



Otolith microchemistry to investigate nursery site fidelity and connectivity of juvenile European sea bass in Ireland

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ABSTRACT: Knowledge of the role that nurseries play in the growth and survival of juvenile European sea bass *Dicentrarchus labrax* is essential to provide information for sustainable stock management. To examine *D. labrax* nursery fidelity, multi-annual geochemical tags from the otoliths of 385 juveniles (0-group, 1-group and 2-group) were collected in 4 estuaries along the south coast of Ireland, close to the northern limit of its range. This study identified high between-estuary discrimination of 0-group otolith geochemical tags over multiple years. It also confirmed that over 91% of captured 1-group *D. labrax* remain close to their original settlement sites irrespective of sampling year. Although the capture in individual estuaries of 2-group *D. labrax* was less common, otolith geochemical tags indicated >95% fidelity to their original settlement sites. Where migration between estuaries was inferred, it was restricted to an adjacent estuary (<50 km coastal distance). The consistency of the multi-annual geochemical tag signatures observed will enable assignment of nursery origin to adult *D. labrax* which disperse around the coast of Ireland and ultimately allow the identification of estuary nursery sites that contribute most to the adult stock.

KEY WORDS: Geochemical tags · Juvenile European sea bass · Estuary nursery fidelity · Laser ablation · Fisheries management

1. INTRODUCTION

Estuaries provide food and shelter to large numbers of juvenile marine fish species for periods ranging from a few months to a number of years (Beck et al. 2001, Able 2005). These highly productive habitats provide nursery areas where young of the year (0-group) juveniles can settle and grow until they are ready to recruit to adult stocks. The importance of a nursery to a particular species should be defined not only by the densities of 0-group juveniles but also by the ability of these habitats within estuaries to support the continued growth of juveniles into their later years as they recruit to the adult stock (Beck et al. 2001, Dahlgren et al. 2006, Glass et al. 2008).

Knowledge gaps exist regarding juvenile fish movement across nursery sites as they mature (Able et al. 2012, Green et al. 2012). A better understanding of how long juvenile fish display fidelity to their original settlement site is also necessary to assess the importance of particular intra-estuarine habitats for recruitment to the adult stock (Beck et al. 2001, Able 2005). If juveniles display high site fidelity over a number of years, it can have implications for habitat conservation (Reis-Santos et al. 2015). Large estuaries tend to be close to urban areas and as a result are often threatened by extensive habitat loss and degradation due to widespread anthropogenic pressures (Kennish 2002, Vasconcelos et al. 2007, Courrat et al. 2009). If it is demonstrated that economically and

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socially valuable fish species require well-functioning estuaries to replenish adult stocks, legislative protections may be easier to develop locally and implementing existing protections, sometimes unpopular, may be more acceptable.

The European sea bass *Dicentrarchus labrax* is a demersal species found throughout the Mediterranean Sea and Eastern Atlantic. An essential stage in its early development is larval settlement within estuarine nurseries (Kennedy & Fitzmaurice 1972, Jennings & Pawson 1992, Pickett et al. 2004, Beraud et al. 2018) prior to recruitment to the adult stock. *D. labrax* stocks are susceptible to highly variable recruitment years (Pickett & Pawson 1994, Henderson & Corps 1997, Pickett et al. 2004) where high settlement rates have been correlated with warm summers (Holmes & Henderson 1990, Pickett & Pawson 1994). As the *D. labrax* population in Irish coastal waters is near the northern limits of the species' range, summer temperatures are likely a major driver of settlement success within Irish estuaries (Kelley 1988, Jennings & Pawson 1992, Pickett et al. 2004). Early research on *D. labrax* recruitment in Ireland identified the occurrence of poor and good growing years, which also appeared to be related to variations in annual temperatures (Kennedy & Fitzmaurice 1972). Evidence from tagging (Pawson et al. 2007) and genetic (Fritsch et al. 2007, Souche et al. 2015) studies suggest that *D. labrax* around Irish coastal waters can be considered a discrete stock component for management purposes. More recent work has also identified spatially structured populations around Europe (de Pontual et al. 2019).

A low frequency of occurrence of 0-group *D. labrax* in Irish waters has been documented (Fahy et al. 2000), so the importance of well-functioning *D. labrax* nurseries to sustain the Irish stock cannot be overstated. As *D. labrax* prefer warmer temperatures, it is not surprising that 0-group specimens are consistently encountered in only a small number of suitable estuaries along the south coast of the country (Kennedy & Fitzmaurice 1972, Fahy et al. 2000, Connor et al. 2019). Therefore, it is thought that most of the *D. labrax* nurseries which contribute to the Irish stock are in these southern estuaries. If these nurseries are to be protected with effective legislation, more knowledge must be gathered to confirm their importance for settlement and continued growth of juvenile *D. labrax* prior to recruitment to the adult stock.

Tagging studies have found that *D. labrax* <32 cm (i.e. pre-adult) are generally recaptured relatively close to their respective tagging areas (Pickett et al.

2004, Pawson et al. 2007), and it is thought that the juveniles remain in their original nursery estuaries until they are ready to recruit to the adult stock (Pawson et al. 1987, Henderson & Corps 1997). However, artificial tagging methods are usually not applicable for small juvenile fish (Gillanders 2002a, Gillanders et al. 2003), and since juvenile fish mortality rates are high, their recapture rates tend to be very low (Gillanders 2002a). As a result, very little is known about movement patterns of juvenile *D. labrax* over their first 2 yr of life.

An alternative tool for examining connectivity among sites and movement patterns of juvenile fish is the analysis of naturally occurring variations in element concentrations of fish otoliths (Kraus & Secor 2005, Elsdon et al. 2008, Vasconcelos et al. 2008, Reis-Santos et al. 2012, Walther & Limburg 2012, Chang & Geffen 2013, Curtis et al. 2014, Avigliano et al. 2018). Fish otoliths are metabolically inert and grow continuously over the lifetime of the fish. In the process, trace elements from the environment are incorporated into the calcium carbonate matrix of the otolith, where their concentrations are permanently retained (Kalish 1991, Campana 1999, Campana & Thorrold 2001, Veinott & Porter 2005, Higgins et al. 2013). As a result, sections in the otolith of a fish which relate to specific periods during its life can be analysed to investigate its environmental history. To date, this approach has allowed examination of stock discrimination (Thorrold et al. 1998, Rooker et al. 2003, Zitek et al. 2010) and nursery origin in adult fish (Elsdon et al. 2008, Vasconcelos et al. 2008, Reis-Santos et al. 2013, Avigliano et al. 2018) as well as investigating connectivity during juvenile life stages in several exploited species (Reis-Santos et al. 2015, Delerue-Ricard et al. 2019, Toledo et al. 2019, Bounket et al. 2021). Prior to using otolith microchemistry to examine fish movement, it must be established that the otoliths produce nursery-specific geochemical tags, preferably at multi-annual temporal scales (Vasconcelos et al. 2008, Reis-Santos et al. 2012, Tournois et al. 2013, Delerue-Ricard et al. 2019). Juvenile *D. labrax* are thought to exhibit a high level of site fidelity in their early life (Pawson et al. 1987, Henderson & Corps 1997).

In this context, otolith microchemistry was used to investigate nursery site fidelity and connectivity of juvenile *D. labrax* in Ireland. The main aims of this study were to investigate (1) if the estuaries which have been identified as likely to be the most productive *D. labrax* nurseries in Ireland (Watson et al. 2024) have discrete and stable otolith geochemical tags over multiple sampling years and (2) if otolith geochemical tags for the 1- and 2-group *D. labrax*

caught in these estuaries assign to their local nursery site.

2. MATERIALS AND METHODS

2.1. Sampling design

Four estuaries around the South coast of Ireland (Slaney, Barrow, Blackwater and Tralee) (Fig. 1) were selected for this study. Monitoring of fish populations in Irish estuaries has been ongoing since 2008 under the Water Framework Directive (WFD), and it has been established from this and an annual Bass Monitoring Programme, ongoing since 2014, that 0-group European sea bass *Dicentrarchus labrax* encounters are uncommon outside these estuaries (Connor et al. 2019). This pattern is also consistent with previous work (Kennedy & Fitzmaurice 1972, Fahy et al. 2000).

To establish year-specific otolith geochemical signature baselines for *D. labrax* nurseries, each study estuary was sampled over 3 consecutive years (2015–2017). These estuaries are likely to produce nursery-specific geochemical tags due to their contrasting physical characteristics. The Tralee study site is within a small estuary (3.1 km²). The river catchment which drains into the estuary is small (99 km²), and the study site is close to the estuary mouth (0.9 km). This site is strongly influenced by the marine environment (Table 1). In contrast, the Barrow site is heavily freshwater-influenced as it is located 16.6 km from the estuary mouth, and a large river system (catchment size: 3316 km²) drains through the large, complex estuary environment (Table 1). Salinity measurements taken during sampling at the Barrow site corroborate this assessment (Table 1). The Slaney site is more influenced by the marine environment than the Blackwater site due to its proximity to the estuary mouth and the area of upstream catchment (Table 1). The same estuaries were sampled for older (1- and 2-group) juvenile *D. labrax*

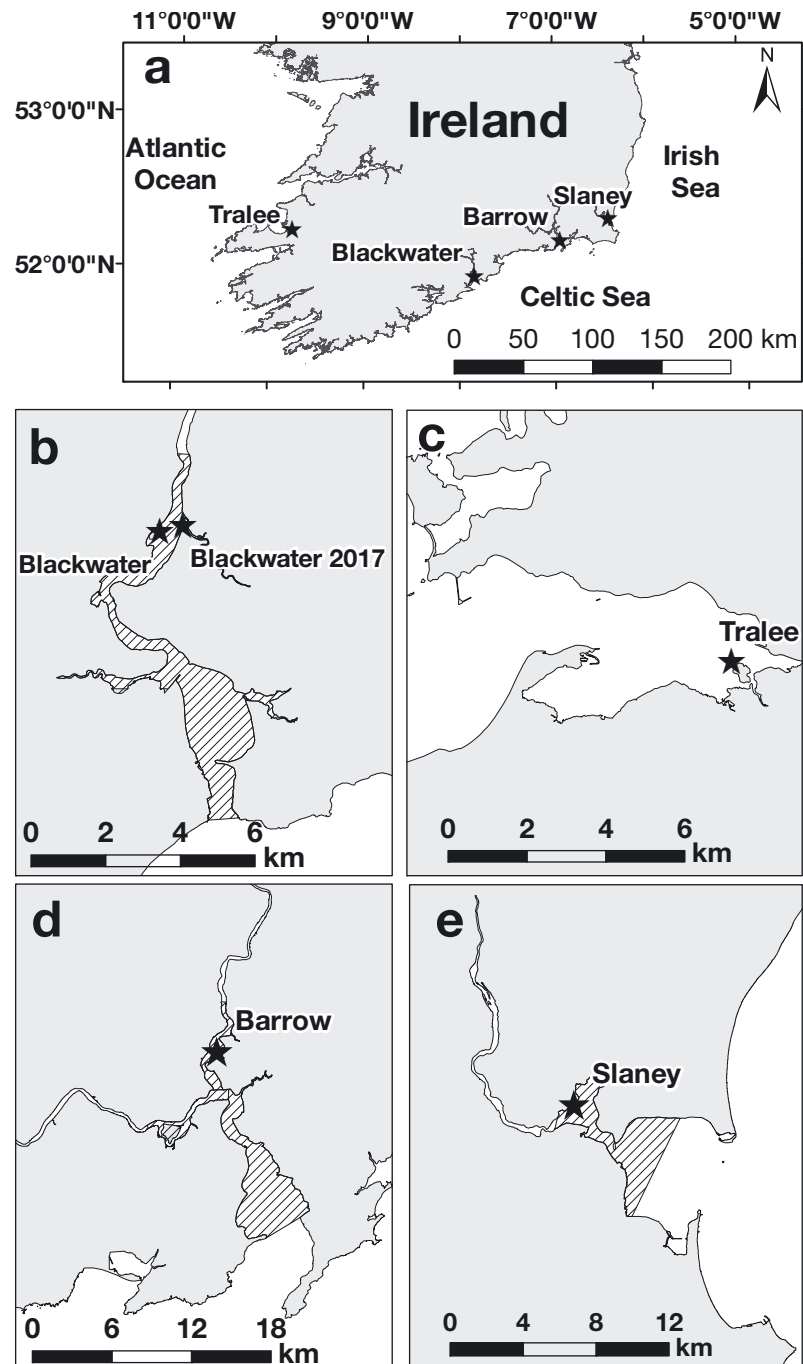


Fig. 1. (a) Southern half of Ireland showing the location of all sampled estuaries for the study. (b–e) Detailed view of each sampled estuary. Each labelled point identifies the juvenile *Dicentrarchus labrax* nursery sampled within each estuary. Sampling took place at the same location each year (2015–2017), with the exception of Blackwater (b), where the opposite bank was sampled in 2017. Hatched sections in (b–e) denote the extent of the trawling footprint used to capture 1 and 2 yr old juvenile *D. labrax* from 2016–2018

over the years 2016–2018, which facilitated investigation of estuary site fidelity during the first 3 yr of life of the local *D. labrax* population.

Table 1. Study site physical characteristics. Salinities and water temperatures (means \pm SD) were recorded continuously during juvenile bass surveys (2016–2018) on high and falling tides. Upstream catchment details were obtained from the EPA Hydrodata Portal (<https://epawebapp.epa.ie/hydronet>)

Site	Distance to mouth (km)	Estuary area (km ²)	Upstream catchment		Salinity (ppt)	Water temp. (°C)
			Area (km ²)	50%ile flow (m ³ s ⁻¹)		
Slaney	6.4	19.2	1746	24.7	23.1 (5.1)	18.9 (0.7)
Barrow	16.6	50.4	5606	55.6	6.1 (2.1)	17.8 (0.2)
Blackwater	9.2	12.8	3316	45.5	17.0 (4.7)	17.4 (0.6)
Tralee	0.9	3.1	99	1.3	20.3 (9.6)	17.3 (3.1)

2.2. Fish sampling

The 0-group and a small number of older juvenile *D. labrax* were captured using a 30 m fine mesh (0.5 cm) beach seine net. Sampling was carried out at specific sites (the Slaney, Barrow, Blackwater and Tralee nurseries) within the 4 estuaries studied. Sampling was conducted between late July and early September over 3 consecutive years (2015–2017). Across all sampling years, except for the 0-group Blackwater cohort in 2017, all 0-group fish were captured at the same site (within 100 m) to limit any potential spatial variation in fish distribution and, consequently, otolith chemistry (Reis-Santos et al. 2015). In 2017, no 0-group fish could be captured within the main Blackwater nursery so additional sampling took place along the opposite bank (Fig. 1b). All captured juvenile *D. labrax* were measured (total length [TL], in cm) onsite and scale samples were taken to determine age at capture (in years). A sample of 0-group (n = 221), 1-group (n = 35) and 2-group (n = 12) fish across all sites and years were retained (Table 2) and transported in a cooler box to the laboratory, where they were frozen at -18°C until dissection. All remaining fish were released at the site of capture.

To sample 1- and 2-group juveniles, a modified herring trawl was deployed from a commercial 10 m stern trawler following techniques developed by the Center for Environment, Fisheries and Aquaculture Science (CEFAS) (Pickett et al. 2002). Between 12 and 16 transect trawls at a speed of 3–5 knots were conducted for 10 min intervals within a 7 km radius of each 0-group sampling site (Fig. 1b–e). Tralee was not trawled due to logistical constraints, as it is particularly shallow. All captured fish were measured (TL, cm) onsite, and scale samples were taken to determine age at capture (in years). A sample of 1-group (n = 104) and 2-group (n = 13) fish were retained for processing (Table 2); all remaining fish were released. Sampling was carried out between late August and early September over 3 consecutive years (2016–2018).

In the Slaney estuary, the 1-group *D. labrax* were captured on average 1.3 km from the 0-group sampling site. In the Barrow and Blackwater estuaries, average distances to the 0-group sampling sites were 2.7 and 1.4 km respectively. All 2-group *D. labrax* retained in the Barrow in 2018 were captured 6 km downstream from the 0-group sampling site. Those from the Slaney in 2017 were caught an average of 0.6 km from the 0-group sampling site (maximum distance: 3.4 km; minimum distance: 0 km). Some

Table 2. Mean total length (TL, in cm) and sample size (n) of all juvenile European sea bass *Dicentrarchus labrax* retained across all study sites and years. Age 0-group fish (n = 221) were used to create site-specific baselines used for analysis; 1-group (n = 139) and 2-group (n = 25) fish were used as mixed stocks for estimating site fidelity during juvenile life

Nursery	Age group	n	TL (\pm SD)
2015			
Barrow	0	2	6.2 (0.5)
Slaney	0	20	6.8 (0.9)
Tralee	0	19	4.7 (0.5)
Blackwater	0	20	4.8 (0.3)
2016			
Barrow	0	20	6.0 (1.2)
Slaney	0	20	8.2 (1.2)
Tralee	0	19	6.8 (1.4)
Blackwater	0	20	5.4 (0.5)
Barrow	1	22	18.5 (2.7)
Slaney	1	18	14.9 (2.9)
2017			
Barrow	0	20	7.5 (1.3)
Slaney	0	20	7.4 (1.0)
Tralee	0	20	8.6 (0.8)
Blackwater	0	21	5.8 (0.9)
Barrow	1	19	18.0 (1.4)
Slaney	1	14	18.5 (1.3)
Blackwater	1	15	15.5 (0.9)
Slaney	2	12	24.2 (1.4)
2018			
Barrow	1	6	23.7 (1.7)
Slaney	1	20	20.9 (1.0)
Blackwater	1	25	17.2 (1.0)
Barrow	2	13	30.2 (2.1)

2-group fish were caught in the Blackwater estuary; however, numbers were not deemed sufficient to retain for otolith microchemical analysis.

Juvenile *D. labrax* abundance at study sites is presented as numbers caught per 100 m² for 0-group fish and numbers caught per 10 min of trawling for older (1- and 2-group) juveniles. To compare observed length-at-age of juvenile *D. labrax* recorded in the 4 estuaries, lengths (TL, cm) of all fish caught (retained and released) at each site were pooled across years. A subsample was aged to validate length-at-age assignments. Univariate analyses (ANOVAs) were performed to investigate differences in growth between sites for each age class, followed by Tukey's post hoc comparison tests. Average lengths were also compared with lower latitude conspecific data available in the literature.

2.3. Otolith preparation and analysis

All laboratory equipment used to extract or prepare otoliths was non-metallic, acid-washed in 10% nitric acid, dried overnight in a laminar flow hood and sealed prior to fish dissection and sample preparation. Sagittal otoliths were extracted from the fish and placed in a Petri dish. The surface of each otolith was cleared of any adhering organic tissue using plastic forceps. Samples were then ultrasonically cleaned for 4 min in trace-element grade 3% hydrogen peroxide and then triple rinsed in 18.2 M Ω deionised water, placed in acid-washed plastic containers and dried overnight in a laminar flow hood prior to being sealed until ready for analysis.

A single sagittal otolith from each fish was randomly chosen for analysis. Each otolith was embedded in a transparent epoxy resin (Struers epofix) using 25 mm disc-shaped moulds. Subsequently, they were cut in a transverse plane, close to the core, using a Buehler Isomet low-speed saw equipped with a diamond blade. A range of silicon-carbide abrasive papers was used to expose the plane containing the otolith core. Finally, individual otolith sections were polished using a polishing cloth with a 3 μ m diamond suspension. A final sonication was carried out to remove any surface con-

tamination before storage in dust-free conditions until analysis.

Otolith elemental concentrations of ⁴³Ca, ²³Na, ²⁴Mg, ³⁹K, ⁵⁵Mn, ⁸⁸Sr and ¹³⁸Ba were analysed with a NWR-213 New Wave Research laser ablation system coupled to a Perkin Elmer DRC-e inductively coupled plasma mass spectrometer (LA-ICP-MS). Otolith sections were analysed in batches of 7, all randomly selected to remove any bias related to potential machine drift. The laser was fired at a rate of 10 Hz, with a spot size of 25 μ m and a moving speed of 10 μ m s⁻¹. Laser energy during the analyses was 10–12 J cm⁻¹. A rectangular raster ablation (approximately 2000 μ m²) was carried out on the ventral edge for each 0-group otolith to obtain the average trace elemental concentrations of the otolith section formed at or close to the final site of capture (Fig. 2a). A raster ablation was made in the otolith corresponding to the first summer's growth for 1- and 2-group fish (Fig. 2b). Blanks were measured by pausing laser ablation for 30 s between each otolith analysis. The external standard NIST 612 (synthetic glass trace-element reference standard) was analysed at the start, middle and end of every batch of otolith samples. Microanalytical, carbonate standard MACS-

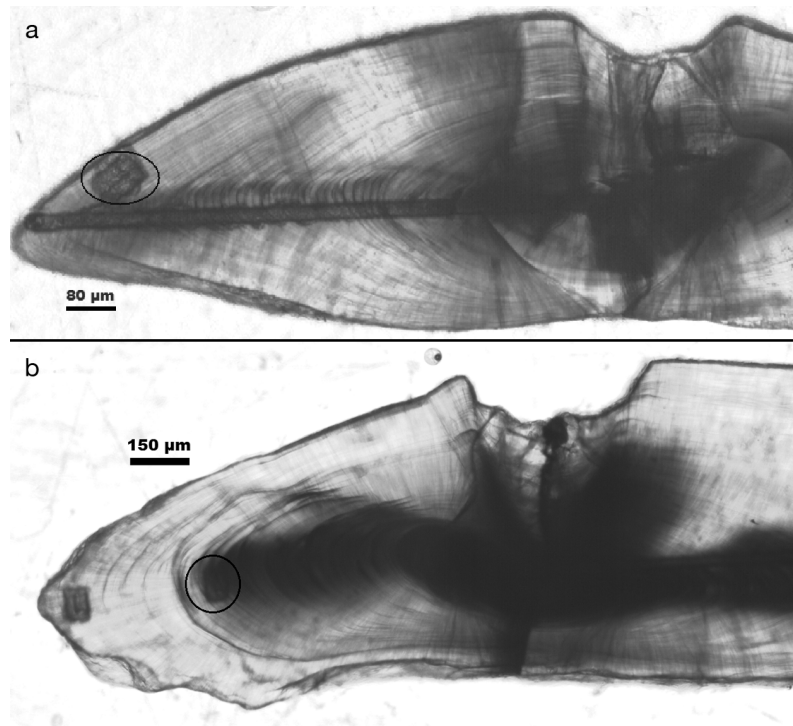


Fig. 2. Sample images of *Dicentrarchus labrax* (a) 0-group otolith illustrating an ablation raster (circled) used to collect geochemical tag data corresponding to the location of capture and (b) 1-group otolith illustrating location of an ablation raster (circled) used to collect geochemical tag data corresponding to its area of residency during the first summer of growth

3 (United States Geological Survey) was also analysed at the start and end of each otolith batch to assess external precision (relative standard deviation (%): Na = 7.2, Mg = 6.2, K = 18.2, Mn = 4.4, Sr = 7.4 and Ba = 9.5). All elements selected for analyses were consistently above the limits of detection.

All ablated material was swept from the sample chamber with a constant flow of helium carrier gas and mixed with a constant flow of argon prior to entering the ICP-MS. The gas mix was optimised daily to provide the highest possible isotope counts while keeping the rate of oxide formation to an acceptable level ($\leq 1\%$) by monitoring the $\text{ThO}^+:\text{Th}^+$ ratio on NIST 612 (Ulrich et al. 2009).

Raw LA-ICP-MS data were processed using the data reduction software 'Iolite' and accompanying data reduction scheme 'Trace Elements' (Paton et al. 2011). Ca^{43} was used as the internal standard to correct for variations in ablation yield during analysis. All elements were reported in parts per million of aragonite and subsequently converted to molar concentrations and standardised to calcium (Element: Ca). All otolith elemental data were \log_{10} transformed to meet the assumptions of normality (Anderson-Darling test) and homoscedasticity (Levene's test) required for the statistical analyses described below.

2.4. Data analyses

2.4.1. Establishing 0-group nursery baselines

Multivariate analyses (MANOVAs) were performed for each sampling year on the multi-elemental geochemical tags of all 0-group fish (Table 2) to investigate differences in *D. labrax* otolith chemistry. Univariate analyses (ANOVAs) were also performed to investigate otolith elemental variation between sites and years followed by Tukey's post hoc comparison tests on all elements displaying significant differences.

To further characterise spatial differences in otolith geochemical tags for 0-group *D. labrax*, a stepwise linear discriminant function analysis (L DFA) procedure (Vasconcelos et al. 2007, Mercier et al. 2011, Jones et al. 2017) was applied, grouping individual geochemical tags according to estuary nursery site. Sampling years were analysed separately. To test the robustness of estuary-specific geochemical tags over multiple years, all data across years was also pooled and analysed. To visualise results, the first 2 discriminant functions were plotted in discriminant space, and 90% confidence ellipses were drawn

around each group. Finally, the validity of each annual model was assessed by means of a leave-one-out cross-validation procedure. For this approach, samples are removed sequentially from the original data set prior to calculating the discriminant functions and testing their ability to successfully assign 'unknown' geochemical tags to the correct capture site (Venables & Ripley 2002, R Core Team 2022).

2.4.2. Assessing connectivity of juvenile stages between sites

To examine potential fidelity to recruitment sites in older juveniles, all 1- and 2-group *D. labrax* were grouped according to their age class, year of capture and estuary of capture (Table 2). The proportion of each mixed stock which could be assigned back to a nursery of origin was assessed by evaluating them against their corresponding year-specific baselines as described above. Following Reis-Santos et al. (2013) and Avigliano et al. (2018), a maximum likelihood estimation approach (MLE) (Millar 1987, 1990a) was performed with the program 'HISEA' (Millar 1990b). This programme generates MLEs and standard deviations of the proportion of each mixed stock originating from each characterised baseline group. Bootstrapping with a 1000 resampled baseline was used. To assess how the HISEA procedure performed over multiple years, all otolith geochemical tag data across years was pooled prior to analysis

3. RESULTS

3.1. Average length and density of Irish juvenile European sea bass

Significant differences in length over the 3 sampling years combined were found between 0-group European sea bass *Dicentrarchus labrax* captured at the study sites ($F_{3,2694} = 245.2$, $p < 0.001$) (Table 3). The 0-group *D. labrax* caught at the Blackwater site were significantly smaller (Tukey's test, $p < 0.001$) than those captured at all other sites (Table 3). The 0-group fish caught in the Slaney were significantly longer ($p < 0.001$) than those caught at the Tralee sampling site. Although 0-group fish caught at the Slaney site were, on average, longer than those caught at the Barrow site over the 3 sampling years (Table 3), this difference was not significant. No significant differences in length were found between 0-group fish caught at the Tralee and Barrow sites

Table 3. Body size (total length [TL], in cm) and density of all the 0-group *Dicentrarchus labrax* caught in the present study over all sampling years. Examples of 0-group *D. labrax* body sizes from other studies at different latitudes also presented. (–) No data

Country	Waterbody	Month	Sampling year(s)	Geographic location (°)	n	TL (cm)		Density (no. per 100 m ²)	Reference
						Mean (±SD)	Range		
Ireland	Slaney	Aug	2015–2017	52.36, –6.48	2340	7.4 (1.2)	4.1–12.0	7.1	This study
Ireland	Barrow	Aug	2015–2017	52.29, –7.01	83	7.1 (1.5)	4.0–10.5	3.7	This study
Ireland	Blackwater	Aug	2015–2017	51.99, –7.88	197	5.0 (1.7)	3.0–7.2	1.1	This study
Ireland	Tralee	Aug	2015–2017	52.26, –9.77	78	6.7 (1.7)	4.1–10.4	0.4	This study
Ireland	Slaney	Aug	1999	52.36, –6.48	63	4.7 (1.1)	3.5–7.5	–	Fahy et al. (2000)
Ireland	Slaney	Aug	1996	52.36, –6.48	110	7.5 (0.8)	3.5–9.0	–	Fahy et al. (2000)
UK	Solent	Sept	2000	50.83, –1.82	–	–	2.0–7.0	–	Pickett et al. (2002)
Portugal	Ria de Aveiro	July	2005	40.64, –8.76	30	–	5.4–9.0	–	Vinagre et al. (2009)
Portugal	Mondego	July	2005	40.14, –8.83	33	–	5.1–8.5	–	Vinagre et al. (2009)
Portugal	Tagus	July	2005	38.87, –9.03	40	–	4.8–9.0	–	Vinagre et al. (2009)
Portugal	Mira	July	2005	37.72, –8.79	31	–	5.3–8.5	–	Vinagre et al. (2009)
France	Mont St Michel	Sept	1998	48.64, –1.50	62	6.4 (–)	4.3–8.4	–	Laffaille et al. (2001)
France	Mont St Michel	Sept	1997	48.64, –1.50	165	7.4 (–)	5.1–10.2	–	Laffaille et al. (2001)

(Table 3). The size ranges observed were comparable to those from an older study from the Slaney estuary (Fahy et al. 2000) and the UK (Pickett et al. 2002). Although available data is limited, there is no clear difference in sizes of 0-group *D. labrax* between those individuals at lower latitudes caught in July (Vinagre et al. 2009) and September (Laffaille et al. 2001) and those sampled during the course of this study (Table 3). *D. labrax* reported from the Slaney from 1996 and 1999 were of a smaller size range than those we encountered in the Slaney during 2015–2018, and the maximum values were 3 cm less than the maximum measured in this study (Table 3). On average, 0-group fish were more abundant at the Slaney site than at the other sampling sites (Table 3).

The average length (TL) of 1-group *D. labrax* caught during 2016–2018 also depended on estuary of capture ($F_{2,515} = 252.6$, $p < 0.001$). Fish caught in the Blackwater estuary ($n = 144$, $TL = 17.3 \pm 1.0$ cm) remained significantly smaller ($p < 0.001$) than fish caught in the Barrow ($n = 256$, $TL = 21.5 \pm 2.4$ cm) and the Slaney ($n = 118$, $TL = 21.1 \pm 1.5$ cm). No significant difference in length was observed between

1-group fish caught in the Barrow or the Slaney, and 1-group fish were present in the highest density in the Barrow estuary (Table 4).

The 2-group fish also exhibited significant differences in length between estuary of capture over both sampling years combined (2017–2018) ($F_{2,278} = 174.4$, $p < 0.001$). These fish were, on average, longer in the Barrow ($n = 165$, $TL = 28.4 \pm 2.6$ cm) than the Slaney ($n = 43$, $TL = 27.8 \pm 2.0$ cm), although this difference was not significant. The 2-group fish caught in the Blackwater were significantly smaller ($n = 73$, $TL = 22.5 \pm 1.4$ cm, $p < 0.001$) than those caught in the other 2 sampled estuaries and were present in the highest density in the Barrow estuary (Table 4).

3.2. Nursery discrimination success using geochemical tags

Otolith element:Ca ratios of baseline samples from all sampling sites across all sampling years are provided in Table 5. Year-specific differences in otolith geochemical tags of *D. labrax* were highly significant

Table 4. Body size (total length [TL], in cm) and density of all 1- and 2-group *Dicentrarchus labrax* caught in the present study from trawling surveys over all sampling years

Waterbody	Sampling years	Juvenile age					
		1-group		2-group			
		n	TL (±SD)	No. bass per 10 min trawled	n	TL (±SD)	No. bass per 10 min trawled
Barrow	2016–2018	256	21.5 (2.4)	4.4	165	28.4 (2.6)	2.8
Slaney	2017–2018	118	21.1 (1.5)	3.9	43	27.8 (2.0)	1.4
Blackwater	2016–2018	144	17.3 (1.0)	2.8	73	22.5 (1.4)	1.4

Table 5. Mean (\pm SD) of elemental ratios in cores of all young of year juvenile *Dicentrarchus labrax* for each nursery across all sampling years. All ratios are in $\mu\text{mol}:\text{mol}$, except for Na:Ca and Sr:Ca ($\text{mmol}:\text{mol}$)

Nursery	n	Year	Na:Ca	Mg:Ca	K:Ca	Mn:Ca	Sr:Ca	Ba:Ca
Barrow	2	2015	16.14 (0.03)	172.55 (2.62)	1850.24 (90.6)	22.8 (12.36)	1.35 (0)	5.66 (0.96)
Barrow	20	2016	16.66 (1.06)	187.11 (53.05)	1458.92 (607.63)	16.93 (4.79)	1.36 (0.18)	5.55 (1.62)
Barrow	20	2017	18.28 (1.67)	177.83 (31.1)	1789.63 (323.35)	18.21 (5.84)	1.14 (0.27)	6.22 (2.79)
Tralee	19	2015	16.52 (1.36)	153.77 (36.72)	1576.84 (326.04)	29.46 (6.97)	1.53 (0.11)	1.04 (0.18)
Tralee	19	2016	17.05 (1.4)	154.53 (33.56)	1494.16 (481.84)	31.56 (7.05)	1.77 (0.16)	1.16 (0.24)
Tralee	20	2017	17.14 (1.19)	136.39 (20.95)	1466.86 (189.84)	19.32 (7.14)	1.72 (0.25)	0.97 (0.32)
Blackwater	20	2015	16.47 (1.73)	167.65 (43.26)	1561.81 (313.88)	14.54 (3.78)	1.34 (0.17)	2.07 (0.43)
Blackwater	20	2016	17.67 (1.2)	179.5 (27.98)	1510.43 (565.71)	28.02 (8.68)	1.05 (0.34)	2.58 (0.58)
Blackwater	21	2017	17.98 (1.51)	171.91 (27.55)	1689.28 (283.33)	18.29 (3.84)	1.11 (0.13)	2.52 (0.41)
Slaney	20	2015	16.95 (1.57)	224.9 (74.28)	1468.91 (245.49)	38.91 (11.77)	1.73 (0.11)	2.61 (0.78)
Slaney	20	2016	16.93 (1.15)	169.34 (24.48)	1258.65 (449.69)	27 (9.15)	1.81 (0.14)	2.51 (0.68)
Slaney	20	2017	17.9 (1.96)	191.79 (44.51)	1448.67 (192.24)	35.24 (10.01)	1.74 (0.32)	2.61 (0.68)

(MANOVA; 2015: Wilks' $\lambda = 0.04$, $F_{3,18} = 18.49$, $p < 0.001$; 2016: Wilks' $\lambda = 0.05$, $F_{3,18} = 20.75$, $p < 0.001$; 2017: Wilks' $\lambda = 0.05$, $F_{3,18} = 21.36$, $p < 0.001$) between sampling sites irrespective of sampling year. Univariate analyses (ANOVA, Tukey's post hoc) of all elements examined found significant differences ($p < 0.01$) in otolith elemental concentrations between each 0-group sampling site for all elements examined, except for Na, across the 4 estuary sites for at least one sampling year (Table 6, Fig. 3). Although K exhibited significant variation between sites ($p < 0.001$) in 2017, high within-site variability, as indicated by low F -ratios, implies that the use of this element for site discrimination is limited. Mg exhibited significant variation ($p < 0.001$) between sites in 2 of 3 sampling years (2015 and 2017). Mn, Sr and Ba exhibited far lower within-site variability than other elements analysed (Table 6). Mn concentrations in otoliths displayed significant differences between 2 or 3 estuary pairs depending on sampling year (Table 6). Sr concentrations in otoliths displayed significant differences between 4 or 5 estuary pairs depending on sampling year (Table 6). Ba concentrations displayed both the highest between-estuary variability (5 out of 6 pairs) and the lowest within-site variability regardless of sampling year (Table 6).

L DFA carried out for each sampling year revealed that the 4 nurseries largely separated out into different groups across discriminant space, regardless of sampling year (Fig. 4). From the 3 discriminant functions in each of the year-specific models, the first 2 consistently explained $>95\%$ of total variance (2015: 96%; 2016: 98%; 2017: 97%).

The leave-one-out cross-validation procedure correctly classified an average of 93% of all 0-group fish to their nursery of capture across sampling years

(Table 7). Correct classification percentages ranged from 80% (for the fish caught in the Blackwater and Slaney nurseries in 2016) to 100% (for the fish caught in the Barrow and Blackwater nurseries in 2015).

Analysis of pooled baseline data across the 3 sampling years found significant differences (MANOVA:

Table 6. One-way analysis of variance (ANOVA) results for differences in elemental concentrations between otoliths of 0-group *Dicentrarchus labrax* captured across 4 nursery estuaries over 3 sampling years (2015–2017). Data are analysed for each year and pooled across years. Only elements which were significantly different are presented. All data was \log_{10} transformed. Post hoc pairs: number of pairs of sites (out of 6) between which there were significant differences ($p < 0.01$) in element concentrations, using Tukey's post hoc comparisons

Element	F	p	Post hoc pairs
2015 (df = 3, 57)			
Mg	7.176	<0.001	2
Mn	41.69	<0.001	2
Sr	26.87	<0.001	4
Ba	73.71	<0.001	5
2016 (df = 3, 75)			
Mn	16.65	<0.001	3
Sr	36.82	<0.001	5
Ba	90.91	<0.001	5
2017 (df = 3, 77)			
Mg	13.31	<0.001	3
K	8.213	<0.001	2
Mn	22.55	<0.001	3
Sr	33.22	<0.001	4
Ba	120.6	<0.001	5
Years pooled (df = 3, 217)			
Mg	16.38	<0.001	3
Mn	34.12	<0.001	5
Sr	71.48	<0.001	4
Ba	292.4	<0.001	5

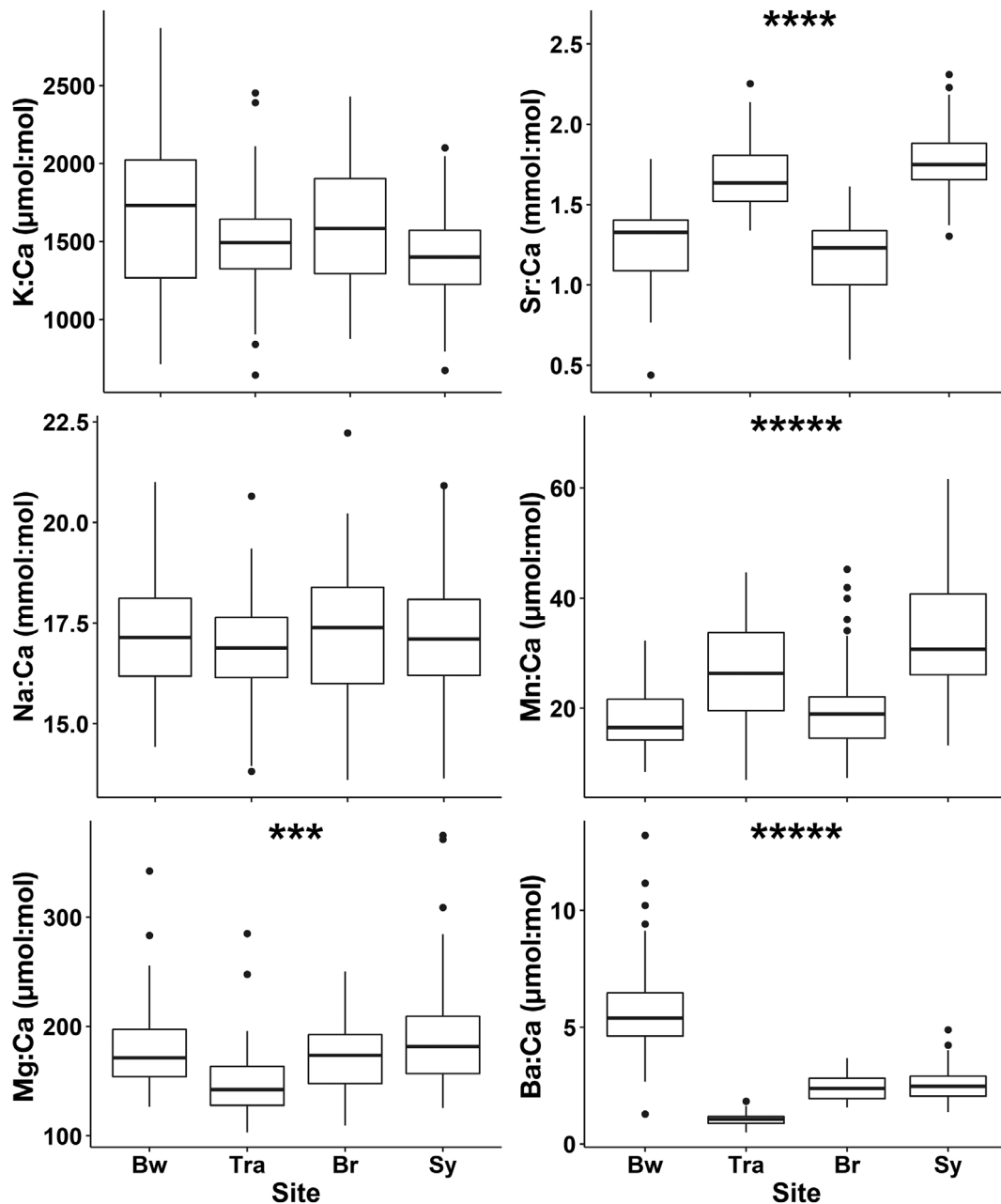


Fig. 3. Comparison of element:Ca ratios in otoliths from 0-group *Dicentrarchus labrax* captured in the 4 study sites (Bw: Barrow, n = 42; Tra: Tralee, n = 58; Br: Blackwater, n = 61; Sy: Slaney, n = 60) pooled across 3 study years (2015–2017). Number of asterisks above each panel denotes the number of pairs of sites (out of 6) between which there were significant differences ($p < 0.01$) in element concentrations, using Tukey's post hoc comparisons. Boxes: interquartile ranges; horizontal lines: medians; whiskers: 5th and 95th percentiles; dots: outliers

Wilks' $\lambda = 0.07$, $F_{3,18} = 53.64$, $p < 0.001$) in otolith geochemical tags between the 4 study nursery sites. The leave-one-out cross-validation procedure correctly classified an average of 93% of all 0-group fish to their nursery of capture (Table 7), showing that the pooled LDFA had good spatial discrimination between the study estuaries (Fig. 5).

3.3. Connectivity and site fidelity to estuarine nursery sites during juvenile life

3.3.1. 1 yr old juveniles

The vast majority of the 139 1-group *D. labrax* analysed were captured close to their original nursery

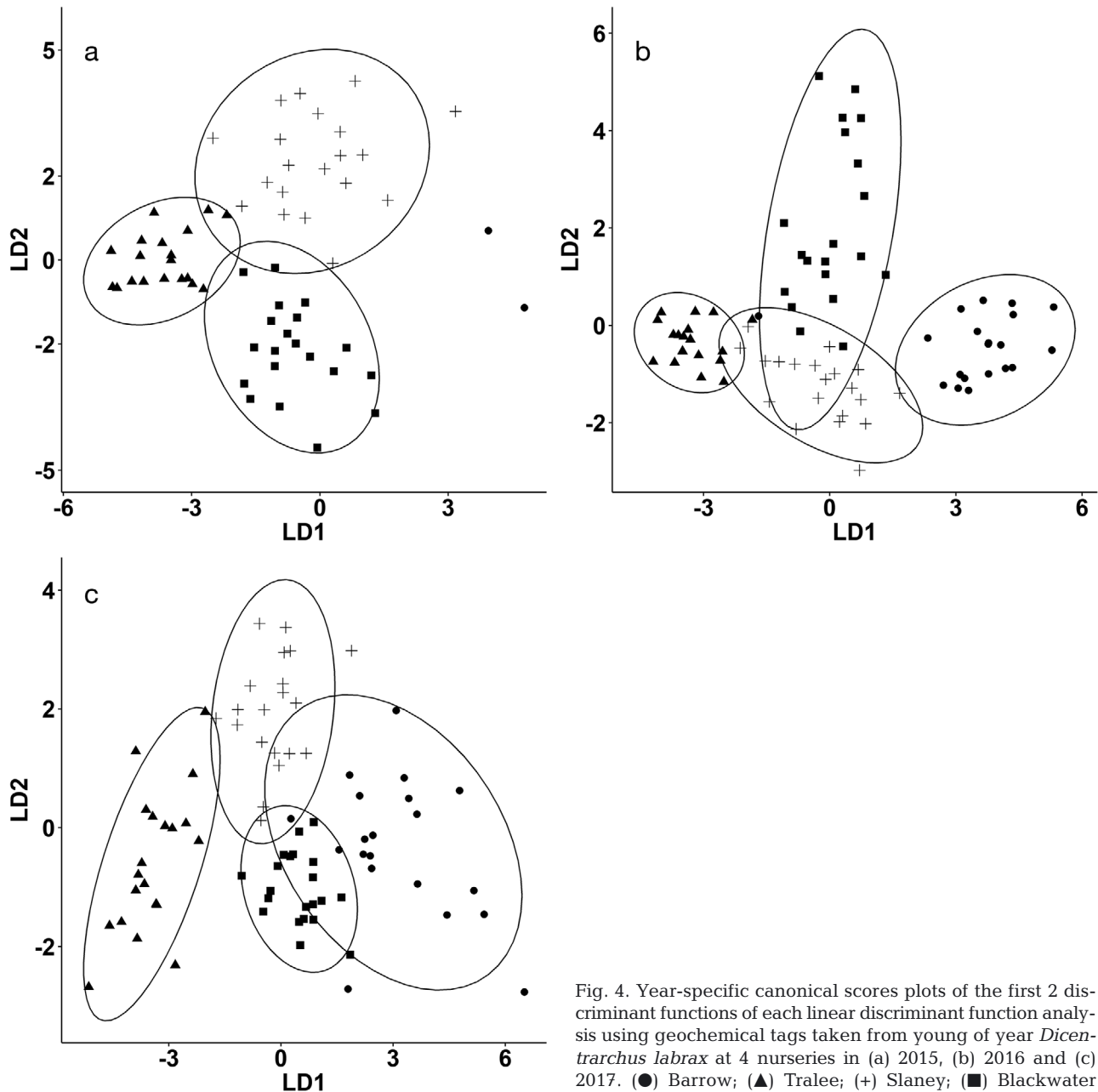


Fig. 4. Year-specific canonical scores plots of the first 2 discriminant functions of each linear discriminant function analysis using geochemical tags taken from young of year *Dicentrarchus labrax* at 4 nurseries in (a) 2015, (b) 2016 and (c) 2017. (●) Barrow; (▲) Tralee; (+) Slaney; (■) Blackwater

Table 7. Stepwise cross-validation linear discriminant function analysis (LDFA) classification of 0-group *Dicentrarchus labrax* to their site of capture. Percentage of fish classified to their site of capture are presented

Nursery	2015	2016	2017	2015–2017 (pooled)
Barrow	100	95	90	93
Tralee	95	100	95	97
Blackwater	100	80	95	92
Slaney	85	80	90	92
Total	95.0	88.8	92.5	93.2

site (Fig. 1). In the Barrow estuary, $86 \pm 6\%$ of the fish sampled ($n = 47$) over the 3 sampling years (Table 8) were predicted to have originated there. In 2016, $73 \pm 10\%$ of the 1-group fish captured ($n = 22$) were predicted to have settled in the Barrow nursery in 2015. Most remaining fish were predicted to have originated from the Blackwater nursery, and a low percentage of this cohort may have originated in the Slaney nursery (Table 8). HISEA analysis found that $97 \pm 4\%$ of 1-group cohorts sampled in Barrow in 2017 ($n = 19$) and all cohorts sampled in 2018 ($n = 6$) were predicted to originate from this estuary (Table 8).

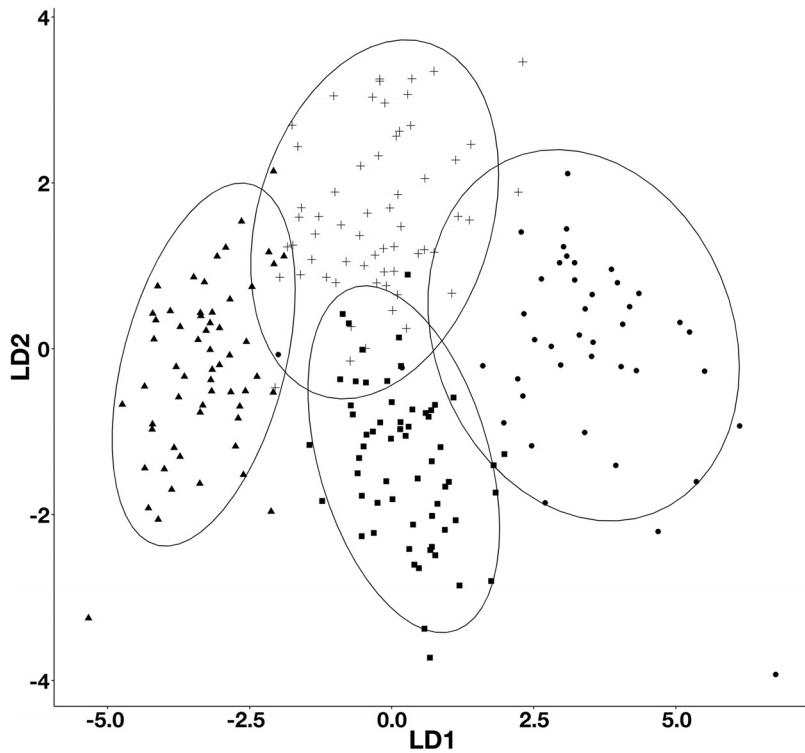


Fig. 5. Canonical scores plots of the first 2 discriminant functions of each linear discriminant function analysis using geochemical tags taken from young of year *Dicentrarchus labrax* at 4 nurseries. Data pooled across 3 sampling years (2015–2017) (●) Barrow; (▲) Tralee; (+) Slaney; (■) Blackwater

Similarly, $95 \pm 4\%$ of the total 1-group fish caught in the Slaney estuary were predicted to have settled at this nursery site in the previous year (Table 8). In the cohort from 2016 ($n = 18$), the model estimated that $85 \pm 10\%$ of the fish originated in the Slaney nursery. The remaining fish were assigned to the other three 0-group sampling sites. However, confidence in correct designation was low due to high standard deviations (Table 8). Of the 1-group Slaney estuary cohorts sampled in the Slaney estuary in 2017 ($n = 14$) and 2018 ($n = 20$), all fish sampled had settled there the previous year according to the MLE procedure (Table 8).

Conversely, in the Blackwater estuary, only $7 \pm 13\%$ of 1-group fish captured were predicted to have settled locally. This relatively low value was driven by the cohort sampled in 2018, for which only $36 \pm 15\%$ was predicted to have originally settled in the Blackwater nursery site. A further $50 \pm 15\%$ of these fish were predicted to have settled in the Barrow nursery site. The

remainder may have originated from the Slaney nursery; however, errors were too high to make confident predictions (Table 8). In 2017, $92 \pm 10\%$ of the 1-group cohort sampled in the Blackwater were predicted to have originally settled in the Blackwater sampling site (Table 8). The remaining fish from this cohort were assigned to the Barrow and Slaney estuaries. However, errors were too high to make confident predictions.

When pooling the baseline geochemical tags for all 1-group fish across sampling years, fidelity to local nurseries remained high, reaching $97 \pm 3\%$ for 1-group fish sampled in the Slaney, $98 \pm 3\%$ for the Barrow and $75 \pm 8\%$ for the Blackwater estuary (Table 8).

3.3.2. 2 yr old juveniles

Only twelve 2-group *D. labrax* individuals were sampled in the Slaney estuary in 2017. The MLE model corresponding to the 2015 age class estimated that $68 \pm 15\%$ of these fish originated from the local nursery site. Another $23 \pm 14\%$ originated from the Blackwater nursery, and we estimated that the

Table 8. Maximum likelihood estimates of the relative contributions of sampling year and estuary-specific 1-group *Dicentrarchus labrax* cohorts to nursery of origin. Otolith geochemical tags of 0-group juveniles collected in specific nurseries over 3 yr (2015–2017) were used as baseline data to identify nurseries of origin of each 1-group cohort. Analyses were both year-class matched and pooled across sampling years. n: total number of 1-group *D. labrax* used for each analysis; na: not available. Percent contribution from local nursery in **bold**

Sampling year	Estuary of capture	n	Predicted nursery of origin (% , \pm SD)			
			Barrow	Slaney	Blackwater	Tralee
2016 ^a	Slaney	18	2 (4)	85 (10)	6 (7)	7 (7)
2016 ^a	Barrow	22	73 (10)	5 (5)	23 (9)	0
2016 ^a	Blackwater	na	na	na	na	na
2017 ^a	Slaney	14	0	100 (0)	0	0
2017 ^a	Barrow	19	97 (4)	0	3 (4)	0
2017 ^a	Blackwater	15	3 (6)	6 (9)	92 (10)	0
2018 ^a	Slaney	20	0	100 (0.3)	0	0
2018 ^a	Barrow	6	100 (0)	0	0	0
2018 ^a	Blackwater	25	50 (15)	13 (12)	36 (15)	1 (3)
2016–18 ^b	Slaney	52	1 (2)	97 (3)	0	2 (2)
2016–18 ^b	Barrow	47	98 (3)	0	2 (3)	0
2017–18 ^b	Blackwater	40	25 (8)	0	75 (8)	0

^aBaseline year-class matched; ^bPooled baseline

remaining fish originated from the Barrow nursery (Table 9). The entire 2-group cohort captured in the Barrow estuary in 2018 ($n = 13$) settled in the Barrow nursery site in 2016 (Table 9).

Pooling baseline geochemical tags across all sampling years increased assignment rates and decreased assignment errors to local nursery sites for all 2-group cohorts (Slaney: $91 \pm 8\%$; Barrow: $100 \pm 0\%$) when baseline data was pooled across all sampling years (Table 9). All $23 \pm 14\%$ of the 2-group Slaney cohort which were assigned to the Blackwater nursery using the year-class matched baseline were reassigned to their local nursery when the pooled baseline was used.

4. DISCUSSION

Using otolith microchemistry to study the movements of fish can only be successful if there is spatial variability in geochemical tags between the habitats of interest. There is considerable evidence globally that fish captured in different catchments exhibit different otolith geochemical tags (Gillanders 2002b, Elsdon & Gillanders 2004, Avigliano et al. 2017, Moll et al. 2019). This study found that otolith geochemical tags in European sea bass *Dicentrarchus labrax* from 4 Irish nurseries provide strong discrimination for future connectivity studies. Pooling baseline geochemical tags from 0-group fish across sampling years indicates that geochemical tags for the nurseries of interest are consistently robust over several years. We also found evidence to suggest that although juvenile *D. labrax* move away from their original settlement sites—as demonstrated by the small number of older juveniles (>0-group) captured amongst the 0-group cohorts during this study—the vast majority remain in the vicinity of their settlement estuary for at least 2 yr. This is likely because *D. labrax* juveniles are specialist feeders which, as they grow, must forage further afield to find larger prey with greater energy content (Selleslagh & Amara 2015). The essential role which key estuaries can play in providing suitable habitat for juvenile *D. labrax*, not only to settle but also to develop and eventually recruit to the adult stock, is evidenced by this finding. Three of the 4 estuaries are extensive transitional waterbodies, in an Irish context, and all are located on the south coast of Ireland.

Although 1-group *D. labrax* were generally captured close to their original settlement sites, some

Table 9. Same as Table 8, but for 2-group *Dicentrarchus labrax*

Sampling year	Estuary of capture	n	Predicted nursery of origin (% , \pm SD)		
			Barrow	Slaney	Blackwater
2017 ^a	Slaney	12	9 (8)	68 (15)	23 (14)
2018 ^a	Barrow	13	100 (1)	0	0
2017 ^b	Slaney	12	9 (8)	91 (8)	0
2018 ^b	Barrow	13	100 (0)	0	0

^aBaseline year class matched; ^bPooled baseline

were captured over 6 km away but within the estuary environs. This study has shown that for the most part, 0-group *D. labrax* settlement sites, identified through regular sampling programmes, contribute the majority of older juveniles residing in each estuary. Although there may be other as yet unidentified 0-group settlement sites within each of the studied nursery estuaries, the high level of classification accuracy of older juveniles to the characterised settlement sites suggests that contribution from other sites is limited. Indeed, estuary systems such as those in this study are often heterogeneous in terms of freshwater inputs and surrounding geology (Reis-Santos et al. 2015, Connor et al. 2019). This usually results in intra-estuarine variation of otolith geochemical tags, depending on where separate larval cohorts of the same species settle and grow (Reis-Santos et al. 2015). Therefore, the results may imply that there are likely limited suitable areas for settlement within the characterised estuaries. This observation agrees with previous research which considered *D. labrax* settlement an active process whereby once larvae reach a particular developmental stage, they actively seek suitable nursery habitat (Jennings & Pawson 1992). These observations give confidence that the geochemical tags developed here can be used in future *D. labrax* connectivity studies in Ireland.

Surprisingly, the otolith geochemical tags for the 1-group *D. labrax* caught in the Blackwater estuary in 2018 did not match those from the local 0-group in 2017. As the majority of those fish were assigned to the Barrow site and otolith geochemical signatures from the Barrow and Blackwater nurseries were more similar in 2017 than in other sampling years, this discrepancy is probably due to errors in re-assignment linked to geochemical tag similarities between these systems. It is also possible that the 1-group 2018 *D. labrax* cohort originated from an as yet uncharacterised nursery area within the Blackwater estuary. Indeed, 0-group *D. labrax* were not encountered within the regular nursery site during sampling in 2017, so 0-group *D. labrax* were caught

on the opposite bank of the estuary instead, 1 km away from the regular sampling location. Although there is little spatial separation between the regular sampling location and that sampled in 2017, the 0-group 2017 sampling site is adjacent to a small freshwater input. This may have affected the otolith geochemical tags of the 0-group *D. labrax* caught in the Blackwater in 2017 if they grew under the influence of this freshwater input (Reis-Santos et al. 2015). It has been suggested that fish must reside in a particular area for more than 20 d to form a resolvable geochemical tag (Elsdon & Gillanders 2004, Russell et al. 2021), so it is possible that this cohort settled at this site and remained there until sampling. If this was the case, the freshwater influence should increase Ba:Ca ratios (Elsdon & Gillanders 2004, Walther & Limburg 2012) and decrease Sr:Ca ratios (Engstedt et al. 2012, Walther & Limburg 2012, Sturrock et al. 2014) in the geochemical tags. Although an increase in otolith Ba:Ca and a decrease in otolith Sr:Ca was detected between the Blackwater nursery geochemical tags of 2015 and 2017, the aforementioned elemental ratios were stable between 2016 and 2017. Thus, it is unlikely that the 2017 sampling location was the sole reason for assignment of a large proportion of the 1-group 2018 Blackwater cohort to the Barrow nursery. The 0-group otolith Sr:Ca ratio in the Barrow 2017 geochemical tag was lower than previous years and similar to the 0-group otolith Sr:Ca ratio in the Blackwater 2017 geochemical tag. This observation likely also contributed to the assignment of 1-group fish caught in the Blackwater estuary in 2017 to the Barrow nursery site. Irrespective of this variability, the Blackwater 2017 geochemical tag will be included in future *D. labrax* recruitment studies and likely pooled with geochemical tags from other sampling years to investigate the importance of potential Blackwater *D. labrax* nurseries for recruitment to the adult stock. Developing multi-annual geochemical tags can account for the variability in environmental conditions within estuarine habitats (Tournois et al. 2013).

When planning connectivity studies using otolith geochemical tags, a key assumption is that inter-estuarine variation is greater than intra-estuarine variation (Elsdon et al. 2008). This was the case for all sampling sites and years within this study, with the exception of the baseline developed for the Blackwater 2017 year class. The year-specific discriminant analysis indicates that the Barrow geochemical tags encompass a relatively large region of discriminant space and that the 2017 Barrow and Blackwater confidence ellipses overlap considerably

in 2-dimensional space. This is an important consideration for future connectivity studies, as it is probable that some adult *D. labrax* of the 2017 year class that recruited from the Blackwater will be misclassified. One method which can improve the discriminatory power of baselines is to pool across sampling years. Previous studies have found that although there are often differences in geochemical tags within estuaries between space and time, these differences are not as strong as between estuaries (Mercier et al. 2011, Reis-Santos et al. 2012, Tournois et al. 2013, 2017, Ryan et al. 2016). To test this observation, the multi-annual baselines developed for this study were pooled, and all 1-group *D. labrax* caught in the Blackwater across each sampling year were re-run using the HISEA (Millar 1990b) procedure. Classification of this cohort to the Blackwater was $75 \pm 8\%$, indicating that pooling multi-annual baselines can improve accuracy in assignment prediction. However, it is still important to characterise all possible settlement sites to account for the possibility of errors caused by intra- or inter-estuarine variation between otolith geochemical tags.

Multi-annual baselines for 0-group *D. labrax* settlement sites in the Tralee estuary were included in the HISEA models even though no 1- or 2-group juveniles were collected from this location. No 1- or 2-group fish were predicted with any confidence to originate in Tralee. Due to its spatial separation (>300 km) from the other settlement sites characterised in this study, there is no plausible scenario where a 1- or 2-group fish analysed in this study could have originated in the Tralee estuary. This finding suggests that the multi-annual baseline developed during this study for this estuary has good discrimination for future *D. labrax* connectivity studies.

The 2-group fish analysed showed a greater level of dispersion from settlement sites than 1-group fish, and this pattern was estuary-specific. All 2-group fish caught in the Barrow estuary were predicted to have first settled at the 0-group sampling site, some 6 km from where they were captured, using both the pooled and year-class matched otolith geochemical baselines. The Barrow estuary is a large body of water providing many foraging opportunities within the relative protection of the estuary, whereas the Slaney estuary is relatively small and shallow. When otolith geochemical baselines were year-class matched, $68 \pm 15\%$ of Slaney 2-group fish were caught no further than 3.4 km (average of 600 m) from their identified settlement site. Most of the remainder ($23 \pm 14\%$) were assigned to the Blackwater nursery, over 130 km away. It is possible that 2-group *D. labrax*

could migrate this distance. However, we consider this scenario to be unlikely due to the size and immaturity of these fish. It is notable that when the baselines were pooled across years, all the Blackwater-assigned fish were reassigned to the Slaney nursery site. These results suggest that pooling the geochemical data across years improves assignment predictions of 2-group fish caught in the Slaney estuary. The remaining 2-group Slaney-caught fish were assigned to the Barrow nursery whether geochemical baselines were pooled across years or not. Migration of 2-group fish between these estuaries is more likely due to the reduced distance (68 km) involved; however, confidence in correct assignment is low due to the high relative error ($9 \pm 8\%$) in the output.

Although recruitment of marine fish is thought to be largely influenced by density-independent factors (Henriques et al. 2017, Pinto et al. 2021), there is evidence to show that *D. labrax* recruitment can be negatively affected by intraspecific competition between juvenile age classes (Henderson & Corps 1997). Regular sampling since 2014 has shown that the Slaney is potentially a highly productive nursery estuary for 0-group *D. labrax*, as highlighted by the high density of 0-group captures compared to the other estuaries sampled over the study period. However, the more limited foraging opportunities combined with the potential for increased intra-specific competition in the Slaney estuary may constrain the level of recruitment to the adult stock. The observation that 0-group *D. labrax* caught in the Slaney were generally larger than cohorts from the other study estuaries but that 1- and 2-group fish from the Slaney were, on average, smaller than those caught in the Barrow provides some evidence for this hypothesis. It is also notable that older juveniles (1- and 2-group) were present at a higher density in the Barrow estuary than the Slaney estuary during sampling. The geochemical tags established during this study will allow this observation to be investigated further in future studies.

Northern stocks of *D. labrax* are slow-growing compared to their southern counterparts (Beraud et al. 2018) due largely to lower water temperatures in this region (Almeida et al. 2015). Mean water temperatures recorded at the Slaney 0-group site during sampling were higher than the other estuary sites. The Slaney site is in a region of the estuary which widens to a shallow, sheltered bay. This characteristic is likely the main reason for the higher average temperatures and may be related to the larger average size of 0-group fish here compared to the other sites sampled in this study. 0-group bass are sensitive to low winter temperatures (Pickett & Pawson 1994);

therefore, extreme winter conditions in Irish nurseries may have a deleterious effect on the survival of juvenile *D. labrax*, particularly during their first winter. As a result, 0-group recruitment success around Ireland is likely to be highly dependent on climatic variability (Pawson 1992, Pinto et al. 2021). To increase the likelihood of survival over their first winter, fish size at the end of the first summer of growth may be particularly important in Ireland. 0-group juvenile condition can enhance survival during their first winter and subsequently improve recruitment rates to the adult stock (Sogard & Olla 2000, Tournois et al. 2017). Juvenile *D. labrax* sampled in the Blackwater were significantly smaller than conspecifics from the other study estuaries. This difference may be a result of a combination of lower temperatures and fewer foraging opportunities due to the relatively small size of the estuary. The Tralee estuary is also small, and the average temperature recorded during sampling was relatively low (although variation was high between samples). However, 0-group *D. labrax* from this site were significantly larger than those captured in the Blackwater. This may be due to a combination of the collective influences of low freshwater input and the wide, shallow nature of the Tralee estuary compared to the Blackwater estuary. No 1- or 2-group *D. labrax* were collected from the Tralee estuary due to logistical constraints, so the effect of estuary size cannot be assessed for older juvenile growth. Currently, it seems likely that the Blackwater may not be as productive a *D. labrax* nursery as the neighbouring estuaries examined in this study. Future connectivity studies will investigate this possibility.

This study confirmed that 1-group and 2-group *D. labrax* captured in estuaries displayed high site fidelity to their original nursery site, even though it is possible that some of these fish migrated from their estuary of capture and subsequently returned during sampling (Mignucci 2021). Using the otolith geochemical tag baselines developed in this study, in the future, adult *D. labrax* that have recruited to Irish stocks along the south coast of Ireland can be classified to their nursery estuaries with confidence, even if year-classes are not matched. Several findings from this study suggest that the variation in otolith elemental concentrations between fish captured in different nurseries are influenced by estuary-specific physiochemical characteristics. Further connectivity studies, combined with information on juvenile densities at different life stages, will allow the development of evidence-based management plans for estuaries that provide the most recruits to the adult *D. labrax* stock.

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