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

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4  
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9  
10          ABSTRACT: Nursery grounds are valuable habitats providing sources of food and refuge  
11          during early life stages for many commercially caught marine fish. Distinguishing  
12          between different nursery grounds and identifying habitat origin using trace elemental  
13          concentrations in aragonite structures of teleost fish have proved valuable in fish ecology  
14          and fisheries. This study aimed to: (1) compare chemical signatures (elemental  
15          fingerprints) within sagittal otoliths of juvenile plaice (*Pleuronectes platessa*) sampled  
16          from known nursery habitats in the SE Irish Sea; and (2) assess their potential and  
17          robustness as natural tags for identifying nursery grounds for the putative SE Irish Sea  
18          plaice stock. Otoliths from 1-group juvenile plaice (6-15 cm total length) were obtained  
19          from 8 nursery grounds in coastal areas off North West England and North Wales  
20          (including Anglesey) between June and August 2008. Solution-based inductively-coupled  
21          plasma mass spectrometry determined the concentrations of 10 elements (Li, Na, Mg, K,  
22          Mn, Zn, Rb, Sr, Sn, Ba), with significant differences in otolith element composition  
23          observed between all nursery grounds. Cross-validation linear discriminant function  
24          analysis (CV-LDFA) classified fish to their nursery ground of capture (46.2% to 93.3%),  
25          with a total group CV-LDFA accuracy of 71.0%. CV-LDFA between regions (North West  
26          England and North Wales) classified fish with 82% accuracy. The discrimination of  
27          juvenile plaice from all 8 nursery grounds within the southeast Irish Sea using otolith  
28          microchemistry offers significant opportunities in the development of future effective  
29          fisheries management strategies through understanding the supply of juveniles from  
30          specific nursery grounds and adult plaice in the Southeast Irish Sea.

31  
32          KEY WORDS: Nursery grounds· Otolith microchemistry· Natural tag· Juvenile plaice·  
33          *Pleuronectes platessa*

## INTRODUCTION

34

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

56 The use of otolith microchemistry can be a valuable alternative to manual tagging in  
57 distinguishing between the habitats of origin in juvenile marine fishes (Thorrold et al.  
58 2001, Gillanders 2005, Brown 2006b). Due to the nature and composition of otoliths,

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

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

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

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

90

91

## MATERIALS AND METHODS

92 **Sample Collection.** Juvenile plaice (1-group) with a total length (TL) between 6 and  
93 15 cm were collected from 8 sites identified as main nursery grounds around the north  
94 coast of Wales and North West England (Dunn & Pawson 2002) during June and August  
95 2008 (Figure 1). 1-group plaice were chosen (as opposed to 0-group) to represent an  
96 integrated signal over 12 months and to account for any possible seasonal fluctuations or  
97 movements made during the first year within their chosen nursery ground. Sampling  
98 sites were selected due to their recognised importance as major nursery grounds for  
99 juvenile plaice within the putative South-East Irish Sea stock (Dunn & Pawson 2002, Fox  
100 et al. 2007). Fish were collected using two techniques: a push-net was used in water  
101 depths of < 1m; and, a nylon beach-seine net (Dimensions: depth 2.2 m cod end mesh 5  
102 mm), used in water > 1 m in depth. On capture, juvenile plaice were immediately  
103 euthanized using the Home Office Schedule 1 method and stored on ice within a portable  
104 refrigeration unit for transportation back to the laboratory where fish were frozen at -20  
105 °C until otolith extraction.

106 **Otolith Preparation.** All equipment used in extraction, cleaning, and storage of the  
107 sagittal otoliths were non-metallic and pre-acid-washed in analytical grade 10% HNO<sub>3</sub>  
108 (>69% HNO<sub>3</sub>, 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<sub>3</sub> / 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<sub>2</sub>O<sub>2</sub> 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<sub>3</sub> / 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<sup>-1</sup> for the  
133 multi-element assessments. Elements observed at a higher concentration in otolith

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

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

154 **Statistical Analysis.** Elemental concentrations were expressed as  $\mu\text{g g}^{-1}$  otolith and  
155 were transformed to an element: Ca ratio (Forrester & Swearer, 2002, Swearer et al.  
156 2003, Brown 2006a b). Data for each element were analysed for univariate normality  
157 (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test) (Minitab  
158 v.14.0), with the assumptions being met following  $\text{Log}_{10}$  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 ( $\pm 0.1$   
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 ( $\pm 0.1$ CI) 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%)

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

260 Some studies using biogeochemical tags to discriminate between geographical  
261 locations have tended to focus on a small suite of elements that have similar ionic radii  
262 and ionic charge to calcium, e.g. Mn, Sr and Ba (Swearer et al. 2003, Hedges et al. 2004,  
263 Clarke et al. 2007) and which substitute for Ca in the otolith matrix e.g. Mg (Rooker et al.  
264 2001a, Swan et al. 2006). However, focusing solely on the use of those elements which  
265 are the primary drivers determining classification in microchemistry studies of  
266 freshwater and diadromous fishes (e.g. Sr and Ba, Table 4) may not be as robust for  
267 microchemistry analysis for fish sampled from marine waters (e.g. Mg, Mn, Sr, Ba: CV-  
268 LDFA: 31.8% this study) (Brown & Severin 2009).

269 To determine which elements are the primary drivers of spatial discrimination using  
270 otolith microchemistry in differing waterbodies is beyond the scope of this paper.  
271 However, a review of the elements used in such studies (Table 4) suggests that certain  
272 metals may contribute more to spatial discrimination within fresh, estuarine and marine  
273 waters. For instance, in estuarine environments, Mg, Mn, Sr and Cd are significant in  
274 discrimination between sites (Table 4) whilst studies identifying the movement between  
275 estuarine and coastal waters have identified Li, Mn, Rb and Sc as significant in  
276 discriminant analyses (Table 4). In the marine environment, Mn, Mg, Sr, Ba, Li, K and Pb  
277 have been identified as significant in discrimination (Table 4). Using elements such as  
278 lithium (due to its fluvial inputs from continents) and Rb (due to higher dissolved  
279 concentrations in marine waters) may be advantageous in discriminating fish from  
280 coastal/marine habitats from fish collected from freshwater/estuarine habitats (Brown  
281 2006a,b, Leakey et al. 2009). Similarly, Mn (due to its elevated particulate phase within  
282 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 ( $\leq 500\text{Km}$  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 ( $> 500\text{Km}$ )  
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

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

#### 314 **Site fidelity of *Pleuronectes platessa***

315 One explanation for the high classification observed for the present study may be due  
316 to the life history patterns observed for juvenile plaice with their prolonged residency  
317 times on defined nursery grounds (Dunn & Pawson 2002) during their first years of  
318 growth. Juvenile (0-group) plaice have been found to exhibit both site fidelity and homing  
319 behavior for their chosen nursery ground (Burrows et al. 2004, Gibson et al. 2011), with  
320 tag and release studies indicating when displaced juvenile plaice will return to their site  
321 of capture (Riley 1973, Burrows et al. 2004). Although it is known that both 0-group and  
322 1-group plaice enter relatively deeper water to avoid colder temperatures during  
323 October-November, they return to shallower depths the following spring (Wennhage et  
324 al. 2001). In addition, Riou et al. (2001) has shown that 1-group plaice individuals are  
325 more numerous close to shore during spring and autumn. Total residency times on  
326 nursery grounds for juvenile plaice can range between 1 and 3 years before juveniles  
327 migrate into deeper water as they enter the sub-adult phase and begin the process of  
328 sexual maturity (Nash et al. 1994, Dunn & Pawson 2002, Fox et al. 2007).

329 Thus, the spatial distribution patterns of juvenile plaice, combined with their site  
330 fidelity make them a perfect species to show spatial signals using otolith microchemistry.  
331 The utilization of integrated chemical signals from the various trace metals within the  
332 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 plaice 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

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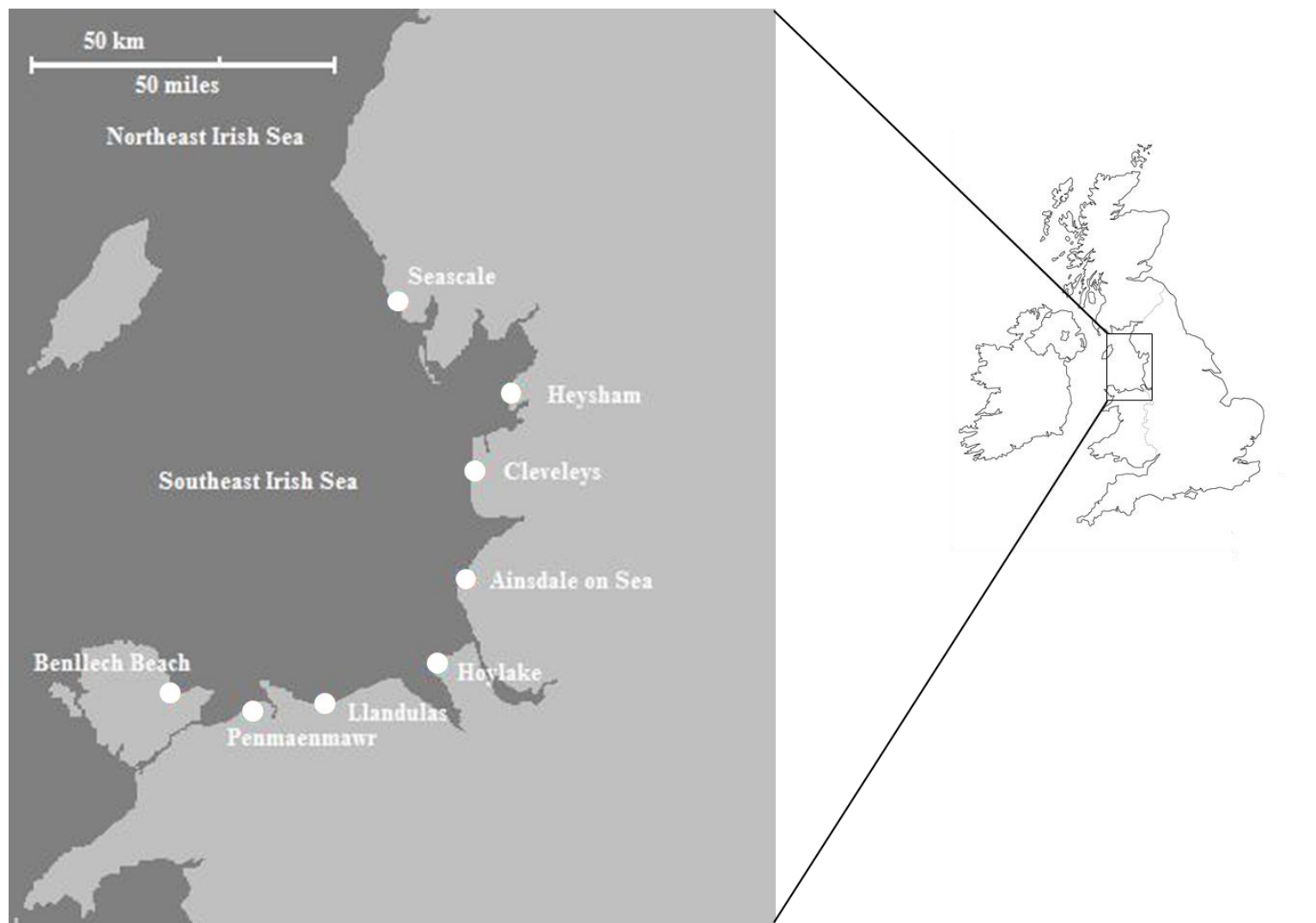


Figure 1. Geographical locations of the 8 juvenile plaice *Pleuronectes platessa* nursery grounds (recognised by Dunn and Pawson 2002) along the North West coasts of England and North Wales sampled during the present study.

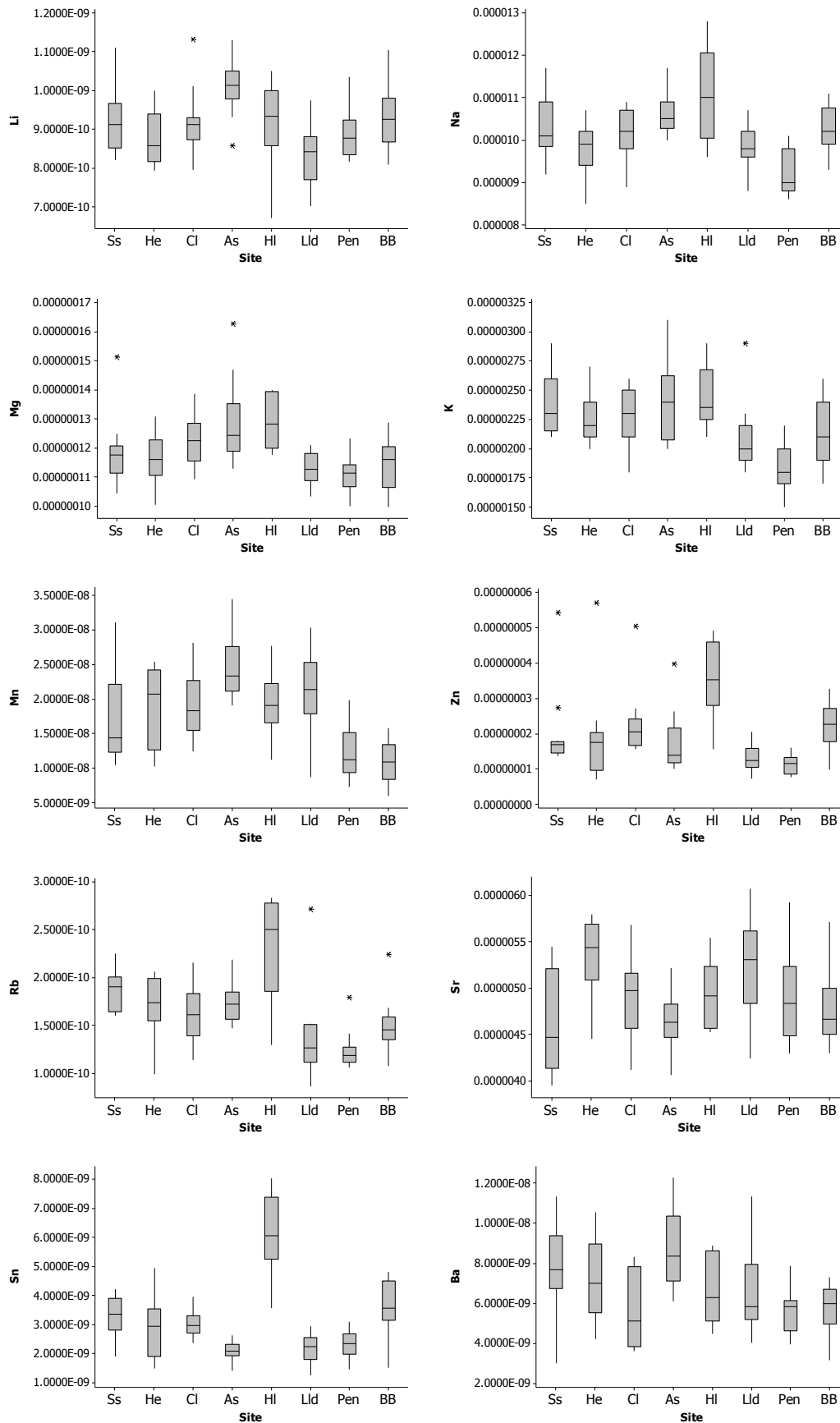


Figure 2. Box-plots for the 10 elements measured ( $\mu\text{g g}^{-1}$ ) in otoliths of juvenile plaice *Pleuronectes platessa* collected from the 8 nursery grounds located in the south-eastern Irish Sea. Nursery grounds are defined as: **Ss**- Seascale (n = 13), **He**- Heysham (n = 15), **Cl**- Cleveleys (n = 15), **As**- Ainsdale on Sea (n = 14), **HI**-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 preceded 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 <sub>7,99</sub> =	P	Post hoc Pairs#	Significance between-site differences
Li	6.11	<0.05	6	<b>As</b> > He, <b>Lld</b> , Pen; Lld > Ss, Cl, BB
Na	8.75	<0.05	9	<b>Pen</b> > <b>Ss</b> , Cl, <b>As</b> , <b>Hl</b> , BB; As, Hl > He, Lld
Mg	6.77	<0.05	8	<b>As</b> > He, <b>Lld</b> , <b>Pen</b> , BB; Hl > Lld, Pen, BB; Pen > Cl
K	9.20	<0.05	7	<b>Pen</b> > <b>Ss</b> , <b>He</b> , <b>Cl</b> , <b>As</b> , <b>Hl</b> , BB; Lld > As
Mn	12.58	<0.05	11	<b>Pen</b> > He, Cl, <b>As</b> , <b>Lld</b> ; <b>BB</b> > Ss, <b>He</b> , <b>Cl</b> , <b>As</b> , Hl, <b>Lld</b> ; <b>As</b> > Ss
Zn	9.56	<0.05	10	<b>Hl</b> > Ss, He, As, <b>Lld</b> , <b>Pen</b> ; Lld > Cl, BB; Pen > Ss, Cl, BB
Rb	12.20	<0.05	12	<b>Hl</b> > He, Cl; <b>Lld</b> , <b>Pen</b> , BB; Lld > Ss, He, As; <b>Pen</b> > <b>Ss</b> , He, Cl, <b>As</b>
Sr	4.51	<0.05	4	He > Ss, As, BB; Ss > Lld
Sn	18.09	<0.05	16	<b>Hl</b> > <b>ALL</b> ; <b>As</b> >, Ss, Cl, <b>BB</b> ; <b>Lld</b> > Ss, Cl, <b>BB</b> ; 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 ( $\mu\text{g g}^{-1}$ ). 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								Total $n$
	Seascale	Heysham	Cleveleys	Ainsdale on Sea	Hoylake	Llandulas	Penmaenmawr	Benllech Beach	
<b>Cross Validation Count</b>									
Seascale	<b>6 (46.2%)</b>	0	2	2	0	0	0	3	13
Heysham	2	<b>8 (53.3%)</b>	2	0	0	3	0	0	15
Cleveleys	2	2	<b>10 (66.7%)</b>	0	0	0	0	1	15
Ainsdale on Sea	1	0	0	<b>13 (92.9%)</b>	0	0	0	0	14
Hoylake	1	0	1	0	<b>4 (66.7%)</b>	0	0	0	6
Llandulas	0	2	0	0	0	<b>11 (73.3%)</b>	2	0	15
Penmaenmawr	0	1	0	0	0	0	<b>14 (93.3%)</b>	0	15
Benllech Beach	0	0	2	0	0	0	2	<b>10(71.4%)</b>	14
									<b>71.0 %</b>

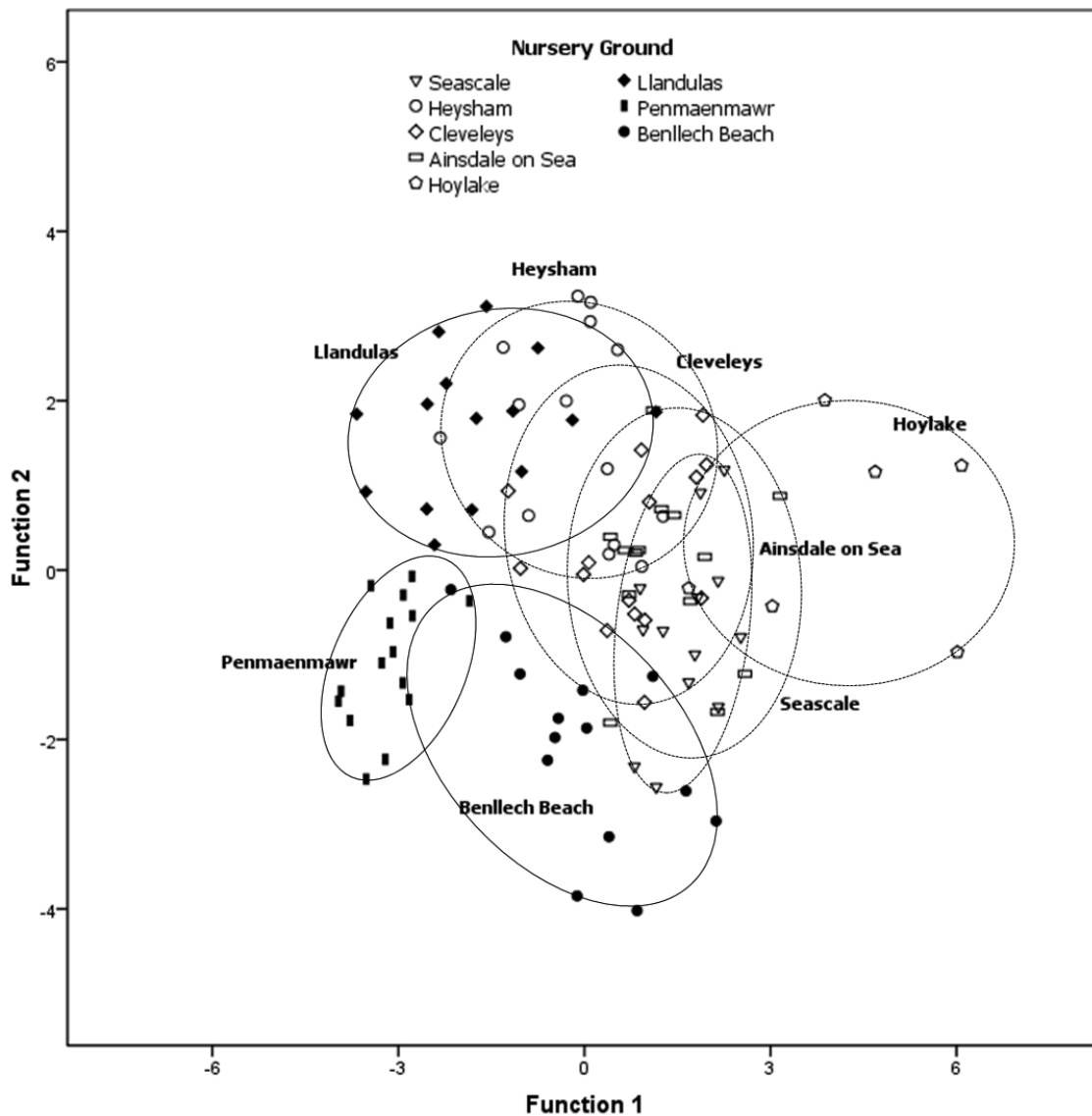


Figure 3. Allocation of juvenile plaice *Pleuronectes platessa* to their sampling sites based on linear discriminant function analysis observed in Table 3 using the elements Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn and Ba.

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	N° Sites	Distance	Elements measured	Tested in DFA	Significant elements	Species	Classification	ICP-MS	Author(s)
Fresh	8	100Km <sup>#</sup>	Na, K, Mg, Mn, Sr, Ba	K, Mg, Mn, Sr, Ba	K, Mn, Sr, Ba	<i>Perca flavescens</i>	62% – 100%	sb & AES	Brazner et al. 2004
Fresh	4	130Km	Mg, Mn, Sr, Ba	All	Mg, Mn, Sr, Ba	<i>Salmo salar</i>	84%-100%	LA	Veinott & Porter. 2005
Fresh	4	170 Km	Mg, Mn, Zn, Sr, Ba	All	Mg, Mn, Zn, Sr, Ba	<i>Salmo trutta</i>	95%-97%	LA	Veinott et al. 2012
Fresh	9	600Km	Mg, Mn, Zn, Sr, Ba	All	Mn, Ba	<i>Oncorhynchus mykiss</i>	91-96%	LA	Veinott & Porter. 2013
Estuarine	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	<i>Solea solea</i>	73% 79%	LA	De Pontual et al. 2000
Estuarine	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	<i>Solea solea</i>	89% 91%		
Estuarine	7	500Km	Li, Mg, Mn, Cu, Sr, Ba, Pb	All	Mg, Mn* Mg, Ba*	<i>Solea solea</i> , <i>S. senegalensis</i>	71% – 81%	LA	Tanner et al. 2012
Est-Coast	9	165Km <sup>#</sup>	Mn, Cu, Sr, Ba, Pb	Cu	Cu	<i>Paralichthys californicus</i>	76 & 86%	sb	Forrester & Swearer. 2002
Est-Coast	9	"	Mn, Cu, Sr, Ba, Pb	Pb	Pb	<i>Paralichthys californicus</i>	68 & 87%	sb	Forrester & Swearer. 2002
Est-Coast	9	"	Mn, Cu, Sr, Ba, Pb	Cu, Pb	Cu, Pb	<i>Paralichthys californicus</i>	81 & 84%	sb	Forrester & Swearer. 2002
Est-Coast	18	500Km	Li, Mn, Sr, Ba	All	Li, Sr**	<i>Pleuronectes vetulus</i>	73-87%	sb	Brown. 2006b
Est-Coast	18	"	Li, Mn, Sr, Ba	All	Sr**	<i>Citharichthys stigmaeus</i>	58-89%	sb	Brown. 2006b
Est-Coast	10-10	300Km	Sr, Sc, P, Na, Y, Rb, Mn, Mg, Li	All	Li, Sc, Mn, Rb	<i>Solea solea</i>	100%	sb	Leakey et al. 2009
Est-Coast	10-10	"	Cu, Ni, Sc, Na, Y, Rb, Mn, Li	All	Li, Sc, Mn, Rb	<i>Merlangius merlangus</i>	95%	sb	Leakey et al. 2009
Est-Coast	13-5	"	Sc, Ba, Rb, Mn, Li	All	Li, Sc, Mn, Rb	<i>Dicentrarchus labrax</i>	100%	sb	Leakey et al. 2009
Est-Coast	17	500Km <sup>#</sup>	Li, Ca, Mn, Sr, Ba	All	Ba	<i>Polydactylus macrochir</i>	various	LA	Moore & Simpfendorfer. 2014
Marine	3	1000Km <sup>#</sup>	Li, Mg, Mn, Ca, Sr, Ba	All	Li, Mg, Mn	<i>Thunnus orientalis</i>	75% & 100%	sb	Rooker et al. 2001b
Marine	5	7000Km <sup>#</sup>	Li, Mg, Mn, Ca, Sr, Ba	All	Li, Mg, Mn, Sr	<i>Thunnus thynnus</i>	62% – 80%	sb	Rooker et al. 2003
Marine	5	100Km <sup>#</sup>	B, Mg, Al, Sc, Ti, Cr, Mn, Ni, Cu, Sr, Ba	All	Mg, Al, Sc, Mn, Ni, Sr, Ba	<i>Pleuronectes platessa</i>	92%	sb	Geffen et al. 2003
Marine	8	500Km	Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba, Pb	All	Li, K, Mn, Zn	<i>Solea solea</i>	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	<i>Solea senegalensis</i>	75-100%	sb	Vasconcelos et al. 2007
Marine	8	"	Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba, Pb	All	Li, Na, Mn	<i>Platichthys flesus</i>	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	<i>Diplodus vulgaris</i>	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	<i>Dicentrarchus labrax</i>	67-90%	sb	Vasconcelos et al. 2007
Marine	4	300Km <sup>#</sup>	Na, Mg, Mn, Co, Cu, Zn, Rb, Sr, Ba, Pb	Na, Mg, Mn, Rb, Sr, Ba	Mg, Mn, Ba	<i>Solea solea</i>	72-100%	LA	Cuveliers et al. 2010
Marine	21	200Km	Mg, Mn, Zn, Sr, Ba, Ce, Pb	All	Mg, Zn, Sr, Ba, Ce, Pb***	<i>Stegastes partitus</i>	52% – 99%	LA	Chittaro & Hogan. 2013
Marine	4	200Km	Mg, Mn, Sr, Ba, Pb	All	Mn, Ba	<i>Merluccius productus</i>	59% – 88%	LA	Chittaro et al. 2013
Marine	4	1100Km	Mg, Mn, Sr, Ba	All	Sr, Ba	<i>Gadus morhua</i>	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	<i>Pleuronectes platessa</i>	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 1<sup>st</sup> and 2<sup>nd</sup> canonical variations for both species

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

\*\*\* Data taken from the regions wide scale model