Validation of otolith $\delta^{18}O$ values as effective natural tags for shelf-scale geolocation of migrating fish

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ABSTRACT: The oxygen isotopic ratio of fish otoliths is increasingly used as a 'natural tag' to assess provenance in migratory species, with the assumption that variations in $\delta^{18}O$ values closely reflect individual ambient experience of temperature and/or salinity. We employed archival tag data and otoliths collected from a shelf-scale study of the spatial dynamics of North Sea plaice *Pleuronectes platessa* L., to examine the limits of otolith $\delta^{18}O$-based geolocation of fish during their annual migrations. Detailed intra-annual otolith $\delta^{18}O$ measurements for 1997–1999 from individuals of 3 distinct sub-stocks with different spawning locations were compared with $\delta^{18}O$ values predicted at the monthly, seasonal and annual scales, using predicted sub-stock specific temperatures and salinities over the same years. Spatio-temporal variation in expected $\delta^{18}O$ values (−0.23 to 2.94‰) mainly reflected variation in temperature, and among-zone discrimination potential using otolith $\delta^{18}O$ varied greatly by temporal scale and by time of year. Measured otolith $\delta^{18}O$ values (−0.71 to 3.09‰) largely mirrored seasonally predicted values, but occasionally fell outside expected $\delta^{18}O$ ranges. Where mismatches were observed, differences among sub-stocks were consistently greater than predicted, suggesting that in plaice, differential sub-stock growth rates and physiological effects during oxygen fractionation enhance geolocation potential using otolith $\delta^{18}O$. Comparing intra-annual $\delta^{18}O$ values over several consecutive years for individuals with contrasted migratory patterns corroborated a high degree of feeding and spawning site fidelity irrespective of the sub-stock. Informed interpretation of otolith $\delta^{18}O$ values can therefore provide relatively detailed fisheries-relevant data not readily obtained by conventional means.

KEY WORDS: Fish migration · Oxygen · Stable isotopes · Natural tag · Site fidelity · Plaice · *Pleuronectes platessa*

INTRODUCTION

The distributions of marine species are often discontinuous in time and space, usually as a consequence of spatial and temporal shifts in the physical and biological characteristics of oceanic habitats (Hixon et al. 2002). In fishes, distribution shifts further reflect ontogenetic and/or seasonal migrations driven primarily by the physiological requirements of maturation and subsequent annual reproductive cycles (Kimirei et al. 2013). Understanding them is therefore particularly important for species conserva-

tion and fisheries management (Pulliam 1988, Botsford et al. 2009).

In recent decades, sustained research focus on movement ecology has greatly improved our understanding of population structure and lifetime movements in many commercially exploited and conservation-sensitive fishes (e.g. Righton et al. 2010, Block et al. 2011, Hussey et al. 2015, Hays et al. 2016). However, direct observation of fully marine fishes in their natural environment over annual migration cycles remains challenging (Metcalfe et al. 2008). The mechanical limitations of tracking and tagging tools

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and hardware still largely restrict studies to shallow, mainly inshore areas, and to limited portions of the adult life in large, often predatory, fishes (Hays et al. 2016).

To describe the stock structure and spatial dynamics of fish either too small or too vulnerable to tag using satellite or archival tags, scientists increasingly apply 'natural tags', most notably the continuously accreting and physiologically inert calcified otoliths. Still a cornerstone of assessment biology, the interpretation of otoliths has long surpassed counting the annular rings to estimate fish age, and fine-scale interpretation of otolith chemistry now offers significant insights into life-history characteristics, stock structure and migratory behaviour (Morrongiello et al. 2012, Geffen et al. 2016, Grønkjær 2016). Because otoliths continuously log environmental data throughout life, and because otolith material is rarely resorbed or physiologically altered after deposition (Mugiya & Uchimura 1989, Campana 1999), otoliths provide a precisely dated and seasonally resolved record of fish lifetime environmental history (Thorrold et al. 1997). The interrogation of otolith structure and chemistry can provide coarse estimates of past geographical location ('geolocation'), at least in those species that migrate between water masses with sufficiently different characteristics (Campana 1999).

Since the isotopic ratio ^{18}O:^{16}O (expressed as $\delta^{18}O$) in biocarbonates varies with both water temperature and isotopic composition ($\delta^{18}O$) at the time of deposition (Epstein & Mayeda 1953), otolith $\delta^{18}O$ is a particularly promising marker for studying marine fish migrations. From the first estimations of seawater temperatures derived from the $\delta^{18}O$ of fish otoliths by Devereux (1967), otolith $\delta^{18}O$ has increasingly been applied to identify marine fish origin (e.g. Gao & Bean 2008, Rooker et al. 2008, Trueman et al. 2012), differentiate between residents and migrants (e.g. Northcote et al. 1992, Bastow et al. 2002, Blamart et al. 2002, Ayvazian et al. 2004) and to distinguish between mixing and non-mixing stocks (e.g. Stephenson 2001, Rooker & Secor 2004, Ashford & Jones 2007, Newman et al. 2010). However, otolith $\delta^{18}O$ heterogeneity across water temperature–$\delta^{18}O$ combinations has frequently been observed (Høie et al. 2004, Storm-Suke et al. 2007, Godiksen et al. 2010, Geffen 2012), suggesting that the isotopic fractionation between otolith aragonite and ambient water may vary between and within species. Although this might preclude identifying geographical movement using otolith $\delta^{18}O$, few attempts have been made so far to ground-truth the efficiency of this natural tag as a tool for geolocation.

As a first step toward this goal, migratory and environmental data gathered from hundreds of adult plaice *Pleuronectes platessa* L. tagged with archival tags were recently coupled with the annual $\delta^{18}O$ signatures laid down simultaneously in the otoliths of 24 tagged fish, from 3 'sub-stocks' that have discrete distributions for most of the year (Darnaude et al. 2014). Comparison between measured and expected $\delta^{18}O$ values at this temporal scale provided strong evidence that annual $\delta^{18}O$ signatures are accurate predictors of plaice sub-stock membership in the North Sea, the otolith $\delta^{18}O$ values of wild plaice largely reflecting environmental temperature and salinity at the time of deposition. However, variation in adult physiology among local sub-stocks and at certain seasons could also be partly responsible for the observed $\delta^{18}O$ differences.

Here, we draw on the same sets of otolith and environmental data to further explore the spatial and temporal limits of otolith $\delta^{18}O$ values as a geolocation tool. For individuals representative of the same 3 sub-stocks and their main migration routes, we re-analysed a subset of the original otoliths for intra-annual $\delta^{18}O$ values gathered over multiple years, including those prior to the commencement of tag recording. By comparing these intra-annual $\delta^{18}O$ signatures with detailed multi-annual predictions of $\delta^{18}O$ (from tag-derived bottom temperatures and salinities) across the full geographical range of the sampled plaice, we investigated (1) how precisely intra-annual variations in temperature and salinity predicted seasonal otolith $\delta^{18}O$ differences among the 3 sub-stocks, (2) areas and seasons where otolith $\delta^{18}O$ could be used for geolocation in the North Sea and English Channel, and (3) whether seasonal differences in otolith $\delta^{18}O$ could identify site fidelity to geographically discrete local summer feeding grounds and winter spawning areas. This *in situ* validation approach, although still novel, is an essential pre-requisite if otolith $\delta^{18}O$ is ever to be meaningfully applied as an alternative natural tag for describing fish population dynamics for application in fisheries management.

MATERIALS AND METHODS

The otoliths and environmental data used in this work derive from an extensive study of plaice tagged with archival tags and released at various locations in the North Sea between 1993 and 2000 (see Hunter et al. 2004 for full details, including hardware deployed and tagging methodology). Of 785 mature, predomi-

nantly female plaice released, 194 individuals were recovered, with tag-recorded environmental data of between 2 and 512 d. The archival tags recorded ambient water temperatures (±0.2°C) and pressures at 10 min intervals throughout the period during which the fish were at liberty, providing detailed information on the environmental conditions experienced, but also allowing reconstruction of fish movements. When tagged plaice remained on the seabed for one or more tidal cycles, the times of high water and accompanying tidal ranges measured were used to identify fish geographical position using the tidal location method (see Hunter et al. 2003b for full details). For each individual, a best-fit track was then reconstructed by fitting a piece-wise linear curve through the release position, and any sequential geolocations and the recapture position (full details in Hunter et al. 2003b). This allowed the generation of daily geolocations for every tagged fish, providing long-term knowledge on the local stock structure and adult annual migrations (see Hunter et al. 2004) but also access to location-specific concomitant otolith records and environmental data.

Intact otoliths were returned along with the archival tags for 83 fish, amongst which individuals with data records ≥3 mo were released and recaptured

mainly between 1997 and 1999 (Darnaude et al. 2014). Consequently, we chose these 3 successive years to reconstruct multi-annual environmental conditions prevailing over the full observed distribution range to compare with the seasonal δ¹⁸O signal simultaneously laid down in the otoliths of the same fish (see section 'Prediction of expected otolith δ¹⁸O values per substock or area').

Plaice distribution and behaviour in relation to environmental conditions experienced

Plaice are not randomly distributed in the North Sea, but form 3 separate feeding aggregations ('substocks') which remain geographically discrete from May to October each year (Hunter et al. 2004): substock A in the Northern (NNS), sub-stock B in the Eastern (ENS) and sub-stock C in the Western (WNS) North Sea (Fig. 1). Between November and April, all 3 sub-stocks migrate to and from predominantly southerly located spawning areas where they mix. However, the timing and extent of the spawning migration varies both among and between substocks (Fig. 1). Most plaice remain in the North Sea throughout the year (Hunter et al. 2004). The small
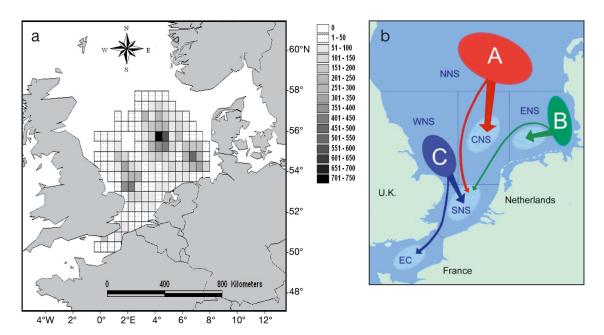

Fig. 1. North Sea areas and plaice *Pleuronectes platessa* sub-stocks studied. (a) The global area over which plaice adults migrate (non-empty grid cells on the map) between their (b) summer feeding aggregations (sub-stocks A, B and C) and winter spawning grounds (light blue areas: Van Neer et al. 2004) was divided into 6 regions: English Channel (EC: <51.00° N), Southern North Sea (SNS: 51.00–52.49° N), Western North Sea (WNS: 52.50–55.49° N; <2.50° E), Central North Sea (CNS: 52.50–55.49° N, 2.50–4.99° E), Eastern North Sea (ENS: 52.50–55.49° N, ≥5.00° E) and Northern North Sea (NNS: ≥55.50° N). The legend in panel a shows the total number of geolocations obtained per grid cell during the 1993–2000 tagging experiment (Hunter et al. 2004), while in b, coloured arrows show migration routes typical of each sub-stock (Fig. 1b modified from Darnaude et al. 2014)

proportion of plaice from sub-stock C that spawn in the English Channel (EC) do so only in December or in January, spending the remainder of the winter in the Southern North Sea (SNS) (Hunter et al. 2004). There, they mix with the individuals of the same sub-stock that spawn in the SNS (mainly in January), but also with fish of sub-stocks A and B that also spawn in this area (in February and March). Similarly, in transit to their spawning grounds in the SNS, the fish from sub-stock A mix with those of sub-stock B in the ENS (from December to April), and some fish from all sub-stocks mix in the Central North Sea (CNS) from January to March, either because they spawn there (sub-stock A) or because their spawning migration takes them through this area (sub-stocks B and C spawning in the SNS or the EC). The environmental conditions encountered should therefore mostly differ among all 3 sub-stocks from May to October. During the rest of the year, environmental conditions are migration-type dependent, but will not necessarily reflect the final location of spawning since the time spent on the spawning grounds (in the EC, SNS, ENS or CNS, depending on the migration type) is limited when compared to the time spent migrating between them (through NNS, CNS and sometimes SNS for sub-stock A; through ENS and sometimes CNS and SNS for sub-stock B; through WNS, SNS and sometimes EC for sub-stock C).

Otolith selection, preparation and analysis

The 2 biggest otoliths (paired sagittae) are not identical in plaice: the left sagitta is symmetric, the right asymmetric. Although the paired otoliths exhibit differential growth from metamorphosis (Lychakov et al. 2008), this does not induce differences in seasonal oxygen isotope signature between them (Geffen 2012). Therefore, to maximize time-resolution when sampling the distal area laid down during tag recording time, frontal sections of right sagittae were used, the fine-scale (intra-annual) δ^{18}O signatures obtained being used to evaluate the potential for otolith δ^{18}O-based seasonal estimates of geolocation. For this, the right otoliths of 24 mature females (8 from each sub-stock), with individual data records ≥78 d (Table 1), were selected. This sub-sample of fish was representative of the principal migration types observed for each sub-stock (Fig. 1). Each individual data record allowed unequivocal identification of spawning areas and feeding grounds for at least one annual cycle, sometimes two (Table 1). Furthermore, for 12 fish repre-

sentative of all migration types (2 each, Fig. 1), tag data records allowed unequivocal identification of both the winter spawning areas and the summer feeding grounds in 1998 (Table 1). The comparison of their otolith δ^{18}O signatures, in particular those for the winters 1997–98 and 1998–99, allowed testing of the extent to which otolith δ^{18}O could identify differences in spawning location within and among sub-stocks.

Otolith preparation was similar for all fish. The right otoliths (sagittae) were cleaned of organic surface debris, embedded in clear epoxy resin and cut along the frontal plane containing the core. Each otolith section (~1000 µm thick) was then ground down to 500–600 µm until the otolith edge was perpendicular to the section surface, and polished until superficial scratches were removed on both sides. All sections were glued onto glass slides using epoxy resin, and their ventral side (facing up) was photographed under reflected light. Glass slides supporting the sections were then rinsed with distilled water, dried overnight in a clean vertical laminar flow workstation and stored in acid-washed plastic envelopes prior to powder sampling for analysis. More details about these initial steps of otolith preparation can be found in Darnaude et al. (2014).

Seasonality of opaque-translucent otolith banding fluctuates with fish age and latitude in North Sea plaice (Van Neer et al. 2004), implying the potential for geographic and individual variation in otolith growth during the year. However, the opaque zone is largely accreted during April to September, and the hyaline (or translucent) zone from October to March (Van Neer et al. 2004, authors' pers. obs.). Therefore, in order to facilitate assessment of otolith δ^{18}O potential for sub-stock discrimination at the sub-annual scale, the year was divided into 4 periods ('seasons'), each of 3 mo: February to April (late winter/early spring: LW/ES), May to July (late spring/early summer: LS/ES), August to October (late summer/early autumn: LS/EA) and November to January (late autumn/early winter: LA/EW). Environmental δ^{18}O records during these 4 seasons are easy to locate on the otoliths, the material laid down during LW/ES being positioned at the translucent–opaque border each year, that for LS/EA occurring on the opaque–translucent border, and those for LS/ES and LA/EW sitting intermediate between these two.

Individual ages for all fish were assessed previously from transverse sections made on the left otoliths (Darnaude et al. 2014). Powder samples corresponding to the 4 seasons (see above) were then

Table 1. Details of female plaice *Pleuronectes platessa* assessed for intra-annual variations in otolith $\delta^{18}O$ signatures. CNS: Central North Sea; ENS: Eastern North Sea; SNS: Southern North Sea; EC: English Channel. For each sub-stock (see Fig. 1), individuals highlighted in grey were used to investigate fidelity to summer feeding grounds and spawning sites. DST: data storage tag. For all fish, the year and age (in yr) analyzed for otolith $\delta^{18}O$ signatures are indicated in **bold** and x2 indicates when the breeding area was identified for 2 consecutive years in the same fish

ID	Recapture date	Size (cm)	Age (yr)	No. days of DST record (start & end dates)	Breeding area (breeding month) identified from DST records	Year(s) analysed for $\delta^{18}O$ (age)
Sub-stock A						
A-1	Jul 99	40	12	386 (Dec 97–Dec 98)	CNS (December)	1995–**1998 (9–11)**
A-2	May 99	44	14	512 (Dec 97–Apr 99)	CNS (January) x2	1997 & **1998 (12–13)**
A-3	Jan 99	40	5	398 (Dec 97–Jan 99)	CNS (January)	**1998 (5)**
A-4	Dec 98	40	8	365 (Dec 97–Dec 98)	CNS (January)	1997 & **1998 (7–8)**
A-5	Sep 98	38	6	272 (Dec 97–Aug 98)	CNS (February)	**1997 (5)**
A-6	Mar 98	38	9	134 (Oct 97–Mar 98)	CNS (January)	1996 & **1997 (7–8)**
A-7	Jun 00	37	6	354 (Feb 99–Feb 00)	SNS (February)	1998–**1999 (5–6)**
A-8	Sep 98	40	6	78 (Oct 97–Jan 98)	SNS (February)	1997 & **1998 (5–6)**
Sub-stock B						
B-1	Dec 98	48	7	382 (Oct 97–Nov 98)	SNS (January) x2	1996 – **1998 (5–7)**
B-2	Feb 99	46	12	317 (Oct 97–Aug 98)	SNS (January)	1997 & **1998 (10–11)**
B-3	Oct 99	38	7	194 (Feb–Aug 99)	SNS (February)	**1999 (7)**
B-4	Feb 98	36	6	104 (Nov 97 – Feb 98)	SNS (January)	**1997 (5)**
B-5	Jan 99	41	7	262 (Oct 97–Jul 98)	ENS (January)	1996 & **1997 (6–7)**
B-6	Jun 98	41	5	202 (Nov 97–May 98)	ENS (February)	1997 & **1998 (4–5)**
B-7	Apr 98	39	6	153 (Nov 97–Apr 98)	ENS (February)	**1997 (5)**
B-8	Feb 98	40	7	97 (Oct 97–Jan 98)	ENS (January)	**1997 (6)**
Sub-stock C						
C-1	Apr 99	52	9	411 (Feb 98–Apr 99)	EC (December) x2	1996– **1998 (6–8)**
C-2	Nov 99	36	7	384 (Oct 98–Oct 99)	EC (January)	1998 & **1999 (6–7)**
C-3	Mar 00	39	12	303 (Feb 98–Dec 99)	EC (January)	**1999 (11)**
C-8	Jun 98	39	11	109 (Feb–May 98)	EC (December)	**1997 (10)**
C-4	Aug 99	38	5	231 (Oct 98–May 99)	SNS (January)	**1999 (5)**
C-5	Oct 99	41	10	223 (Feb–Sep 99)	SNS (January)	1998 & **1999 (9–10)**
C-6	Sep 98	44	10	221 (Dec 97–Jul 98)	SNS (December)	1997 & **1998 (9–10)**
C-7	Sep 99	41	9	182 (Feb–Aug 98)	SNS (January)	**1998 (9)**

collected from the otolith frontal section, using a computer-controlled micro-milling system (New Wave Research 'MicroMill'). Sampling always covered the annual growth band(s), reflecting the life of the fish throughout the archival tag data recording period. For the 12 individuals with tag records allowing unequivocal identification of both spawning area and feeding grounds in 1998 (2 for each migration type, Table 1), seasonal powder samples were also gathered for the 2 yr preceding initial capture to investigate fish multi-annual fidelity to spawning and feeding grounds.

The positions of opaque and translucent zones on magnified images of the sections were used to identify data storage tag (DST) recording periods in the otoliths, and were digitized to provide navigational input to the instrument. Depending on the fish and the year of life sampled, the respective widths of the opaque and translucent bands allowed extraction of 1 to 3 otolith powder samples between the opaque–translucent borders. Therefore, 1 to 12 sequential layers (of ~50–80 μm width and 450 μm depth) were milled per otolith, from the distal edge (most recent growth) inwards. The respective positions of the samples on the otoliths were used to assign them retrospectively to seasons. The corresponding powder samples (40–50 μg in weight) were collected separately and analysed at the Woods Hole Oceanographic Institution, MA, USA, using a Finnigan MAT252 mass spectrometer system with a Kiel III carbonate device. All isotopic values were reported relative to the international carbonate standard Vienna Pee Dee Belemnite (VPDB), using the international standard delta notation:

$$\delta^{18}O = [(R_{sample}/R_{standard}) - 1] \times 1000 \, (‰) \qquad (1)$$

where R is the $^{18}O{:}^{16}O$ ratio in the sample or standard. Analytical precision for $\delta^{18}O$ values, based on the SD of daily analysis of NBS-19 carbonate standard, was $\pm 0.07‰$.

Prediction of expected otolith δ¹⁸O values per sub-stock or area

Using all geolocations derived from the 194 tagged fish recaptured between 1993 and 2000 (n = 13 512), monthly distributions of adult plaice were summarized using grid-maps, showing the cells (0.5° latitude × 0.5° longitude) containing 80% of geolocations for each sub-stock (Fig. 1). Daily seabed temperatures and salinities in 1997, 1998 and 1999 were generated for corresponding grid cells using the general estuarine transport model (GETM), developed and validated for realistic 3-dimensional simulations of temperature and salinity in the North Sea (Stips et al. 2004). The model domain extends from a boundary in the western English Channel (−5° E) into the North Sea with an eastern boundary in the Baltic (16° E) and then northwards as far as the Shetland Isles (60° N) at a resolution of ~6 nautical miles and with 25 terrain-following vertical levels. Meteorological forcing in the model for the 3 studied years was derived from the European Centre for Medium-range Weather Forecasting ERA datasets. Tidal boundaries were calculated from Topex-Poseidon satellite altimetry, and temperature and salinity boundary conditions were taken from the climatologic predictions of the POLCOMS S12 model (http://cobs.pol.ac.uk/modl/metfcst/POLCOMS_DOCUMENTATION/).

For each sub-stock or area in the study zone (Fig. 1), daily GETM temperature and salinity estimates for each grid cell and year were used to predict corresponding otolith δ¹⁸O values. For this, ambient water (w) δ¹⁸O values relative to the Vienna Pee Dee Belemnite standard (VSMOW) were derived from salinity (S) estimates using the equation of Harwood et al. (2008) for the North Sea:

$$\delta^{18}O_w \text{ (VSMOW)} = 0.274 \times S - 9.3 \qquad (2)$$

then converted into $\delta^{18}O_w$ relative to the Vienna Pee Dee Belemnite standard (VPDB) using the equation of Coplen et al. (1983):

$$\delta^{18}O_w \text{ (VPDB)} = 0.97002 \times \delta^{18}O_w \text{ (VSMOW)} - 29.98 \quad (3)$$

Finally, the corresponding temperature estimates (T, in K) were incorporated in order to predict otolith δ¹⁸O values ($\delta^{18}O_o$) using the theoretical equation for inorganic aragonite deposition (Kim et al. 2007):

$$1000 \ln\alpha = \left(17.88 \times \frac{1000}{T}\right) - 31.14,$$
$$\text{with } \alpha = \frac{1000 + \delta^{18}O_o}{1000 + \delta^{18}O_w} \qquad (4)$$

The resulting daily maps of otolith δ¹⁸O values were used to simulate specific range of monthly otolith δ¹⁸O signatures for each sub-stock or area.

Prediction of individual otolith δ¹⁸O values during tag recording time

For the 12 fish representative of the 6 main migration types in 1998 (Table 1), specific daily otolith δ¹⁸O values between release and recapture were predicted using the tag-recorded temperatures. Equivalent ambient salinities at all successive individual daily geolocations were extracted from the CEFAS database for North Sea bottom salinity, where available (16%), or were predicted using the GETM. Water salinities were converted into $\delta^{18}O_w$ (VPDB) values using Eqs. (2) & (3). Daily otolith δ¹⁸O values were predicted from tag-recorded temperatures using Eq. (4) from Kim et al. (2007). Intra-annual otolith growth was assumed to vary slightly among individuals and years. To display predicted and measured otolith δ¹⁸O values over time, therefore, calendar dates were estimated for measured δ¹⁸O values by graphically adjusting profile shape and inflection points to best match the corresponding predicted annual δ¹⁸O profile using Analyseries 2.0 (Paillard et al. 1996).

Data analysis

All statistical analyses and simulations were performed in R (R Development Core Team 2011), taking 0.05 as the limit for statistical significance. For all variables tested (salinity, temperature, predicted and measured otholith δ¹⁸O), data homoscedasticity and the normality and independence of residuals after parametric ANOVAs were investigated using Shapiro-Wilk normality tests, Studentized Breusch-Pagan tests and Durbin-Watson tests of residuals, respectively. When these conditions were not met, even after data transformation, non-parametric statistics were used (see below).

Inter-annual differences in the temperatures, salinities and otolith δ¹⁸O values predicted over the full distributional area of plaice during the study period were tested by a non-parametric 1-way, fixed-effects, unbalanced ANOVA (Kruskal-Wallis tests), followed by post hoc multiple comparisons tests (Wilcoxon-Mann-Whitney tests) with Bonferroni corrections for statistical significance. Since no significant year effect could be detected on either the environmental

data (temperature: χ^2 = 3.24, p = 0.81; df = 2; salinity: χ^2 = 2.06, p > 0.66; df = 2) or the predicted $\delta^{18}O$ signal at this scale (χ^2 = 5.54, p > 0.92; df = 2), the values predicted in each grid cell for all 3 study years (1997, 1998 and 1999) were considered grouped for the rest of the analyses.

Predicted intra-annual variations of salinity, temperature and otolith $\delta^{18}O$ among areas over the 1997–1999 period were investigated at different temporal scales using 2-way (area × month or area × season) fixed-effects, unbalanced ANOVAs with permutations (PERMANOVAs, n = 999 permutations, Anderson 2001) followed by separate Kruskal-Wallis 1-way ANOVAs and post hoc multiple comparisons tests (Wilcoxon-Mann-Whitney tests with Bonferroni corrections for statistical significance) for identifying areas with significantly different conditions at each temporal scale (year, season or month).

To verify the pertinence of the 4 seasons chosen to describe seasonal $\delta^{18}O$ changes in North Sea plaice (see 'Otolith selection, preparation and analysis' above), monthly variations of the predicted otolith $\delta^{18}O$ signals for all sub-stocks were investigated using a 2-way (sub-stock × month) unbalanced PERMANOVA. This was later supplemented by non-parametric Kruskal-Wallis ANOVAs (1 per factor) and post hoc multiple comparison tests (Wilcoxon-Mann-Whitney tests, with Bonferroni corrections) to identify the months expected to produce maximum differences among sub-stocks and those responsible for the maximum and minimum $\delta^{18}O$ signatures recorded during the year for each sub-stock. The same approach (2-way PERMANOVAs, followed by Kruskal-Wallis ANOVAs and post hoc Wilcoxon-Mann-Whitney tests) was used to investigate seasonal differences in predicted and measured otolith $\delta^{18}O$ values among sub-stocks. Lastly, differences between observed and predicted values for each sub-stock and season were tested separately using non-parametric Wilcoxon tests for paired samples.

In the following sections, all average values are provided with corresponding standard deviations (SD).

RESULTS

In addition to providing a detailed description of the distribution area and the spatio-temporal movements of plaice in the North Sea (Fig. 1), the data gathered during the 1993–2000 tagging experiment allowed the generation of 136 875 daily estimates of bottom temperature and salinity over the entire dis-

tribution range of our tagged fish for 1997–1999. Fine-scale analysis of the right-hand sagittae of 24 of the tagged fish returned with intact otoliths generated 117 measures of intra-annual $\delta^{18}O$ to be compared with the $\delta^{18}O$ values calculated from expected temperatures and salinities. The comparison was performed at various temporal scales, for each of the 3 sub-stocks, and for the areas seasonally occupied by the fish within the North Sea and the English Channel.

Environmental conditions and predicted geolocation success

In the areas frequented by adult plaice (Fig. 1), bottom temperatures and salinities were predicted to fluctuate from 1.62 to 21.65°C and from 24.10 to 35.28 over the year, around respective global annual averages of 10.41 ± 3.85°C and 34.58 ± 0.98. Otolith $\delta^{18}O$ signatures in this zone were predicted to range from −2.08 to 3.03‰, around a global annual average of 1.6 ± 0.91‰ (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m598p167_supp.pdf). The ambient environmental conditions encountered varied greatly over time and space, with major consequences for the expected area discrimination success using corresponding otolith $\delta^{18}O$ values.

Spatio-temporal variations in environmental conditions

Temperature and salinity conditions were predicted to differ significantly both by month (temperature: p < 0.001, F = 49863.96, df = 11; salinity: p < 0.001, F = 22.46, df = 11) and by area (temperature: p < 0.001, F = 26301.12, df = 5; salinity: p < 0.001, F = 14395.20, df = 5), the interaction between the 2 factors also being significant (temperature: p < 0.001, F = 1738.22, df = 55; salinity: p < 0.001, F = 8.49, df = 55), as the intra-annual patterns of variations differed among areas (Fig. 2).

Spatial differences in salinity were more consistent than those for temperature. Average salinities for all months differed significantly (p < 0.05) among all areas but the WNS and CNS, with consistently lower (p < 0.05) salinities in the ENS (annual average: 33.45 ± 1.52) and in the SNS (annual average: 34.48 ± 0.97) than in the rest of the study area. Salinities in the EC (annual average: 35.12 ± 0.07) and the NNS (annual average: 34.98 ± 0.25) also differed significantly from October to March (p < 0.05)
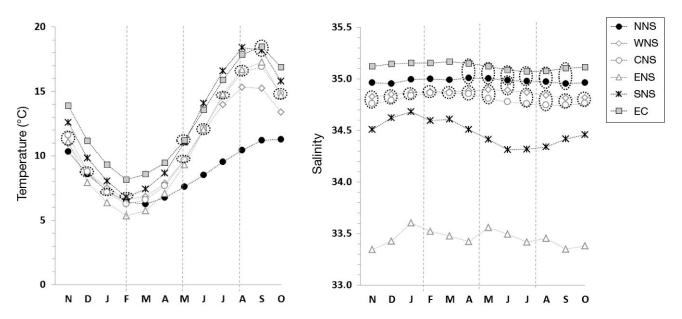
Fig. 2. Intra-annual variations in temperature and salinity within the study zone. For each of the 6 areas defined in Fig. 1 (NNS, WNS, CNS, ENS, SNS and EC), monthly means were calculated from the daily values predicted by the general estuarine transport model (GETM; see 'Materials and methods') in 1997, 1998 and 1999 over the full distribution of plaice *Pleuronectes platessa* in the area. For each month, dotted ellipses regroup areas with average values that did not differ significantly ($p \geq 0.05$)

and were always higher ($p < 0.05$) than those in the WNS (annual average: 34.86 ± 0.17) and CNS (annual average: 34.80 ± 0.45). Intra-annual variations in salinity were significant only in the SNS, with significantly ($p < 0.05$) lower average salinities from June to August (<34.32), and higher average salinities from December to March (>34.59), than during the rest of the year (34.42–34.51; Fig. 2). In the ENS, the average salinity also fluctuated throughout the year (minimum = 33.35 ± 1.53 in November, maximum = 33.60 ± 1.50 in January) but differences among months were not significant due to the high year-round variability in the salinity range (6.29–10.96) within the ENS.

Intra-annual temperature profiles were more similar among areas than salinities, with significant temporal variations ($p < 0.05$) irrespective of zone (Fig. 2). However, the amplitude of intra-annual variation varied among areas, being maximum in the ENS (total range: 1.62–21.65°C) and minimum in the NNS (total range: 4.02–18.00°C). The most contrasted thermal conditions were observed in the NNS (annual average: 8.69 ± 1.54°C), where average monthly temperatures were at least 1.74 to 4.87°C lower than in the rest of the study zone from May to October. Intra-annual variations in water temperature in the NNS, although significant ($p < 0.05$), were less marked, with minima around 6.5°C in February to April and maxima around 11.2°C in

September to October (Fig. 2). For the 5 other areas, intra-annual patterns of variation were alike and more pronounced, with minima consistently observed in February to March, and maxima in August to September (Fig. 2). However, average temperatures between areas differed significantly ($p < 0.05$) for most of the year, with minimum monthly values generally observed in the WNS (annual average: 10.70 ± 1.70°C) or the ENS (annual average: 10.73 ± 4.43°C) and maxima in the EC (annual average: 12.91 ± 2.09°C) or the SNS (annual average: 12.32 ± 2.25°C). The CNS (annual average: 11.08 ± 2.15°C) always had intermediate average temperatures (Fig. 2). Among-area differences depended on the period of the year. Thus, monthly temperatures were at the least 0.82 to 1.35°C greater on average in the EC than in the North Sea, but only from October to April. Similarly, in the WNS, they were at least 2.12 to 4.97°C higher on average than in the NNS and 1.17 to 1.41°C lower on average than in the rest of the study area, but only from August to October.

Area discrimination based on intra-annual otolith $\delta^{18}O$ values

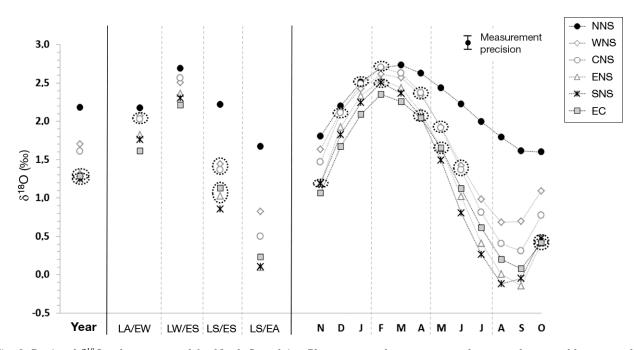As a result of variations in environmental conditions in the study zone, significant differences in

Fig. 3. Regional $\delta^{18}O$ values expected for North Sea plaice *Pleuronectes platessa* at annual, seasonal or monthly temporal scales for the 3 years investigated. For each area defined in Fig. 1, $\delta^{18}O$ means at the annual, 'seasonal' and monthly scales were calculated from the temperature and salinity data gathered for all cells with at least 10 geolocations. For each temporal scale, dotted ellipses regroup areas with average values that did not differ significantly ($p \geq 0.05$), when present. LW/ES: late winter/early spring, LS/EA: late summer/early autumn, LS/ES: late spring/early summer, LA/EW: late autumn/early winter

otolith $\delta^{18}O$ signatures were expected by area ($p < 0.001$, $F = 26\,328.18$, df = 5) and by month ($p < 0.001$, $F = 32\,419.57$, df = 11), the significant interaction of the 2 factors ($p < 0.001$, $F = 1126.69$, df = 55) indicating different patterns of intra-annual variation among areas.

With the exception of December, $\delta^{18}O$ signatures were predicted to differ significantly ($p < 0.05$) all year round among at least the NNS, the WNS and the ENS (Fig. 3). Consistently higher $\delta^{18}O$ values were predicted in the NNS (annual average: 2.18 ± 0.53‰) and lower $\delta^{18}O$ values in the ENS (annual average: 1.32 ± 0.77‰) than in the WNS (annual average: 1.71 ± 1.07‰). The difference in average $\delta^{18}O$ values among these 3 areas was predicted to be greater than 0.4‰ from June to October (Table S1), and seasonal otolith $\delta^{18}O$ signatures were expected to differ significantly ($p < 0.01$) in both LS/ES (with respective means of 1.03 ± 0.88‰ for ENS, 1.44 ± 0.56‰ for WNS and 2.22 ± 0.36‰ for NNS) and LS/EA (with respective means of 0.10 ± 0.88‰ for ENS, 0.83 ± 0.52‰ for WNS and 1.67 ± 0.57‰ for NNS). Seasonal otolith $\delta^{18}O$ values, especially those in LS/EA, were therefore predicted to allow confident area discrimination throughout the period of feeding ground 'residency' (in the NNS, WNS and ENS).

During the spawning migration (from November to April), constant discrimination only of the CNS (annual average: 1.61 ± 0.90‰) was predicted ($p < 0.05$) against the other areas where the spawning occurs (Fig. 3). Monthly $\delta^{18}O$ values in the CNS were always expected to be at least 0.15, 0.20 and 0.32‰ higher than those for the SNS, ENS and EC, respectively (Table S1). Monthly $\delta^{18}O$ signatures were also predicted to differ significantly from November to March between the SNS and the EC ($p < 0.05$), with average values in the EC being 0.11 to 0.16‰ lower, and between the ENS and the SNS ($p < 0.05$) in December, January and March, although average differences in $\delta^{18}O$ signatures for these months were only 0.07–0.10‰ (Fig. 3, Table S1). Consequently, expected seasonal otolith signatures for the 4 potential spawning zones were predicted to differ significantly both in LA/EW ($p < 0.05$) and LW/ES ($p < 0.05$), with mean values increasing from the EC (1.61 ± 0.46‰ in LA/EW and 2.22 ± 0.15‰ in LW/ES) to the CNS (2.03 ± 0.50‰ in LA/EW and 2.57 ± 0.22‰ in LW/ES), with the SNS and ENS having intermediate values (1.76 ± 0.51‰ and 1.82 ± 0.57‰ in LA/EW and 2.30 ± 0.30‰ and 2.38 ± 0.46‰ in LW/ES, respectively). However, otolith $\delta^{18}O$ signatures were expected to differ among all areas only in LW/ES, with the highest

Table 2. Monthly mean (range in parentheses) otolith $\delta^{18}O$ values predicted for each North Sea plaice *Pleuronectes platessa* sub-stock over the 3 yr period investigated (1997–1999) with corresponding temperature and salinity data

Month	Temperature (°C)	Salinity	$\delta^{18}O$ (‰)
Sub-stock A			
1	7.27 (5.16–9.95)	34.94 (34.14–35.17)	2.46 (2.25–2.59)
2	6.31 (3.43–8.12)	34.80 (31.82–35.17)	2.64 (2.34–2.75)
3	6.34 (3.96–7.88)	34.85 (31.27–35.21)	2.64 (2.05–2.79)
4	7.06 (5.81–9.10)	35.01 (34.31–35.18)	2.52 (2.31–2.64)
5	7.65 (6.15–10.47)	35.05 (34.80–35.17)	2.40 (2.05–2.56)
6	8.55 (7.13–11.38)	35.06 (34.82–35.17)	2.20 (1.83–2.42)
7	9.47 (7.03–14.09)	35.02 (34.82–35.16)	1.98 (1.48–2.43)
8	10.76 (7.47–16.43)	35.00 (34.61–35.17)	1.69 (0.76–2.32)
9	12.01 (8.69–16.23)	34.98 (34.64–35.17)	1.41 (0.77–2.06)
10	11.57 (8.69–15.91)	35.04 (34.72–35.17)	1.52 (0.93–2.08)
11	11.18 (8.65–13.95)	34.94 (33.90–35.16)	1.58 (1.32–1.85)
12	8.76 (6.49–11.62)	34.89 (33.67–35.18)	2.11 (1.94–2.35)
Global	8.22 (3.43–16.49)	34.94 (31.27–35.21)	2.58 (0.76–2.79)
Sub-stock B			
1	7.13 (3.25–10.72)	33.43 (31.65–35.15)	2.35 (2.04–2.59)
2	5.67 (1.93–8.07)	33.56 (28.40–35.14)	2.45 (1.81–2.70)
3	6.10 (3.98–7.90)	33.75 (29.67–35.11)	2.40 (1.79–2.71)
4	7.45 (4.87–10.38)	33.28 (25.23–35.13)	1.96 (0.54–2.47)
5	9.40 (6.70–13.25)	33.81 (31.10–34.99)	1.67 (1.13–2.07)
6	11.87 (9.02–15.99)	33.85 (32.15–34.97)	1.13 (0.76–1.60)
7	14.42 (10.42–18.21)	33.49 (30.57–35.00)	0.47 (−0.39–1.11)
8	16.82 (12.93–18.96)	33.45 (30.71–34.82)	−0.06 (−0.77– 0.21)
9	17.29 (15.09–19.04)	32.84 (30.70–34.82)	−0.32 (−0.73– 0.04)
10	14.69 (10.77–18.09)	33.29 (30.88–34.78)	0.36 (0.06–0.65)
11	11.10 (6.61–13.98)	33.32 (30.89–34.82)	1.15 (0.91–1.30)
12	8.44 (5.13–12.24)	34.00 (30.43–35.11)	1.93 (1.73–2.04)
Global	9.42 (1.93–19.04)	33.66 (25.23–35.15)	1.10 (−0.77 –2.71)
Sub-stock C			
1	8.35 (5.08–10.93)	34.99 (34.46–35.26)	2.22 (2.00–2.44)
2	6.97 (5.08–8.88)	34.87 (34.29–35.15)	2.51 (2.37–2.65)
3	6.97 (5.78–8.53)	34.78 (32.53–35.21)	2.48 (2.15–2.63)
4	8.08 (6.68–10.12)	34.89 (34.42–35.14)	2.26 (2.06–2.36)
5	10.17 (8.23–12.97)	34.88 (34.58–35.18)	1.79 (1.62–1.99)
6	15.43 (10.43–15.65)	34.86 (34.61–35.08)	1.21 (0.98–1.39)
7	16.58 (12.85–18.30)	34.85 (34.60–35.11)	0.63 (0.42–0.91)
8	15.77 (12.78–19.40)	34.83 (34.60–35.05)	0.38 (0.03–0.83)
9	12.01 (10.11–18.94)	34.84 (34.57–35.06)	0.57 (0.16–1.61)
10	14.08 (9.41–17.89)	34.83 (34.57–35.12)	0.91 (0.55–1.77)
11	12.60 (899–15.79)	34.90 (33.52–35.18)	1.26 (1.00–1.52)
12	9.06 (6.87–13.26)	34.76 (33.52–35.19)	2.00 (1.64–2.10)
Global	10.96 (5.08–19.40)	34.87 (33.67–35.26)	1.66 (0.03–2.65)

Sub-stock discrimination based on intra-annual otolith $\delta^{18}O$ values

Expected discrimination success at monthly and seasonal scales

Due to migration among areas during the year, the predicted intra-annual variations in otolith $\delta^{18}O$ signatures of the 3 sub-stocks never fully matched those of any single area in the study zone. Nonetheless, the reconstructed environmental conditions experienced by the 3 sub-stocks differed markedly (Table 2), as predicted from their geographically discrete summer feeding locations and winter migration routes (Fig. 1). Annual temperature profiles were comparable for sub-stocks B and C, with important seasonal variations and minima around 6–7°C in February–March while maxima around 16–17°C were observed in August–September (Table 2). By contrast, the average temperatures experienced by sub-stock A peaked at 12°C in September and very rarely exceeded 14°C. Annual salinity profiles were similar for sub-stocks A and C, with relatively constant average salinities (34.8–35.1) throughout the year (Table 2). This contrasted with sub-stock B, for which monthly salinities were <34.0 on average and more variable overall.

Consequently, predicted intra-annual otolith $\delta^{18}O$ values varied greatly among sub-stocks (Table S2A), ranging from 0.76 to 2.79‰ in sub-stock A, from −0.77 to 2.71‰ in sub-stock B and from 0.03 to 2.65‰ in sub-stock C, around average values of 2.58 ± 0.23‰, 1.10 ± 0.17‰ and 1.66 ± 0.16‰, respectively (Table 2). They varied significantly (p < 0.001) by month irrespective of sub-stock (Table S2C) and largely mirrored the seasonal temperature signal observed across the entire study area (Fig. 2): average monthly otolith $\delta^{18}O$ values were consistently expected to be highest (>2.40‰) in February–March (i.e. during the coldest months of the year) and lowest (<1.69‰) in August or September (i.e. during the warmest months), irrespective of the sub-stock (Table 2, Table S2C). However, intra-annual patterns of $\delta^{18}O$ variation (mean value and
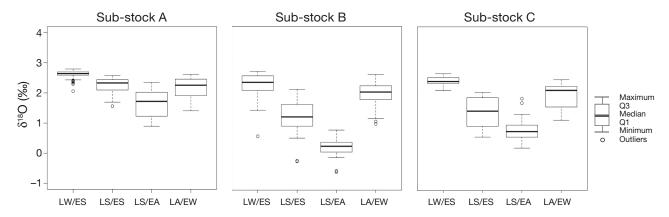
(p < 0.05) average predicted for the NNS (2.69 ± 0.12‰) and intermediate yet significantly different (p < 0.05) values in the WNS (2.51 ± 0.46‰) and the CNS (2.57 ± 0.22‰). In LA/EW, differences in average $\delta^{18}O$ signatures between these 2 latter areas (CNS = 2.03 ± 0.50‰; WNS = 2.07 ± 0.57‰) were too low to be significant (Fig. 3).

Fig. 4. Seasonal expected $\delta^{18}O$ values for each of the 3 North Sea plaice *Pleuronectes platessa* sub-stocks (A, B and C; see Fig. 1). In each case, $\delta^{18}O$ values were predicted from the environmental temperature and salinity ranges experienced by the fish using the equations of Kim et al. (2007) and Harwood et al. (2008). Seasons on the *x*-axis defined as in Fig. 3

amplitude) differed among sub-stocks (Table 2), mainly due to differences in the temperatures and salinities they experienced on their respective feeding grounds, in the NNS, ENS or WNS (Figs. 2 & 3). Consequently, predicted otolith $\delta^{18}O$ values varied significantly (p < 0.001) among sub-stocks irrespective of the month (Table S2B). However, they differed among all 3 sub-stocks only in April (p < 0.05), August (p < 0.05), September (p < 0.01) and October (p < 0.001). During the rest of the year, predicted otolith $\delta^{18}O$ values allowed constant discrimination between the northern and the 2 southern sub-stocks (p < 0.01), except during December, when predicted values were similar for sub-stocks A and C but differed significantly between the 2 southern sub-stocks (p < 0.05).

As a result of these differences, predicted otolith $\delta^{18}O$ values varied significantly (p < 0.001) by both season and sub-stock (Table S3A). With the exception of LS/ES and LA/EW for sub-stock A (Table S3C), otolith $\delta^{18}O$ values were consistently predicted to differ (p < 0.01) among seasons for a given sub-stock. Irrespective of sub-stock, $\delta^{18}O$ values were consistently the highest (p < 0.001) in LW/ES and the lowest (p < 0.001) in LS/EA, the other 2 seasons having intermediate values (Fig. 4, Table S3C). Otolith $\delta^{18}O$ signatures were also predicted to differ significantly (p < 0.01) by sub-stock, irrespective of season (Table S3B). However, they differed among all 3 sub-stocks only in LS/EA (p < 0.01). During the other 3 seasons, discrimination only of the northern from the 2 southern sub-stocks was predicted (p < 0.05) (Fig. 4, Table S3B).
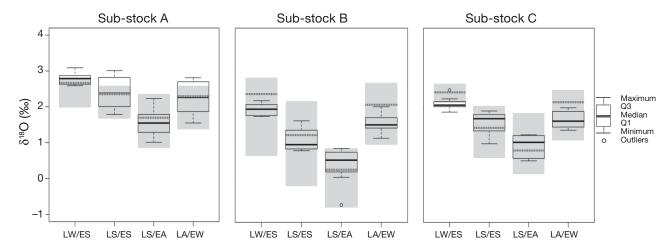

Fig. 5. Potential for 'seasonal' sub-stock discrimination using otolith $\delta^{18}O$ in North Sea plaice *Pleuronectes platessa*. In each case, the boxplot shows the otolith $\delta^{18}O$ values measured for each sub-stock (minimum, Q1, median, Q3 and maximum values, outliers; see Fig. 1 for sub-stocks), and the range of otolith $\delta^{18}O$ values predicted from the corresponding environmental data is indicated (grey shade), the dotted grey line showing the median predicted value. Seasons on the *x*-axis defined as in Fig. 3
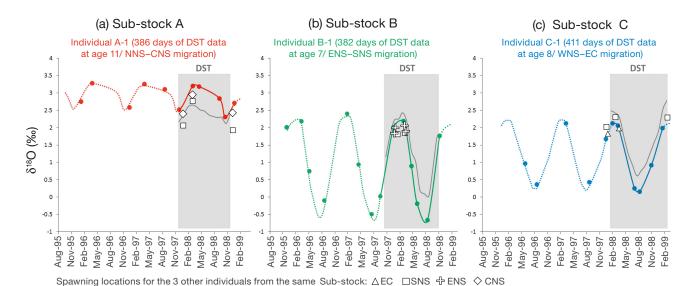
Fig. 6. Potential for seasonal geolocation using otolith $\delta^{18}O$ signatures in plaice *Pleuronectes platessa* from sub-stocks (a) A, (b) B and (c) C (see Fig. 1). Solid circles represent the intra-annual otolith $\delta^{18}O$ values measured during the final 3 yr of life in 3 individuals (1 sub-stock^{-1}) with known spawning locations in 2 successive winters (Table 1). To investigate fidelity to migration routes, intra-annual values are plotted above the repeated 'typical' annual pattern derived from otolith $\delta^{18}O$ values measured during data storage tag (DST) recording time (coloured curve, solid during tag recording time, dotted for the pre-tag recording period). The predicted $\delta^{18}O$ signal based on tag data is plotted as the solid grey line. Open symbols represent otolith $\delta^{18}O$ values measured during the spawning migrations in the same winters (1997–98 or 1998–99) for 3 other individuals from the same sub-stock (Table 1), 1 from each migration type (see legend for spawning locations, defined in Fig. 1)

Consistency between predicted and observed 'seasonal' otolith $\delta^{18}O$ values

For the majority of our observations, measured and predicted seasonal otolith $\delta^{18}O$ values were consistent (Fig. 5); however, small differences between them were observed. For several individuals, $\delta^{18}O$ measures fell outside the expected ranges, with values 0.20‰ lower than the minimum predicted in the LW/ES for sub-stock C, and values 0.21–0.44‰ higher than the maxima predicted for all seasons but LS/EA for sub-stock A. Nonetheless, when observed $\delta^{18}O$ values did not match the predictions for a given season (e.g. in the LW/ES for sub-stocks A and C), the differences in $\delta^{18}O$ among stocks were consistently above the expected values, maintaining the potential for sub-stock discrimination.

As predicted, measured otolith $\delta^{18}O$ values differed significantly by sub-stock irrespective of season (Table S4B), allowing constant discrimination of the northern from the 2 southern sub-stocks during the year (p < 0.05) and robust differentiation of all 3 sub-stocks in LS/EA (p < 0.001). In LS/EA, the differences in average measured otolith $\delta^{18}O$ values among sub-stocks were even greater than expected (Fig. 5). Intra-annual variations in measured $\delta^{18}O$ values also followed those predicted from temperature and salinity conditions irrespective of the sub-stock (Fig. 5).

The $\delta^{18}O$ values were consistently predicted to be the greatest (p < 0.01) in LW/ES and the lowest (p < 0.001) in LS/EA (Fig. 5, Table S4C). Moreover, measured $\delta^{18}O$ signatures differed among all seasons (p < 0.01) for a given sub-stock, except for LS/ES and LA/EW in sub-stock A (Table S4C).

Discriminating migratory behaviours based on otolith $\delta^{18}O$ signatures

By matching the intra-annual $\delta^{18}O$ profiles predicted for 1998 with sampled values from the corresponding annual otolith growth rings (Fig. 6), we were able to confirm the broad timing of deposition of the opaque and translucent bands, irrespective of the sub-stock. Indeed, otolith samples taken at the translucent to opaque transition (deposited in LW/ES, Van Neer et al. 2004) consistently had the highest $\delta^{18}O$ values, matching the maximum values predicted for February–March. The lowest $\delta^{18}O$ values were consistently taken from the opaque to translucent transition (deposited in LS/EA; Van Neer et al. 2004), matching the minimum values predicted for August–October. However, the respective widths of the opaque and translucent bands varied among individuals, depending on the age of the fish at the time of deposition but also, apparently, by sub-stock.

Matching the growth band signatures to predicted monthly $\delta^{18}O$ values for the corresponding sub-stock confirmed this pattern, and suggested more rapid growth over the autumn and winter months in sub-stock A, over the spring and summer months in sub-stock C and constant growth all year long in sub-stock B. Depending on the sub-stock, the measured $\delta^{18}O$ signal was also consistently lower (sub-stocks B and C) or higher (sub-stock A) than that predicted from the environmental conditions, with mismatches between predicted and measured signatures ranging from 0.04 to 0.51‰ (Fig. 6).

In spite of the observed mismatches, the different intra-annual $\delta^{18}O$ profiles observed for 1998 in the 12 fish investigated suggest that otolith oxygen signatures can confidently discriminate most migratory behaviours in North Sea plaice (Fig. 6). The $\delta^{18}O$ signatures recorded in 1998 confirmed the value of LS/EA $\delta^{18}O$ values for geolocation of plaice among the NNS (1.92 to 3.15‰), the WNS (0.33 to 0.89‰) and the ENS (−0.78 to 0.29‰) within the period corresponding to feeding ground residency. Analysis of the $\delta^{18}O$ signatures obtained for all 6 migration types in the migration period in 1997−98 and 1998−99 (N = 27 from 12 fish) further suggested that the fish from sub-stocks A and C with different spawning locations have discriminable otolith signatures in LA/EW and LW/ES (Fig. 6). This observation is consistent with the $\delta^{18}O$ values predicted from the environmental conditions prevailing in the areas traversed for each spawning migration. Consequently, based on the predicted differences in otolith signatures at this time of the year between the CNS (2.03 ± 0.50‰ in LA/EW and 2.57 ± 0.22‰ in LW/ES) and the SNS (1.76 ± 0.51‰ in LA/EW and 2.30 ± 0.30‰ in LW/ES), observed signatures for these 2 seasons were higher in the fish of sub-stock A spawning in the CNS (2.30−2.59‰ and 2.87−3.29‰ for LA/EW and LW/ES, respectively) than for those spawning in the SNS (1.87−2.27‰ and 2.67−3.02‰, respectively). Similarly, the differences in expected otolith signatures in the EC (1.61 ± 0.46‰ in LA/EW and 2.22 ± 0.15‰ in LW/ES) and in the SNS (1.76 ± 0.51‰ in LA/EW and 2.30 ± 0.30‰ in LW/ES) could explain the slightly different otolith signatures recorded by the fish from sub-stock C spawning in the EC (1.77−1.92‰ in LA/EW and 2.02−2.11‰ in LW/ES) and in the SNS (2.02−2.27‰ in LA/EW and 2.27−2.51‰ in LW/ES). Finally, the comparable values predicted for both seasons in the SNS and the ENS (1.76 ± 0.51‰ and 1.82 ± 0.57‰ in LA/EW and 2.30 ± 0.30‰ and 2.38 ± 0.46‰ in LW/ES, respectively) resulted in non-distinguishable $\delta^{18}O$ values for both seasons in the fish of

sub-stock B, with values of 1.71−1.95‰ for LA/EW and of 1.80−2.18‰ for LW/ES.

Site fidelity and repeated patterns of behaviour

Analysis of the $\delta^{18}O$ values in the years prior to tagging suggested a high degree of fidelity to summer feeding and winter spawning grounds (Fig. 6). The pre-tagging summer signatures (N = 25) indicate feeding ground (sub-stock) fidelity for at least 2 successive years in all of our sampled fish. Indeed, the LS/EA pre-tagging $\delta^{18}O$ values measured (all comprised between 1.59 and 2.39‰, −0.46 and 1.24‰ and 0.45 and 1.40‰ in the fish assigned to sub-stocks A, B and C, respectively) remained relatively constant, irrespective of the individual, inter-annual differences for a given fish ranging from 0.02 to 0.28‰.

Fidelity to spawning site was more complicated to interpret, as average differences in $\delta^{18}O$ seasonal signatures between spawning migration types for sub-stocks A and C were only of ~0.2‰, i.e. close to the detection limit (0.07‰). However, observed signatures for pre-tagging LA/EW seasons in fish assigned to sub-stock A and spawning sites in the CNS or in the SNS ranged from 2.21 to 2.70‰ and from 1.87 to 2.27‰, respectively. For sub-stock C fish, signatures ranged between 2.11 and 2.32‰ and between 1.87 and 2.27‰ depending on whether, in 1998, the fish had spawned in the SNS or in the EC, respectively. For sub-stock B fish assigned, signatures ranged between 1.78 and 2.01‰ irrespective of the spawning location identified during DST recording time.

DISCUSSION

Otolith $\delta^{18}O$ signatures have widely been used to infer past environmental and/or migratory histories of fish (e.g. Surge & Walker 2005, Shephard et al. 2007, Rooker et al. 2008, Imsland et al. 2014), and scientists are increasingly seeking applications for tracking fish geographical movements based on oxygen isoscapes (e.g. Wunder 2010, Trueman et al. 2012, Torniainen et al. 2017). Individual experience and environmental variation, however, have not previously been considered to any significant extent in fish studies involving otolith $\delta^{18}O$ (e.g. Stephenson 2001, Imsland et al. 2014, Torniainen et al. 2017). This results primarily from the difficulties in obtaining accurate corresponding positional and environmental data (Begg et al. 2005, Tanner et al. 2016). The first attempt to address this shortcoming (Dar-

naude et al. 2014) demonstrated that otolith $\delta^{18}O$ in adult North Sea plaice largely reflected the differences in ambient temperatures and salinities experienced by wild fish. Furthermore, annual $\delta^{18}O$ values allowed robust re-assignment of the same fish to their 3 local sub-stocks. The data presented here greatly extend the previous study, as location-specific daily estimates of temperature and salinity for the migrations of individual, free-swimming fish in their natural environment have for the first time been matched with oxygen isotope data from the same individuals at a sub-annular scale, linking environmental variation and otolith $\delta^{18}O$ to fish geographical distribution. As far as we are aware, our results provide the first example documenting the scope and limits for applying otolith $\delta^{18}O$ values to reconstruct the past migration histories of a wild marine fish.

Likely causes of mismatches between expected and observed otolith $\delta^{18}O$ values

As in Darnaude et al. (2014), we used measured and modelled environmental data in our study to predict otolith $\delta^{18}O$ values from fish positions estimated from archival tag data. Although this approach generates robust predictions of otolith $\delta^{18}O$ values, both for individual fish and for sub-stocks (Darnaude et al. 2014), we acknowledge 2 inherent and largely unavoidable potential sources of error in this process. The first relates to the estimation of fish location (geolocation) and to the estimation of complementary environmental data in those locations where *in situ* measurements were unavailable. However, these 2 sources of bias have only limited impact (<0.2‰), even when combined, on the majority of monthly otolith $\delta^{18}O$ predictions across the North Sea area (Darnaude et al. 2014). Another potential source of error lies in the estimation of water $\delta^{18}O$ from salinity. However, by applying the North Sea-specific equation developed by Harwood et al. (2008), again we are confident that our dataset is predominantly accurate. Unavoidably, localised inputs from the Rhine and Elbe Rivers can influence the $\delta^{18}O$ salinity relationship (Harwood et al. 2008), but any resulting errors in our estimations would be localised primarily to sub-stock B.

A third possible, and more likely, source of error in our seasonal predictions is linked to sub-stock-specific variation in plaice otolith growth rate during the year. The incorporation of such variations in growth when calculating expected otolith annual $\delta^{18}O$ values by sub-stock, from monthly, environmental-based

$\delta^{18}O$ estimates, has already been shown to have a marked influence on the prediction accuracy (Darnaude et al. 2014). Like the few additional studies that have investigated sub-annual variation in otolith $\delta^{18}O$ signatures (e.g. Høie & Folkvord 2006, Kastelle et al. 2017), we did not apply differential monthly weighting when calculating seasonal $\delta^{18}O$ signals expected from the fish position-linked environmental data. This may have slightly biased our predictions, as some of our observations suggested more rapid growth over autumn and winter in sub-stock A, spring and summer in sub-stock C and constant year-long growth in sub-stock B. An explanation for these differences lies in the distinct annual ambient temperature profiles for sub-stock A (Darnaude et al. 2014) and variation in the timing and duration of migration among the sub-stocks (Hunter et al. 2004). Because otolith growth rate has been shown to reflect fish metabolic rates (Grønkjær 2016) it should reflect the variation in metabolism documented for female plaice (Rijnsdorp & Ibelings 1989, Fonds et al. 1992, Bromley 2000). In plaice, metabolism is mainly related to temperature and feeding (Fonds et al. 1992). Maximum otolith growth is therefore expected in the warmest months for each sub-stock (i.e. from June to October in sub-stocks B and C, but from September to November in sub-stock A). However, female plaice feed intensely during spring to build reserves for gametogenesis commencing in July (Rijnsdorp 1989), then cease feeding and reduce their metabolic rates during their spawning migrations, due to limited metabolic scope that precludes the simultaneous oxygen demands for spawning and feeding (Rijnsdorp & Ibelings 1989). Although no study has yet investigated temporal variation in feeding levels or metabolic rates among the 3 sub-stocks studied here, the timing of otolith edge deposition in North Sea plaice varies by the area of capture, in relation to the interruption of somatic growth in winter (Van Neer et al. 2004). Several studies (e.g. Harding et al. 1978, Bromley 2000) have also shown that spawning occurs progressively later with latitude, with peak spawning generally observed in December–January in the EC, in January–February in the SNS and in February–March in the CNS and ENS. Fish from sub-stock A, which spawn either in the SNS or CNS, are therefore more likely to cease otolith accretion in spring, while those of sub-stock C, which spawn either in the EC or SNS, are more likely to exhibit reduced otolith growth in autumn and winter. Almost constant otolith growth is most likely in sub-stock B otoliths since, as many sub-stock B fish spawn in the ENS (Hunter et al. 2004), in areas

relatively close to their summer feeding grounds, their fasting duration during spawning may be more limited. For individual fish, therefore, we are unable to entirely exclude possible errors in the positioning of some seasonal growth bands on the otolith. Given the 3-dimensional structure of the otoliths, the drilling depth (450 µm) used in this study might also have resulted in partial contamination of our sub-seasonal samples with material from earlier or later periods in the year, particularly since we used frontal sections for this. However, all otoliths in the present study were cut and polished down with the specific aim to obtain 500 to 600 µm thick sections along this plane with the otolith edge (and its most outer growth bands) as perpendicular to the section surface as possible. Therefore we are confident that the bias in sub-seasonal δ¹⁸O induced by such contamination, when present, was limited.

This having been said, position-based errors do not fully explain the divergence between measured and predicted seasonal otolith δ¹⁸O values. Indeed, they would only result in an attenuation of the δ¹⁸O signal when, conversely, some of the δ¹⁸O values measured (for example those observed for sub-stock A in LW/ES) were greater than any of those predicted in the study area, even at the monthly scale. Our intra-annual measured δ¹⁸O values repeatedly diverged from predicted values, even when using *in situ* temperature tag records from the same fish (Fig. 6). This mismatch was previously identified in Darnaude et al. (2014), where it is discussed at length. In North Sea plaice, vital effects appear to alter the δ¹⁸O signal ultimately recorded in otoliths, both through variations in the otolith deposition rate during the year and through changes in the water-otolith oxygen isotopic fractionation, at least during certain seasons. Ideally, multi-stock validation studies across a wide range of ontogenies are required to establish a more comprehensive understanding of the relationship between fish metabolism and otolith δ¹⁸O.

Optimizing δ¹⁸O values to reconstruct environmental and migratory histories

Our results demonstrate how sub-annual otolith δ¹⁸O signatures can successfully reveal the spatial and temporal details of annual migration cycles. Such otolith δ¹⁸O-based geolocation can be achieved only by a comprehensive understanding of the spatial dynamics of the target species, coupled with an adequately detailed accumulation of matching data mapping environmental variation across the species

range. This cross-referencing of datasets further allows optimization of the temporal scale to be adopted for otolith sampling in order to maximize discrimination/geolocation potential. In our case, partitioning the calendar year into LW/ES, (February to April), LS/ES (May to July), LS/EA (August to October) and LA/EW (November to January) instead of traditional seasons optimized our ability to apply otolith δ¹⁸O signatures to discriminate among sub-stocks and spawning migration types, and to capture the maximal amplitude of δ¹⁸O intra-annual fluctuations irrespective of the fish.

δ¹⁸O signatures for LS/EA, LW/ES and LA/EW were the most valuable for characterizing differences in both environmental and migratory histories. In particular, LS/EA included the 3 months (August–October) with the lowest predicted δ¹⁸O values, irrespective of sub-stock. Monthly values in this season were also consistently expected to differ significantly among all sub-stocks (B < C < A) due to their residency, at this time, in discrete areas with contrasted temperatures (especially in the colder NNS) and different salinities (mainly in the ENS). The measured δ¹⁸O values for LS/EA therefore allowed robust discrimination of all 3 sub-stocks, ranging mostly (>75%) between 1.3 and 2.2‰ in sub-stock A, between 0.2 and 0.8‰ in sub-stock B, and between 0.6 and 1.2‰ in sub-stock C. LW/ES included the 2 months (February and March) with the highest expected monthly δ¹⁸O values during the year, irrespective of sub-stock, and 1 month (April) where the expected δ¹⁸O values differed significantly between all sub-stocks (B < C < A). Accordingly, measured otolith δ¹⁸O values for this season allowed discrimination of fish from sub-stock A (>2.5‰) from those of sub-stocks B and C (1.7–2.2‰ and 1.9–2.3‰, respectively). Measured δ¹⁸O signatures for the 6 migration types examined in 1998 further suggest that fish from sub-stocks A and C with different spawning locations have discriminable LA/EW signatures, as predicted from prevailing environmental conditions in their respective migration transit areas. Our results require further validation with larger sample sizes, but suggest LA/EW otolith δ¹⁸O signatures of 1.8–2.3‰ and 2.3–2.6‰ in sub-stock A fish spawning in the SNS and the CNS, respectively. In sub-stock C fish spawning in the EC and the SNS, concomitant signatures appear to range from 1.7 to 2.0‰ and from 2.0 to 2.3‰, respectively, while in sub-stock B fish, they seem to range from 1.7 to 2.0‰, irrespective of the spawning locations (SNS or ENS). We are therefore confident that feeding site location will be identifiable for all plaice females using LS/EA otolith δ¹⁸O

values, and 4 out of the 6 spawning migration types should equally be recognizable using LA/EW and LW/ES ones. Given the fine-scale spatial and temporal variations in $\delta^{18}O$ observed, higher otolith sampling resolution, e.g. by using a secondary ion mass spectrometer (SIMS) ion microprobe (Matta et al. 2013), could further improve this categorisation. However, the time and cost of SIMS is often currently impractical for large sample sizes.

The ground-truth validation approach applied here shows that seasonal samples derived from high-resolution micro-milling represent a non-negligible addition to the information on plaice migratory behaviour in the North Sea beyond the results obtained using annual otolith signatures only (Darnaude et al. 2014). The seasonal otolith $\delta^{18}O$ values laid down during and prior to tagging confirmed inter-annual fidelity to summer feeding sites (N = 25), as previously suggested from multi-annual archival tag records (Hunter et al. 2003a). Otolith $\delta^{18}O$ values further allowed multiple-year evidence of spawning site fidelity, at least in sub-stocks A and C. This is an important observation in that the vulnerability of fish stocks to exploitation by fisheries is often related to the degree of site fidelity exhibited by fish stocks (Sadovy de Mitcheson et al. 2008, Erisman et al. 2017), but this characteristic is often inferred, rather than directly measured.

Otolith $\delta^{18}O$ as a natural tag

As well as providing significant insights into plaice population structure and spatial dynamics (Hunter et al. 2004), the hundreds of plaice released bearing archival tags allowed us to test whether measurement of otolith $\delta^{18}O$ could provide similar population-level information. We were able to correctly identify sub-stock membership for most plaice using otolith $\delta^{18}O$ values, and achieved broad-scale geolocation on a finer temporal scale than is currently applied to most offshore fisheries area-based management (Kell et al. 2004). This fully validates $\delta^{18}O$ as an alternative natural tag for fish geolocation and stock identification in offshore environments, and we suggest that the technique can be applied to study other shelf-species in well-described systems.

Our results emphasize that geolocation accuracy using otolith $\delta^{18}O$ is dependent on the variability of the water masses frequented both in terms of temperature and of salinity. The low assumed variability in offshore salinity in marine studies has led to an assumption in previous studies that otolith $\delta^{18}O$ variations in marine fish otoliths reflect movements between water masses with distinct temperatures (e.g. Dorval et al. 2011, Imsland et al. 2014, Javor & Dorval 2014). However, local variation in sea salinity, especially in the coastal zone (Harwood et al. 2008), can affect otolith $\delta^{18}O$, as already shown for species migrating to coastal brackish or hypersaline habitats (Northcote et al. 1992, Bastow et al. 2002, Walther et al. 2011, Stanley et al. 2015). Our results demonstrate that in regions with very similar salinities, such as the CNS and WNS, differences in temperature of just 2°C induce significant differences in predicted otolith $\delta^{18}O$ values. Spatial differences in salinity of just 1 psu can however result in mismatches in regional classification between temperature and otolith $\delta^{18}O$. We emphasize therefore that a basic knowledge of apparently stable local salinities is imperative for the successful assessment of marine fish spatial movements or stock membership using otolith $\delta^{18}O$.

Because most commercially exploited marine fish are poikilotherms (Carey et al. 1971) that may experience high variability of ocean temperatures (Levitus & Antonov 1995) and often exist as discontinuous sub-stocks (Metcalfe 2006), otolith $\delta^{18}O$ use could have widespread application. However, geolocation efficiency using otolith $\delta^{18}O$ is likely to be species-specific (Stanley et al. 2015). In pelagic thermoregulating fishes such as tunas, where variations in internal temperature are low (Block & Finnerty 1994, Block et al. 2001), otolith $\delta^{18}O$ is thus unlikely to be practicable as a tag. Vertical migrations are also likely to bias otolith $\delta^{18}O$-based estimates of fish geolocation, making the technique difficult to apply to mesopelagic or vertically active species (e.g. Righton et al. 2016). Because plaice, like other flatfish, spend much of their life on or close to the seabed (Hunter et al. 2009), the risk of confounding effects due to vertical movements with geographical migrations is limited. Other potential candidates include cod *Gadus morhua*, which can spend protracted periods of time resting on the seabed (Righton et al. 2001). Cod otolith $\delta^{18}O$ values are already applied in ageing (Kastelle et al. 2017), but broader baseline knowledge of bottom temperature and salinities should be sufficient to produce geographical isoscapes of expected otolith $\delta^{18}O$, in turn allowing the efficacy of otolith $\delta^{18}O$ for fish geolocation to be predicted for the area. Finally, our results support the existence of inter-stock differences in physiology that could sometimes affect the environmental signal recorded in fish otoliths (Darnaude et al. 2014), by shifting $\delta^{18}O$ values upward (like here in sub-stock A) or downward (in sub-stocks B and C), depending on the stock.

The ability to successfully gauge temporal resolution in otolith sampling is also essential for the successful exploitation of otolith δ^{18}O as a natural tag. With few exceptions (e.g. Zazzo et al. 2006, Dufour et al. 2008, Torniainen et al. 2017), previous isotopic studies examining otolith δ^{18}O have been based, at best, on results from annual otolith samples. Our results demonstrate, for plaice at least, that geolocation potential is high at a broad spatial scale, but that spatial discrimination based on annual otolith δ^{18}O is poor when compared to monthly or even seasonal values. Furthermore, because the spatial discriminatory power of otolith δ^{18}O varies over the course of the year, the timing and extent of spatial movements in migrating fish will inevitably bias assessments of spatial distribution based on annual δ^{18}O alone. In our study, the season with maximal inter-regional differences in otolith δ^{18}O (here the summer) corresponded with the period of fish residency in geographically distinct areas (here from June to October), greatly enhancing sub-stock discrimination, even from annual δ^{18}O values. Where no prior knowledge of seasonal movements and environmental histories is available, otolith analysis at the highest possible level of resolution will clearly be of benefit for accurate stock separation or geolocation studies.

CONCLUSION

Beyond sub-stock discrimination based on annual otolith δ^{18}O, sub-annual sampling of the otolith allows regional geolocation with a seasonally dependent, but relatively accurate degree of resolution, commensurate to the scale of migration in our study species. This technique can potentially yield simple geo-referenced data that may be valuable for fish stock conservation and sustainable fisheries management. Successful application of δ^{18}O values as a natural tag does, however, require baseline knowledge of temperature and salinity across the geographical range of the target species and of potential population-specific vital effects during oxygen uptake/incorporation into otolith aragonite. For plaice, otolith δ^{18}O values act as an effective low-cost natural tag, the results from which can complement and extend observations from other methodologies used to describe population structure. These data have an immediate application for the description of stock movements and management areas occupied, for example to predict the potential impacts of management strategies, such as area closures to fishing.

LITERATURE CITED

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Ashford J, Jones C (2007) Oxygen and carbon stable isotopes in otoliths record spatial isolation of Patagonian toothfish (*Dissostichus eleginoides*). Geochim Cosmochim Acta 71:87–94

Ayvazian SG, Bastow TP, Edmonds JS, How J, Nowara GB (2004) Stock structure of Australian herring (*Arripis georgiana*) in southwestern Australia. Fish Res 67:39–53

Bastow TP, Jackson G, Edmonds JS (2002) Elevated salinity and isotopic composition of fish otolith carbonate: stock delineation of pink snapper, *Pagrus auratus*, in Shark Bay, Western Australia. Mar Biol 141:801–806

Begg GA, Campana SE, Fowler AJ, Suthers IM (2005) Otolith research and application: current directions in innovation and implementation. Mar Freshw Res 56:477–483

Blamart D, Escoubeyrou K, Juillet-Leclerc A, Ouahdi R (2002) Composition isotopique δ^{18}O – δ^{13}C des otolithes des populations de poissons récifaux de Taiaro (Tuamotu, Polynésie française): implications isotopiques et biologiques. C R Biol 325:99–106

Block BA, Finnerty JR (1994) Endothermy in fishes: a phylogenetic analysis of constraints, predispositions, and selection pressures. Environ Biol Fishes 40:283–302

Block BA, Dewar H, Blackwell SB, Williams TD and others (2001) Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. Science 293:1310–1314

Block BA, Jonsen ID, Jorgensen SJ, Winship AJ and others (2011) Tracking apex marine predator movements in a dynamic ocean. Nature 475:86–90

Botsford LW, Brumbaugh DR, Grimes C, Kellner JB and others (2009) Connectivity, sustainability, and yield: bridging the gap between conventional fisheries management and marine protected areas. Rev Fish Biol Fish 19:69–95

Bromley PJ (2000) Growth, sexual maturation and spawning in central North Sea plaice (*Pleuronectes platessa* L.), and the generation of maturity ogives from commercial catch data. J Sea Res 44:27–43

Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 188:263–297

Carey FG, Teal JM, Kanwisher JW, Lawson KD (1971) Warm-bodied fish. Am Zool 11:137–145

Coplen TB, Kendall C, Hopple J (1983) Comparison of stable isotope reference material. Nature 302:236–238

Darnaude AM, Sturrock A, Trueman CN, Mouillot D, Craven JA, Campana SE, Hunter E (2014) Listening in on the past: What can otolith δ¹⁸O values really tell us about the environmental history of fishes? PLOS ONE 9:e108539

Devereux I (1967) Temperature measurements from oxygen isotope ratios of fish otoliths. Science 155:1684–1685

Dorval E, Piner K, Robertson L, Reiss CS, Javor B, Vetter R (2011) Temperature record in the oxygen stable isotopes of Pacific sardine otoliths: experimental vs. wild stocks from the Southern California Bight. J Exp Mar Biol Ecol 397:136–143

Dufour E, Höök TO, Patterson WP, Rutherford ES (2008) High-resolution isotope analysis of young alewife *Alosa pseudoharengus* otoliths: assessment of temporal resolution and reconstruction of habitat occupancy and thermal history. J Fish Biol 73:2434–2451

Epstein S, Mayeda T (1953) Variations in ¹⁸O contents of waters from natural sources. Geochim Cosmochim Acta 4:213–224

Erisman B, Heyman W, Kobara S, Ezer T, Pittman S, Aburto-Oropeza O, Nemeth RS (2017) Fish spawning aggregations: where well-placed management actions can yield big benefits for fisheries and conservation. Fish Fish 18: 128–144

Fonds M, Cronie R, Vethaak AD, Van Der Puyl P (1992) Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. J Sea Res 29: 127–143

Gao YW, Bean D (2008) Stable isotope analyses of otoliths in identification of hatchery origin of Atlantic salmon (*Salmo salar*) in Maine. Environ Biol Fishes 83:429–437

Geffen AJ (2012) Otolith oxygen and carbon stable isotopes in wild and laboratory-reared plaice (*Pleuronectes platessa*). Environ Biol Fishes 95:419–430

Geffen AJ, Morales-Nin B, Gillanders BM (2016) Fish otoliths as indicators in ecosystem based management: results of the 5th International Otolith Symposium (IOS2014). Mar Freshw Res 67:i–iv

Godiksen JA, Svenning MA, Dempson JB, Marttila M, Storm-Suke A, Power M (2010) Development of a species-specific fractionation equation for Arctic charr (*Salvelinus alpinus* (L.)): an experimental approach. Hydrobiologia 650:67–77

Grønkjær P (2016) Otoliths as individual indicators: a reappraisal of the link between fish physiology and otolith characteristics. Mar Freshw Res 67:881–888

Harding J, Nichols JH, Tungate DS (1978) The spawning of plaice (*Pleuronectes platessa* L.) in the southern North Sea and English Channel. Rapp P-V Réun Cons Int Explor Mer 172:102–113

Harwood AJP, Dennis PF, Marca AD, Pilling GM, Millner RS (2008) The oxygen isotope composition of water masses within the North Sea. Estuar Coast Shelf Sci 78:353–359

Hays GC, Ferreira LC, Sequeira AMM, Meekan MG and others (2016) Key questions in marine megafauna movement ecology. Trends Ecol Evol 31:463–475

Hixon MA, Pacala SW, Sandin SA (2002) Population regulation: historical context and contemporary challenges of open vs. closed systems. Ecology 83:1490–1508

Høie H, Folkvord A (2006) Estimating the timing of growth rings in Atlantic cod otoliths using stable oxygen isotopes. J Fish Biol 68:826–837

Høie H, Andersson C, Folkvord A, Karlsen O (2004) Precision and accuracy of stable isotope signals in otoliths of pen-reared cod (*Gadus morhua*) when sampled with a high-resolution micromill. Mar Biol 144:1039–1049

Hunter E, Metcalfe JD, Reynolds JD (2003a) Migration route and spawning area fidelity by North Sea plaice migration. Proc Biol Sci 270:2097–2103

Hunter E, Aldridge JN, Metcalfe JD, Arnold GP (2003b) Geolocation of free-ranging fish on the European continental shelf as determined from environmental variables. I. tidal location method. Mar Biol 142:601–609

Hunter E, Metcalfe JD, Arnold GP, Reynolds JD (2004) Impacts of migratory behaviour on population structure in North Sea plaice. J Anim Ecol 73:377–385

Hunter E, Cotton RJ, Metcalfe JD, Reynolds JD (2009) Large-scale variation in seasonal swimming patterns of plaice in the North Sea. Mar Ecol Prog Ser 392:167–178

Hussey NE, Kessel ST, Aarestrup K, Cooke SJ and others (2015) Aquatic animal telemetry: a panoramic window into the underwater world. Science 348:1255642

Imsland AK, Ólafsson K, Skírnisdóttir S, Gunnarsson S and others (2014) Life history of turbot in Icelandic waters: intra- and inter-population genetic diversity and otolith tracking of environmental temperatures. Fish Res 155: 185–193

Javor B, Dorval E (2014) Geography and ontogeny influence the stable oxygen and carbon isotopes of otoliths of Pacific sardine in the California Current. Fish Res 154:1–10

Kastelle CR, Helser TE, McKay JL, Johnston CG, Anderl DM, Matta ME, Nichol DG (2017) Age validation of Pacific cod (*Gadus macrocephalus*) using high-resolution stable oxygen isotope (δ¹⁸O) chronologies in otoliths. Fish Res 185:43–53

Kell LT, Scott R, Hunter E (2004) Implications for current management advice for North Sea plaice: Part I. Migration between the North Sea and English Channel. J Sea Res 51:287–299

Kim ST, O'Neil JR, Hillaire-Marcel C, Mucci A (2007) Oxygen isotope fractionation between synthetic aragonite and water: influence of temperature and Mg²⁺ concentration. Geochim Cosmochim Acta 71:4704–4715

Kimirei IA, Nagelkerken I, Trommelen M, Blankers P and others (2013) What drives ontogenetic niche shifts of fishes in coral reef ecosystems? Ecosystems 16:783–796

Levitus S, Antonov J (1995) Observational evidence of inter-annual to decadal-scale variability of the subsurface temperature-salinity structure of the World Ocean. Clim Change 31:495–514

Lychakov DV, Rebane YT, Lombarte A, Demestre M, Fuiman LA (2008) Saccular otolith mass asymmetry in adult flatfishes. J Fish Biol 72:2579–2594

Matta ME, Orland IJ, Ushikubo T, Helser TE, Black BA, Valley JW (2013) Otolith oxygen isotopes measured by high-precision secondary ion mass spectrometry reflect life history of a yellowfin sole (*Limanda aspera*). Rapid Commun Mass Spectrom 27:691–699

Metcalfe JD (2006) Fish population structuring in the North Sea: understanding processes and mechanisms from studies of the movements of adults. J Fish Biol 69: 48–65

Metcalfe JD, Righton D, Eastwood P, Hunter E (2008) Migration and habitat choice in marine fishes. In: Magnhagen C, Braithwaite VA, Forsgren E, Kapoor BG (eds) Fish behaviour. Science Publishers, Enfield, NH, p 187–133

Morrongiello JR, Thresher RE, Smith DC (2012) Aquatic biochronologies and climate change. Nat Clim Chang 2: 849–857

Mugiya Y, Uchimura T (1989) Otolith resorption induced by anaerobic stress in the goldfish, *Carassius auratus*. J Fish Biol 35:813–818

Newman SJ, Wright IW, Rome BM, Mackie MC and others (2010) Stock structure of grey mackerel, *Scomberomorus semifasciatus* (Pisces: Scombridae) across northern Australia, based on otolith stable isotope chemistry. Environ Biol Fishes 89:357–367

Northcote TG, Hendy CH, Nelson CS, Boubee JAT (1992) Tests for migratory history of the New Zealand common smelt (*Retropinna retropinna* (Richardson)) using otolith isotopic composition. Ecol Freshw Fish 1:61–72

Paillard D, Labeyrie L, Yiou P (1996) Macintosh program performs time-series analysis. EOS Trans Am Geophys Union 77:379

Pulliam HR (1988) Sources, sinks, and population regulation. Am Nat 132:652–661

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Righton D, Metcalfe J, Connolly P (2001) Fisheries: different behaviour of North and Irish Sea cod. Nature 411:156

Righton DA, Andersen KH, Neat F, Thorsteinsson V and others (2010) Thermal niche of Atlantic cod *Gadus morhua*: limits, tolerance and optima. Mar Ecol Prog Ser 420:1–13

Righton D, Westerberg H, Feunteun E, Økland F and others (2016) Empirical observations of the spawning migration of European eels: the long and dangerous road to the Sargasso Sea. Sci Adv 2:e1501694

Rijnsdorp AD (1989) Maturation of male and female North Sea plaice (*Pleuronectes platessa* L.). ICES J Mar Sci 46: 35–51

Rijnsdorp AD, Ibelings B (1989) Sexual dimorphism in the energetics of reproduction and growth of North Sea plaice, *Pleuronectes platessa* L. J Fish Biol 35:401–415

Rooker JR, Secor DH (2004) Stock structure and mixing of Atlantic bluefin tuna: evidence from stable δ¹³C and δ¹⁸O isotopes in otoliths. Collect Vol Sci Pap ICCAT 56: 1115–1120

Rooker JR, Secor DH, DeMetrio G (2008) Natal homing and connectivity in Atlantic bluefin tuna populations. Science 322:742–744

Sadovy de Mitcheson Y, Cornish A, Domeier M, Colin PL, Russell M, Lindeman KC (2008) A global baseline for spawning aggregations of reef fishes. Conserv Biol 22: 1233–1244

Shephard S, Trueman C, Rickaby R, Rogan E (2007) Juvenile life history of NE Atlantic orange roughy from otolith stable isotopes. Deep Sea Res I 54:1221–1230

Stanley RRE, Bradbury IR, DiBacco C, Snelgrove PVR, Thorrold SR, Killen SS (2015) Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*). ICES J Mar Sci 72:2350–2363

Stephenson P (2001) Analysis of stable isotope ratios to investigate stock structure of red emperor and Rankin cod in northern Western Australia. J Fish Biol 58:126–144

Stips A, Bolding K, Pohlmann T, Burchard H (2004) Simulating the temporal and spatial dynamics of the North Sea using the new model GETM (general estuarine transport model). Ocean Dyn 54:266–283

Storm-Suke A, Dempson JB, Reist JD, Power M (2007) A field-derived oxygen isotope fractionation equation for *Salvelinus* species. Rapid Commun Mass Spectrom 21: 4109–4116

Surge D, Walker KJ (2005) Oxygen isotope composition of modern and archaeological otoliths from the estuarine hardhead catfish (*Ariopsis felis*) and their potential to record low-latitude climate change. Palaeogeogr Palaeoclimatol Palaeoecol 228:179–191

Tanner SE, Reis-Santos P, Cabral HN (2016) Otolith chemistry in stock delineation: a brief overview, current challenges and future prospects. Fish Res 173:206–213

Thorrold SR, Jones CM, Campana SE (1997) Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). Limnol Oceanogr 42:102–111

Torniainen J, Lensu A, Vuorinen PJ, Sonninen E and others (2017) Oxygen and carbon isoscapes for the Baltic Sea: testing their applicability in fish migration studies. Ecol Evol 7:2255–2267

Trueman CN, MacKenzie KM, Palmer MR (2012) Identifying migrations in marine fishes through stable-isotope analysis. J Fish Biol 81:826–847

Van Neer W, Ervynck A, Bolle LJ, Millner RS (2004) Seasonality only works in certain parts of the year: the reconstruction of fishing seasons through otolith analysis. Int J Osteoarchaeol 14:457–474

Walther BD, Dempster T, Letnic M, McCulloch MT (2011) Movements of diadromous fish in large unregulated tropical rivers inferred from geochemical tracers. PLOS ONE 6:e18351

Wunder MB (2010) Using isoscapes to model probability surfaces for determining geographic origins. In: West JB, Bowen GJ, Dawson TE, Tu KP (eds) Isoscapes: understanding movement, pattern, and process on Earth through isotope mapping. Springer Netherlands, Dordrecht, p 251–270

Zazzo A, Smith GR, Patterson WP, Dufour E (2006) Life history reconstruction of modern and fossil sockeye salmon (*Oncorhynchus nerka*) by oxygen isotopic analysis of otoliths, vertebrae, and teeth: implication for paleoenvironmental reconstructions. Earth Planet Sci Lett 249: 200–215