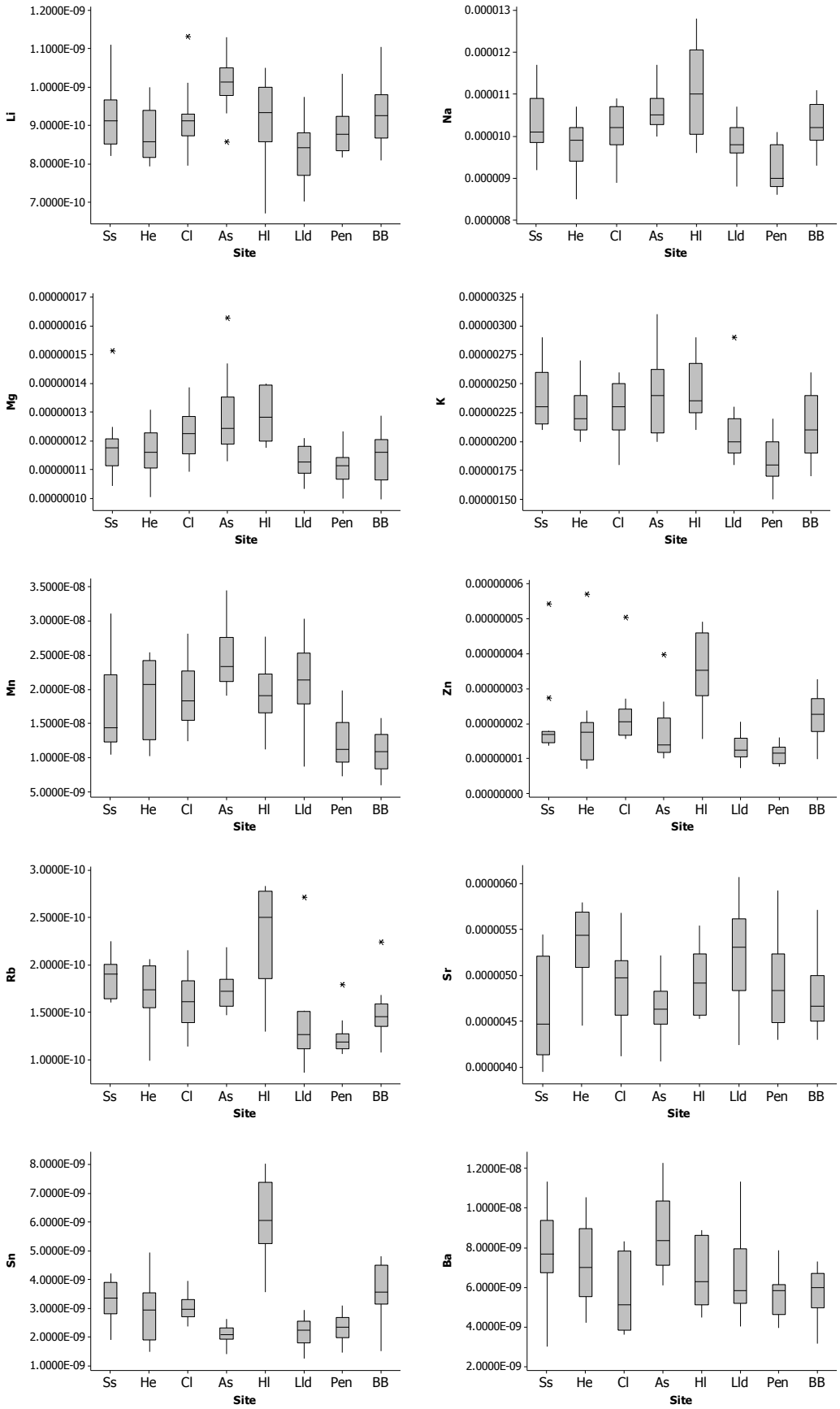

Figure 2. Box-plots for the 10 elements measured (μg g^{-1}) in otoliths of juvenile plaice *Pleuronectes platessa* collected from the 8 nursery grounds located in the south-eastern Irish Sea. Nursery grounds are defined as: **Ss**- Seascale (n = 13), **He-** Heysham (n = 15), **Cl-** Cleveleys (n = 15), **As-** Ainsdale on Sea (n = 14), **Hl-**Hoylake (n = 6), **Lld-** Llandulas (n = 15), **Pen-** Penmaenmawr (n = 15) and **BB-** Benllech Beach (n = 14).

Table 1. MANOVA results of comparisons of mean element: Ca ratios (Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn, Ba) in the otoliths of juvenile plaice *Pleuronectes platessa* from 8 nursery grounds along the eastern Irish Sea coast. *P < 0.01; ** P < 0.001

Site	DF		Seascale	Heysham	Cleveleys	Ainsdale on Sea	Hoylake	Llandulas	Penmaenmawr	Benllech Beach
Seascale	10, 90	F		6.878	4.811	3.492	6.956	12.356	15.880	4.706
		P		**	**	**	**	**	**	**
Heysham	10, 90	F	6.878		4.456	9.044	10.440	3.515	11.388	9.770
		P	**		**	**	**	*	**	**
Cleveleys	10, 90	F	4.811	4.456		6.750	6.908	7.464	12.961	5.106
		P	**	**		**	**	**	**	**
Ainsdale on Sea	10, 90	F	3.492	9.044	6.750		11.594	12.415	18.570	10.015
		P	**	**	**		**	**	**	**
Hoylake	10, 90	F	6.956	10.440	6.908	11.594		17.039	24.204	10.730
		P	**	**	**	**		**	**	**
Llandulas	10, 90	F	12.356	3.515	7.464	12.415	17.039		7.569	12.214
		P	**	*	**	**	**		**	**
Penmaenmawr	10, 90	F	15.880	11.388	12.961	18.570	24.204	7.569		7.999
		P	**	**	**	**	**	**		**
Benllech Beach	10, 90	F	4.706	9.770	5.106	10.015	10.730	12.214	7.999	
		P	**	**	**	**	**	**	**	

F values are given for the MANOVA test for pairwise element: Ca ratios (Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn, Ba).
DF, degrees of freedom.

Table 2. ANOVA results for comparisons of elemental concentrations in the otoliths of juvenile plaice from the 8 nursery grounds sampled in the eastern Irish Sea. Sites which are significant from others are proceeded by >, sites in **bold** indicate significant difference at P < 0.001. Site codes (Ss, He, Cl, As, Hl, Lld, Pen and BB) are described in Figure 2.

Element	Site effect $F_{7, 99}$ =	*P*	Post hoc Pairs#	Significance between-site differences
Li	6.11	<0.05	6	**As** > He, **Lld**, Pen; Lld > Ss, Cl, BB
Na	8.75	<0.05	9	**Pen** > **Ss**, Cl, **As**, **Hl**, BB; As, Hl > He, Lld
Mg	6.77	<0.05	8	**As** > He, **Lld**, **Pen**, BB; Hl > Lld, Pen, BB; Pen > Cl
K	9.20	<0.05	7	**Pen** > **Ss**, **He**, **Cl**, **As**, **Hl**, BB; Lld > As
Mn	12.58	<0.05	11	**Pen** > He, Cl, **As**, **Lld**; **BB** > Ss, **He**, **Cl**, **As**, Hl, **Lld**; **As** > Ss
Zn	9.56	<0.05	10	**Hl** > Ss, He, As, **Lld**, **Pen**; Lld > Cl, BB; Pen > Ss, Cl, BB
Rb	12.20	<0.05	12	**Hl >** He, Cl; **Lld**, **Pen**, BB; Lld > Ss, He, As; **Pen** > **Ss**, He, Cl, **As**
Sr	4.51	<0.05	4	He > Ss, As, BB; Ss > Lld
Sn	18.09	<0.05	16	**Hl** > **ALL**; **As** >, Ss, Cl, **BB**; **Lld** > Ss, Cl, **BB**; Pen > Ss, Cl, BB
Ba	5.64	<0.05	5	As > Cl, Lld, Pen, BB; Cl > Ss

#The number of pairs of sites (out of a total of 28 pairs) which indicated significant differences (P < 0.05) in element concentrations using Bonferroni post hoc comparisons.
F values are given for the ANOVA test for site effects.

Table 3. Percentage classification of juvenile plaice *Pleuronectes platessa* between nursery grounds using cross validation linear discriminate function analysis (CV-LDFA) using multi-elemental fingerprints Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn and Ba (μg g^{-1}). Numbers in **bold** indicate percentage of correctly classified fish to their nursery ground of capture. Total *n* = number of individuals analysed with their total accumulated percentage correctly classified fish in parenthesis. Shaded panels indicate adjacent sites to which fish were attributed from their original site of capture.

	Predicted nursery ground								
	Seascale	Heysham	Cleveleys	Ainsdale on Sea	Hoylake	Llandulas	Penmaenmawr	Benllech Beach	Total *n*
Cross Validation Count									
Seascale	**6** (**46.2**%)	0	2	2	0	0	0	3	13
Heysham	2	**8** (**53.3**%)	2	0	0	3	0	0	15
Cleveleys	2	2	**10** (**66.7**%)	0	0	0	0	1	15
Ainsdale on Sea	1	0	0	**13** (**92.9**%)	0	0	0	0	14
Hoylake	1	0	1	0	**4** (**66.7**%)	0	0	0	6
Llandulas	0	2	0	0	0	**11** (**73.3**%)	2	0	15
Penmaenmawr	0	1	0	0	0	0	**14** (**93.3**%)	0	15
Benllech Beach	0	0	2	0	0	0	2	**10**(**71.4**%)	14
									71.0 %

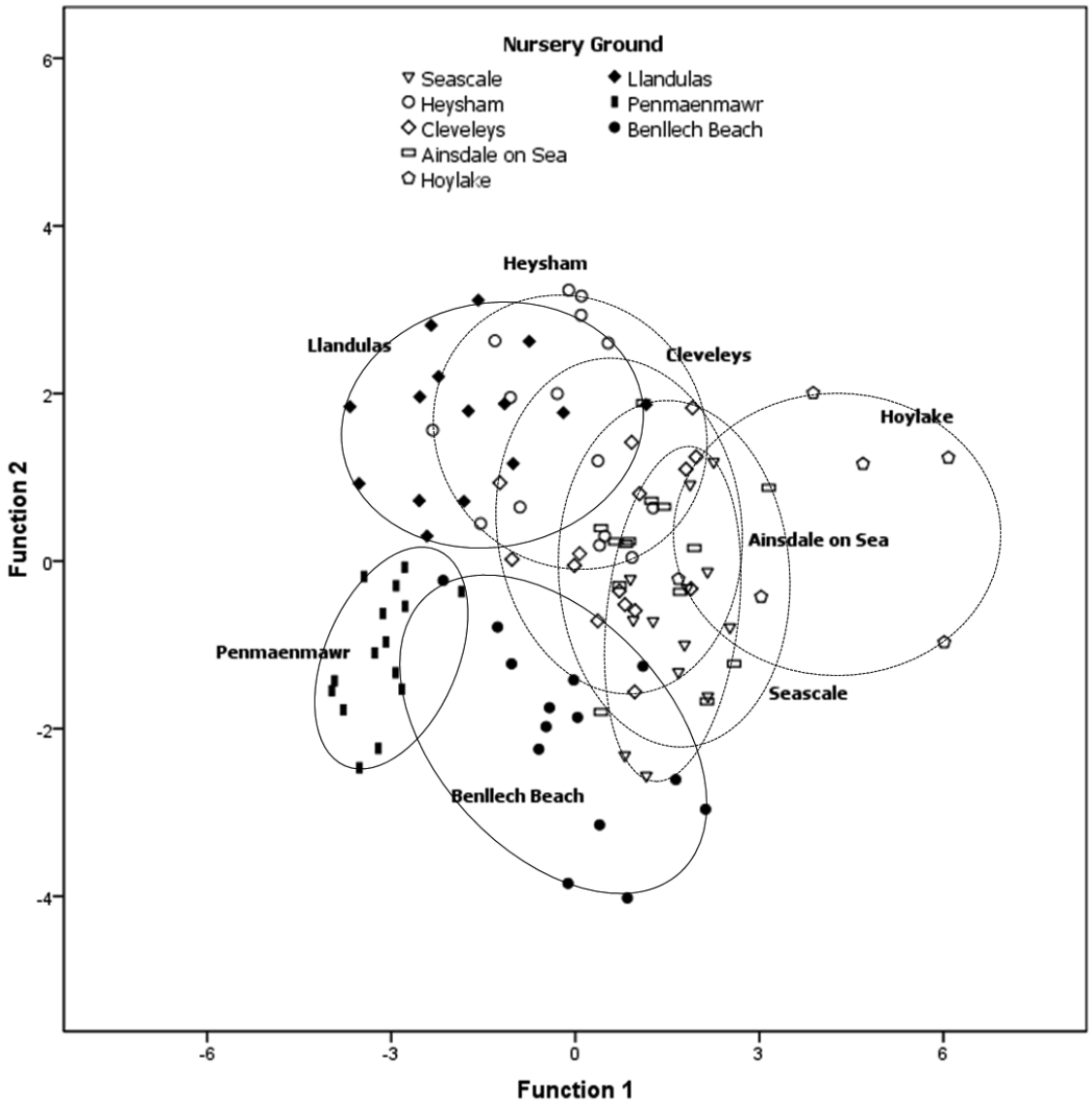

Figure 3. Allocation of juvenile plaice *Pleuronectes platessa* to their sampling sites based on linear discriminant function analysis observed in Table 3 using the elements Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn and Ba.

Table 4. Summary of recently published data examining the number of elements used in otolith microchemistry, the number tested and those significant to discriminate between movement patterns of fish from fresh, estuarine, coastal and marine waters using inductively-coupled plasma mass spectrometry (**ICP-MS**). Data are organised by water bodies. **Est-Coast** = Estuarine and Coastal water. **DFA** = Discriminant function analysis. **OES/AES** = atomic emission spectrometry; **LA** = Laser Ablation; **sb** = Solution based.

Water	Nº Sites	Distance	Elements measured	Tested in DFA	Significant elements	Species	Classification	ICP-MS	Author(s)
Fresh	8	100Km#	Na, K, Mg, Mn, Sr, Ba	K, Mg, Mn, Sr, Ba	K, Mn, Sr, Ba	*Perca flavescens*	62% – 100%	sb & AES	Brazner et al. 2004
Fresh	4	130Km	Mg, Mn, Sr, Ba	All	Mg, Mn, Sr, Ba	*Salmo salar*	84%-100%	LA	Veinott & Porter. 2005
Fresh	4	170 Km	Mg, Mn, Zn, Sr, Ba	All	Mg, Mn, Zn, Sr, Ba	*Salmo trutta*	95%-97%	LA	Veinott et al. 2012
Fresh	9	600Km	Mg, Mn, Zn, Sr, Ba	All	Mn, Ba	*Oncorhynchus mykiss*	91-96%	LA	Veinott & Porter. 2013
Estuarine	2 2	200Km	Li, Mg, Mg, Al, Fe, Mn, Co, Ni, Cu, Zn, Cu, Zn, As, Rb, Mo, Cd, Sn, Ba, Hg, Tl, Pb, Th, U.	Mn, Sr As, Fe, Sr	Mn, Sr As, Fe, Sr	*Solea solea*	73% 79%	LA	De Pontual et al. 2000
Estuarine	2 2	"	Li, Mg, Mg, Al, Fe, Mn, Co, Ni, Cu, Zn, Cu, Zn, As, Rb, Mo, Cd, Sn, Ba, Hg, Tl, Pb, Th, U.	Mg, Cd Li, Mg, Rb, Cd, Th	Mg, Cd Li, Mg, Rb, Cd, Th	*Solea solea*	89% 91%	sb	De Pontual et al. 2000
Estuarine	7	500Km	Li, Mg, Mn, Cu, Sr, Ba, Pb	All	Mg, Mn* Mg, Ba*	*Solea solea,* *S. senegalensis*	71% – 81%	LA	Tanner et al. 2012
Est-Coast	9	165Km#	Mn, Cu, Sr, Ba, Pb	Cu	Cu	*Paralichthys californicus*	76 & 86%	sb	Forrester & Swearer. 2002
Est-Coast	9	"	Mn, Cu, Sr, Ba, Pb	Pb	Pb	*Paralichthys californicus*	68 & 87%	sb	Forrester & Swearer. 2002
Est-Coast	9	"	Mn, Cu, Sr, Ba, Pb	Cu, Pb	Cu, Pb	*Paralichthys californicus*	81 & 84%	sb	Forrester & Swearer. 2002
Est-Coast	18	500Km	Li, Mn, Sr, Ba	All	Li, Sr**	*Pleuronectes vetulus*	73-87%	sb	Brown. 2006b
Est-Coast	18	"	Li, Mn, Sr, Ba	All	Sr**	*Citharichthys stigmaeus*	58-89%	sb	Brown. 2006b
Est-Coast	10-10	300Km	Sr, Sc, P, Na, Y, Rb, Mn, Mg, Li	All	Li, Sc, Mn, Rb	*Solea solea*	100%	sb	Leakey et al. 2009
Est-Coast	10-10	"	Cu, Ni, Sc, Na, Y, Rb, Mn, Li	All	Li, Sc, Mn, Rb	*Merlangius merlangus*	95%	sb	Leakey et al. 2009
Est-Coast	13-5	"	Sc, Ba, Rb, Mn, Li	All	Li, Sc, Mn, Rb	*Dicentrarchus labrax*	100%	sb	Leakey et al. 2009
Est-Coast	17	5000Km#	Li, Ca, Mn, Sr, Ba	All	Ba	*Polydactylus macrochir*	various	LA	Moore & Simpfendorfer. 2014
Marine	3	1000Km#	Li, Mg, Mn, Ca, Sr, Ba	All	Li, Mg, Mn	*Thunnus orientalis*	75% & 100%	sb	Rooker et al. 2001b
Marine	5	7000Km#	Li, Mg, Mn, Ca, Sr, Ba	All	Li, Mg, Mn, Sr	*Thunnus thynnus*	62% – 80%	sb	Rooker et al. 2003
Marine	5	100Km#	B, Mg, Al, Sc, Ti, Cr, Mn, Ni, Cu, Sr, Ba	All	Mg, Al, Sc, Mn, Ni, Sr, Ba	*Pleuronectes platessa*	92%	sb	Geffen et al. 2003
Marine	8	500Km	Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba, Pb	All	Li, K, Mn, Zn	*Solea solea*	67-100%	sb	Vasconcelos et al. 2007
Marine	8	"	Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba, Pb	All	Na, Mg, Mn, Cu, Sr	*Solea senegalensis*	75-100%	sb	Vasconcelos et al. 2007
Marine	8	"	Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba, Pb	All	Li, Na, Mn	*Platichthys flesus*	80-100%	sb	Vasconcelos et al. 2007
Marine	8	"	Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba, Pb	All	Li, K, Mn, Ba, Pb	*Diplodus vulgaris*	77-100%	sb	Vasconcelos et al. 2007
Marine	8	"	Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba, Pb	All	Mg, Mn, Sr, Ba, Pb	*Dicentrarchus labrax*	67-90%	sb	Vasconcelos et al. 2007
Marine	4	300Km#	Na, Mg, Mn, Co, Cu, Zn, Rb, Sr, Ba, Pb	Na, Mg, Mn, Rb, Sr, Ba	Mg, Mn, Ba	*Solea solea*	72-100%	LA	Cuveliers et al. 2010
Marine	21	200Km	Mg, Mn, Zn, Sr, Ba, Ce, Pb	All	Mg, Zn, Sr, Ba, Ce, Pb***	*Stegastes partitus*	52% – 99%	LA	Chittaro & Hogan. 2013
Marine	4	200Km	Mg, Mn, Sr, Ba, Pb	All	Mn, Ba	*Merluccius productus*	59% – 88%	LA	Chittaro et al. 2013
Marine	4	1100Km	Mg, Mn, Sr, Ba	All	Sr, Ba	*Gadus morhua*	66% – 78%	LA	D'Avignon & Rose. 2013
Marine	8	200Km	Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn, Ba	All	Li, K, Mn, Sr, Sn	*Pleuronectes platessa*	46-93%	sb	This Study

Distances are approximate linear measurements and are taken from the two furthest sampling locations.

* Data taken from the inter-annual variability observed from the 1st and 2nd canonical variations for both species

** Data taken from the regions reduced model for both species

*** Data taken from the regions wide scale model