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

A. L. Marriott[1,2,*], I. D. McCarthy[1], A. L. Ramsay[1], S. R. N. Chenery[2]

[1]School of Ocean Sciences, College of Natural Sciences, Bangor University, Anglesey LL59 5AB, UK

[2]Inorganic Geochemistry, Centre for Environmental Geochemistry, British Geological Survey, Keyworth NG12 5GG, UK

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*Corresponding author: anma@bgs.ac.uk

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 European plaice *Pleuronectes platessa* sampled from known nursery habitats in the south-eastern Irish Sea and (2) assess their potential and robustness as natural tags for identifying nursery grounds for the putative south-eastern Irish Sea plaice stock. Otoliths from juvenile plaice ('1-group', 6 to 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 south-eastern 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 south-eastern 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 (Dickey-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 fish migrate 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). Although mark and recapture studies on juvenile fish have provided some insight (e.g. Burrows et al. 2004, Pickett et al. 2004, Tupper 2007), these methods can be labour intensive and 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 to be tagged in order 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 fish are providing a wealth of information on population dynamics, movement patterns and early life history strategies (see reviews by 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 European plaice *Pleuronectes platessa* is among 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 south-eastern Irish Sea, the main nursery grounds for juvenile plaice have been identified along the coastal waters of north-west England and north Wales (Dunn & Pawson 2002, Ellis et al. 2012), where the newly benthic-orientated juveniles spend between 1 and 3 yr 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 was therefore our aim 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 juveniles 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 along the coasts of north-west England and north Wales (Dunn & Pawson 2002) during June and August 2008 (Fig. 1). We chose 1-group plaice (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-eastern Irish Sea stock (Dunn & Pawson 2002, Fox et al. 2007). Fish were collected using 2 techniques: a push-net was used in water depths of <1 m, and a nylon beach-seine net (depth 2.2 m, cod end mesh 5 mm) was used in water >1 m in depth. On capture, juvenile plaice were immediately euthanized using the Home Office Schedule 1 method www.gov.uk/government/ publications/the-humane-killing-of-animals-code-of-practice) and stored on ice within a portable refrigeration unit for transportation back to the laboratory where fish were frozen at –20°C until otolith extraction.

Otolith preparation

All equipment used in extracting, cleaning and storing the sagittal otoliths was 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 referred to as Milli-Q) and dried under a laminar flow hood for 24 h prior to use. Similarly, analytical tubes were prepared as outlined above with one minor alteration in that they were acid-cleaned using a solution of 1% HNO_3 / 0.5% HCl (both analytical grade). To prevent the possible risk of zinc contamination, powder-free vinyl gloves (Shermond) were used during all procedures (Batley 1989, Friel et al. 1996, Dugan et al. 2008).

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

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

Calibration solutions were prepared using a commercial multi-element standard (SPEX-CertiPrep) diluted with Milli-Q to give concentrations of 100, 10 and 1 ng ml^{-1} for the 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 and 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 analysed 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× 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, 4 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 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 elements. Prior to the analysis of elemental concentrations observed in juvenile plaice otoliths between nursery grounds, an assessment of both left and right sagittal otoliths was performed. Results showed no significant differences in the elemental concentrations of the 10 elements between otolith pairs (paired *t*-test; all $p > 0.05$). A combination of both univariate and multivariate statistical techniques was used to investigate single and multi-elemental fingerprints of the otoliths from each of the 8 nursery grounds. To analyse and quantify the variation in elemental composition of juvenile plaice otoliths within and between the 8 nursery grounds, a multivariate analysis of variance (MANOVA) using Wilks' criterion was performed followed by pairwise comparisons between nursery sites. Examination of the differences in otolith chemical composition for each element between the 8 nursery grounds was conducted using a 1-way ANOVA. Where the ANOVA indicated significant differences, pairwise comparisons (Bonferroni test) were used to identify which sampling locations differed from the others. Cross-validation linear discriminant function analysis (CV-LDFA, SPSS v.16.0) was used to determine the accuracy with which juvenile plaice could be classified back to their nursery ground of capture and through geographical separation by region, i.e. north-west England (NWE) and north-west Wales (NWW), based on the element concentrations within their otoliths (Clarke et al. 2007, Ramsay et al. 2011). Canonical score plots were used to provide a visual representation of the classification of individual fish back to their nursery ground. To evaluate the chance-corrected agreement between the actual and predicted site of capture, Cohen's kappa statistic was calculated. Scores range between 0 and 1, with 0 indicating no improvement to that achieved by pure chance and 1 indicating perfect agreement in classification to site (Titus et al. 1984, Ramsay et al. 2011).

RESULTS

Observations of the elemental box plots (Fig. 2) indicated apparent differences between nursery grounds. Some elements indicated elevated concentrations at some sites, most notably Zn, Rb and Sn at Hoylake and Zn at Benllech Beach. Similarly, elevated peaks of Mn and Ba were observed at Ainsdale on Sea. Conversely, decreased Zn concentrations were detected at Penmaenmawr and Llandulas, and decreased concentrations of Mg, K and Rb were observed at the 3 most westerly sites, Llandulas, Penmaenmawr and Benllech Beach.

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

Using CV-LDFA, 71.0% of juvenile plaice were correctly classified back to their nursery ground of origin based on their elemental composition, with classification results ranging from 46.2% for Seascale to 93.3% for Penmaenmawr (Table 3). The first 2 canonical discriminant functions of the CV-LDFA explained 73.2% of the total variance and were based on the differences in Li, K, Mn, Sr and Sn amongst the nursery grounds. Cohen's kappa statistic indicated the chance corrected CV-LDFA classification was 0.66 (±0.1 confidence intervals, CIs) for all elements between sites. Classification results showed that where incorrectly classified, many of the fish were assigned to an adjacent nursery ground (Table 3). For example, for fish collected from Heysham, 2 juvenile plaice were assigned to Seascale and 2 to Cleveleys, both adjacent sites to Heysham. Similarly, 2 juvenile plaice from Cleveleys were assigned to the adjacent site at Heysham. Two sites along the North Wales coast, Llandulas and Benllech Beach, both had 2 juvenile plaice assigned to Penmaenmawr (Table 3). Differences among the 8 nursery grounds can be seen when the first 2 discriminant functions are plotted (Fig. 3).

Graphical separation using the 8 nursery grounds within the first 2 discriminant functions is more apparent in Fig. 3 when the multi-element fingerprints of the 107 juveniles sampled were separated by region, with sites sampled from NWW becoming distinguishable from those juvenile fish sampled from NWE. CV-LDFA results indicated high classification accuracy of juvenile *P. platessa*, with 82.2% (NWE: 53/63; NWW: 35/44) of cases correctly assigned to their regional location of capture for the NWE and NWW (Fig. 3). Cohen's kappa statistic indicated the CV-LDFA classification was 0.64 (±0.1 CI) for all elements between regional boundaries.

DISCUSSION

The use of otolith microchemistry in the present study allowed for the accurate classification of an inshore population of juvenile plaice collected from 8 nursery grounds along the north-western coast of England and Wales. Using a multi-element approach (Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn and Ba), significant differences were found among sites, indicating the potential use of these natural tags in distinguishing between individual nursery grounds for a coastal marine species (Rooker et al. 2001b, Forrester & Swearer 2002, Brown 2006b). Similarly, using a multi-element approach (11 elements; Table 4), Geffen et al. (2003) reported high classification success for post-juvenile plaice collected from 5 sites in the

eastern Irish Sea, with their results revealing separation between groups of plaice that related to previously identified spawning grounds within the Irish Sea (Dunn & Pawson 2002). In general, otolith microchemistry in flatfishes has been very successful at identifying both individual fish back to site and between sites over differing geographical ranges, i.e. 10s to 100s of km (see Table 4). Furthermore, the results attained during this study are comparable with classification rates observed in similar otolith microchemistry studies in flatfish (range 70–92%, see Table 4) over a similar spatial scale (100s of km, see Table 4).

A multi-element approach in discriminating between populations in different geographical locations has been regularly used in fishes (see Table 4). However, otolith microchemistry studies in fishes have adopted 2 approaches, where the discriminant function analysis used to classify fish back to source has used all measured elements or has selected a reduced set of elements which were found to be statistically significant in discriminating between areas. A comparison between these 2 analytical approaches was conducted by Vasconcelos et al. (2007), who obtained high classification accuracies using a multi-element approach (Li, Na, Mg, K, Mn, Cu, Zn, Sr, Ba and Pb) that allowed discrimination between populations (Table 4). However, reducing the set of elements in their discriminant analysis failed to improve classification success, and Vasconcelos et al. (2007) concluded that the best outcome was to use the larger dataset in the discrimination model. Adopting a similar analytical approach, the data from the present study were re-analysed to determine whether classification success could be improved by analysing a reduced set of statistically significant elements (in our case; Li, K, Mn, Sr, Sn). However, we also found no improvement in our classification success (CV-LDFA: 65.4%) compared to our initial analysis using all 10 elements, which provided the most accurate discrimination among 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 being 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 Li (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 other non-marine environments (Leakey et al. 2009). Additionally, Mn may be particularly useful in discriminating flatfish habitats due to the nature of their benthic lifestyle and their close proximity to the sediment. The resuspension of those sediments via

bioturbation (Geffen et al. 2003) and the heavy metals associated with them may allow benthic fluxes of Mn to be reflected in their otolith chemistry (Leakey et al. 2009).

One of the main obstacles found to limit the use of otolith microchemistry to identify movement patterns in marine fish appears to be the homogeneous distribution of the more reliably identified elements (Sturrock et al. 2012). However, the use of a larger suite of elements such as Na, Mg, K, Zn, Rb, Sr and Sn and those elements deemed likely to prove reliable geographical markers, such as Li, Mn and Ba (Sturrock et al. 2012), may increase the complexity of the otolith elemental signature and extend the scope of those spatially explicit low-level elements to allow for better classification results for fish sampled from marine environments (Geffen et al. 2003, Vasconcelos et al. 2007, Leakey et al. 2009, Sturrock et al. 2012, this study). This was apparent when looking at marine studies conducted within close proximity of each other (≤500 km, Table 4), where a larger set of elements (between 5 and 11) was necessary to discriminate between sampling locations compared to studies conducted over larger geographical ranges (>500 km), where 4 to 6 elements were used. However, caution must be taken in using the elements just described in future studies as primary drivers and should only be used in the context of the results for individual sites where all elements measured from natural and anthropogenic inputs have been taken into account.

As analytical costs decrease the application of a multi-tag approach, using a combination of trace elements and stable isotopes to observe movement patterns and assign origin of fish over geologically diverse environments is becoming increasingly 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) have also adopted 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).

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 behaviour 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 and November, they return to shallower depths the following spring (Wennhage et al. 2007). In addition, Riou et al. (2001) showed that 1-group plaice 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 yr 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 Anglesey) suggest that both 1-group (present study) and 2/3-group plaice (Geffen et al. 2003) move little from their chosen sites. However, if juvenile place were found to move, evidence would suggest they move to sites which are in close proximity of each other, e.g. within a chosen region, and have similar geologies and therefore similar chemical signals, a factor which

seems evident when we take into account the high classification accuracy observed within the regional areas for this study.

Thorrold et al. (1998) stated that in order to identify fish back to source, all source locations need to be sampled. By way of explanation, within the context of the present study, to assess which nursery areas contribute the greatest proportions of juvenile fish to the adult stock requires the sampling of all possible sources of recruits. For the present study, it was not possible to sample all sources of juvenile plaice in the southeast Irish Sea, as it is likely that these are not known. In addition, licensing conditions restricted how many sites could be sampled, and accessibility to some sites was difficult (e.g. within Morecambe Bay). However, fish were sampled from the major nursery grounds identified by previous studies (Dunn & Pawson 2002, Fox et al. 2007, Ellis et al. 2012) which are likely to produce the majority of recruits for the putative south-eastern Irish Sea stock. It is possible that plaice larvae derived from spawning grounds in the western Irish Sea may be transported onto nursery grounds in the eastern Irish Sea (Fox et al. 2009). However, we targeted 1-group plaice in our study to ensure that the dominant chemical signal measured in the otolith would be derived from the residency period on the nursery ground itself and any signal derived from the mother or the pelagic larval phase would be significantly diluted.

Determining the connectivity between juvenile nursery grounds is critical if we are to understand recruitment patterns and the relative importance of different nursery grounds to the adult stocks (see review by Gillanders et al. 2003). The use of a multi-elemental otolith tag in the present study suggests that it may be possible to identify adults to nursery ground or region of origin by looking at the juvenile portion of the adult otoliths (Forrester & Swearer 2002, Cuveliers et al. 2010). Given the relative sizes of the otoliths derived from juvenile and adult plaice, it is likely that solution-based ICP-MS would be used on juvenile otoliths whilst laser ablation ICP-MS would be used to assess the otolith core of adults. The former approach would be used to obtain an integrated 'signature' for the juvenile, whereas the latter would be used to derive the juvenile 'signature' for that fish. However, one must be cautious when using 2 different analytical techniques to determine otolith elemental concentrations, as both methods will vary in their sensitivity and detection limits (see Campana 1999, de Pontual et al. 2000, Ludsin et al. 2006), which may affect which elements are available for inclusion in the discriminant analysis.

The understanding of a stock's structure, ecology and, more importantly, the exchange rates between spatially separated sub-populations of both juvenile fish and adults is essential for future management programmes if we are to continue sustainable fishing (Tanner et al. 2012). To effectively manage a species, a clear understanding of habitat importance and therefore its productivity in maintaining the population has to be identified (Chittaro et al. 2009). The use of otolith microchemistry has helped in classifying juvenile plaice to individual nursery grounds for this study and possibly identifying a regional split hitherto unknown. Although the role of dispersal in marine population dynamics is still incomplete (Cook 2011), the use of natural chemical tags has enabled researchers to quantify these movements. Furthermore, the use of established baselines based on the elemental chemistry of these otoliths would further the understanding of movement and connectivity between nursery grounds. In doing so, future assessments of those nursery grounds combined with changes over temporal scales may assist in the understanding of their relative importance to adult stocks and assist in the prioritization of management and conservation of the more productive nursery grounds.

The site fidelity observed in juvenile plaice suggests that they are likely to experience the same physical and biological conditions since settlement, and this, combined with their

natural homing trait (Burrows et al. 2004), makes them an ideal model to study inter-annual variability (i.e. temporal stability) of the elemental 'tag' for local nursery grounds using otolith microchemistry. A recent study using otoliths extracted from juvenile plaice collected from 2 sites in north Wales found that the elemental concentrations of Mg, Na, K, Sr and Ba varied little over an inter-annual (3 to 4 yr) period (Marriott 2014), further strengthening the use of plaice as a study species to assess elemental changes over temporal scales.

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

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Fig. 1. Geographical locations of the 8 juvenile European plaice *Pleuronectes platessa* nursery grounds (recognised by Dunn & Pawson 2002) along the north-west coasts of England and north Wales sampled during the present study

Fig. 2. Ten elements measured ($\mu g\ g^{-1}$) in otoliths of juvenile European plaice *Pleuronectes platessa* collected from the 8 nursery grounds located in the south-eastern Irish Sea (see Fig. 1). Nursery grounds are defined as Ss: Seascale (n = 13 fish sampled), 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)

Fig. 3. Allocation of juvenile European 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 1. MANOVA results (*F*-values) of comparisons of mean element:Ca ratios (Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn, Ba) in the otoliths of juvenile European 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	**	**	**	**	**	**	**	

Table 2. ANOVA results (*F*-values) for comparisons of elemental concentrations in the otoliths of juvenile European plaice *Pleuronectes platessa* from the 8 nursery grounds sampled in the eastern Irish Sea. Post hoc pairs indicate the number of pairs of sites (out of a total of 28 pairs) which showed significant differences ($p < 0.05$) in element concentrations using Bonferroni post hoc comparisons. Sites that significantly differ from others are proceeded by >, sites in **bold** indicate a significant difference at $p < 0.001$. Site codes (Ss, He, Cl, As, Hl, Lld, Pen and BB) are defined in Fig. 2

Element	Site effect $F_{7, 99}$ =	p	Post hoc pairs	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

Table 3. Allocation of juvenile European plaice *Pleuronectes platessa* among nursery grounds by 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 fish correctly classified to their nursery ground of capture, with percentage correct in parentheses. Total n = number of individuals analysed with total percentage of correctly classified fish in parentheses. Shaded panels indicate adjacent sites to the original site of capture to which fish were attributed

	Predicted nursery ground						
	Seascale	Heysham	Cleveleys	Ainsdale on Sea	Hoylake	Llandulas	Penm
Actual nursery ground							
Seascale	**6** (**46.2**%)	0	2	2	0	0	
Heysham	2	**8** (**53.3**%)	2	0	0	3	
Cleveleys	2	2	**10** (**66.7**%)	0	0	0	
Ainsdale on Sea	1	0	0	**13** (**92.9**%)	0	0	
Hoylake	1	0	1	0	**4** (**66.7**%)	0	
Llandulas	0	2	0	0	0	**11** (**73.3**%)	
Penmaenmawr	0	1	0	0	0	0	**14** (
Benllech Beach	0	0	2	0	0	0	

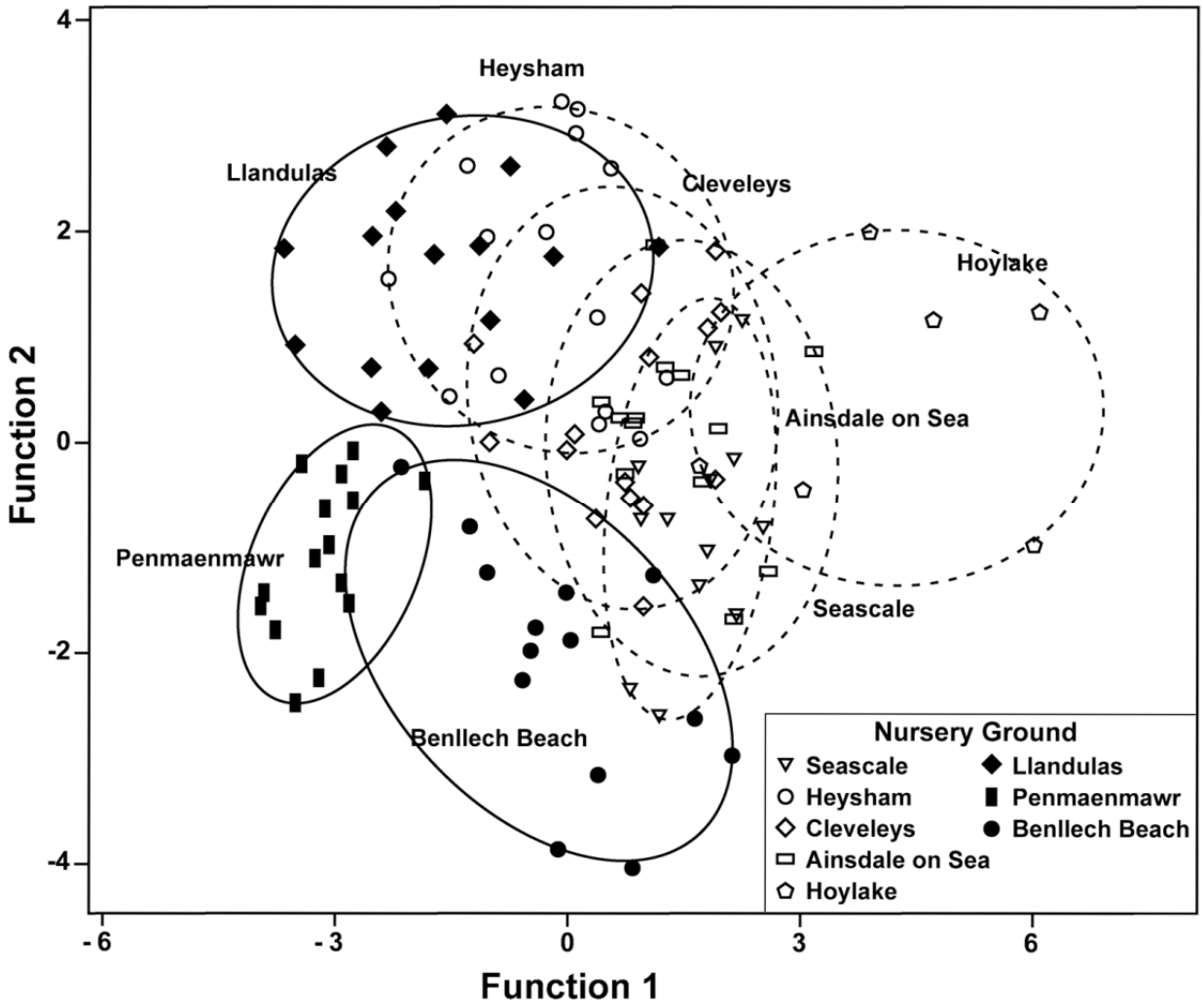

Fig. 3. Allocation of juvenile European plaice Pleuronectes platessa to their sampling sites based on linear discriminant function analysis observed in Table 3 using the elements Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn and Ba

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: optical/atomic emission spectrometry, LA: laser ablation, sb = solution based

Water	No. Sites	Distance (km)	Elements measured	Tested in DFA	Significant elements	Species	Classification to site (%)	ICP-MS	Author(s)
Fresh	8	100[a]	Na, K, Mg, Mn, Sr, Ba	K, Mg, Mn, Sr, Ba	K, Mn, Sr, Ba	*Perca flavescens*	62–100	sb and AES	Brazner et al. (2004)
Fresh	4	130	Mg, Mn, Sr, Ba	All	Mg, Mn, Sr, Ba	*Salmo salar*	84–100	LA	Veinott & Porter (2005)
Fresh	4	170	Mg, Mn, Zn, Sr, Ba	All	Mg, Mn, Zn, Sr, Ba	*Salmo trutta*	95–97	LA	Veinott et al. (2012)
Fresh	9	600	Mg, Mn, Zn, Sr, Ba	All	Mn, Ba	*Oncorhynchus mykiss*	91–96	LA	Veinott & Porter (2013)
Estuarine	2 2	200	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	[a]	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	500	Li, Mg, Mn, Cu, Sr, Ba, Pb	All	Mg, Mn[b] Mg, Ba[b]	*Solea solea,* *S. senegalensis*	71–81	LA	Tanner et al. (2012)
Est-Coast	9	165[a]	Mn, Cu, Sr, Ba, Pb	Cu	Cu	*Paralichthys californicus*	76 and 86	sb	Forrester & Swearer (2002)
Est-Coast	9	[a]	Mn, Cu, Sr, Ba, Pb	Pb	Pb	*Paralichthys californicus*	68 and 87	sb	Forrester & Swearer (2002)
Est-Coast	9	[a]	Mn, Cu, Sr, Ba, Pb	Cu, Pb	Cu, Pb	*Paralichthys californicus*	81 and 84	sb	Forrester & Swearer (2002)
Est-Coast	18	500	Li, Mn, Sr, Ba	All	Li, Sr[c]	*Pleuronectes vetulus*	73–87	sb	Brown (2006b)
Est-Coast	18	[a]	Li, Mn, Sr, Ba	All	Sr[c]	*Citharichthys stigmaeus*	58–89	sb	Brown (2006b)
Est-Coast	10-10	300	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	“a”	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	“a”	Sc, Ba, Rb, Mn, Li	All	Li, Sc, Mn, Rb	*Dicentrarchus labrax*	100	sb	Leakey et al. (2009)
Est-Coast	17	5000[a]	Li, Ca, Mn, Sr, Ba	All	Ba	*Polydactylus macrochir*	Various	LA	Moore & Simpfendorfer (2014)
Marine	3	1000[a]	Li, Mg, Mn, Ca, Sr, Ba	All	Li, Mg, Mn	*Thunnus orientalis*	75 and 100	sb	Rooker et al. (2001b)
Marine	5	7000[a]	Li, Mg, Mn, Ca, Sr, Ba	All	Li, Mg, Mn, Sr	*Thunnus thynnus*	62–80	sb	Rooker et al. (2003)
Marine	5	100[a]	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	500	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	“a”	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	“a”	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	“a”	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	“a”	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	300[a]	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	200	Mg, Mn, Zn, Sr, Ba, Ce, Pb	All	Mg, Zn, Sr, Ba, Ce, Pb[d]	*Stegastes partitus*	52–99	LA	Chittaro & Hogan (2013)
Marine	4	200	Mg, Mn, Sr, Ba, Pb	All	Mn, Ba	*Merluccius productus*	59–88	LA	Chittaro et al. (2013)
Marine	4	1100	Mg, Mn, Sr, Ba	All	Sr, Ba	*Gadus morhua*	66–78	LA	D’Avignon & Rose (2013)
Marine	8	200	Li, Na, Mg, K, Mn, Zn, Rb, Sr, Sn, Ba	All	Li, K, Mn, Sr, Sn	*Pleuronectes platessa*	46–93	sb	This study

[a]Distances are approximate linear measurements and are taken from the 2 farthest sampling locations
[b]Data taken from the inter-annual variability observed from the 1st and 2nd canonical variations for both species
[c]Data taken from the region-reduced model for both species
[d]Data taken from the region-wide scale model