




Original Article

Otolith $\delta^{18}\text{O}$ and microstructure analyses provide further evidence of population structure in sardine *Sardinops sagax* around South Africa

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Sardine *Sardinops sagax* is an ecologically and economically important Clupeid found off the entire South African coast that includes both coastal upwelling and western boundary current systems. Although the management of the sardine fisheries historically assumed a single, panmictic population, the existence of three, semi-discrete subpopulations has recently been hypothesized. We conducted otolith $\delta^{18}\text{O}$ and microstructure analyses to investigate nursery habitat temperatures and early life growth rates, respectively, of sardine collected from three biogeographic regions around South Africa's coast to test that hypothesis. Analyses indicated that for both summer- and winter-captured adults and summer-captured juveniles, fishes from the west coast grew significantly slower in water that was several degrees cooler than those from the south and east coasts. This suggests that mixing of sardines between regions, particularly the west and other coasts, is relatively limited and supports the hypothesis of semi-discrete subpopulations. However, the west-south differences disappeared in the results for winter-captured juveniles, suggesting that differences in early life conditions between regions may change seasonally, and/or that all or most winter-captured juveniles originated from the west coast. Further elucidating the interactions between South African sardine subpopulations and the mechanisms thereof is important for sustainable harvesting of this species.

Keywords: coastal upwelling, otolith $\delta^{18}\text{O}$, otolith microstructure, population structure, South African sardine, western boundary current

Introduction

The South African coastline extends between the Southeast Atlantic and the Southwest Indian oceans and is bordered by

both eastern and western boundary current systems (Figure 1). The west coast has a relatively narrow shelf and forms the southern part of the Benguela Current upwelling system, where

seasonal, wind-driven coastal upwelling results in a cool shelf habitat characterized by high primary and secondary production, particularly during austral spring/summer (Hutchings *et al.*, 2009; Kirkman *et al.*, 2016). In contrast, the south and east coasts are dominated by the Agulhas Current that flows south-westwards along the continental shelf and produces warm, oligotrophic, and advective environments. The broad shelf off the south coast known as the Agulhas Bank is an irregular extension of the South African coastal plain, and hydrological conditions there are primarily related to strong forcing by the Agulhas Current on the outer shelf and wind-driven upwelling on the inner shelf (Hutchings *et al.*, 2009; Kirkman *et al.*, 2016). The east coast has a particularly narrow shelf and is markedly impacted by the Agulhas Current (Roberts *et al.*, 2010) although limited local upwelling does occur there (Hutchings *et al.*, 2010).

Sardine *Sardinops sagax* is an abundant Clupeid found off South Africa that is distributed around the coast and in all of South Africa's marine biogeographic provinces except the tropical (Beckley and van der Lingen, 1999). This species is a major target of the South African small pelagic fishery and is caught off the west and south coasts using purse seine nets, and annual sardine catches in that fishery have ranged from 16 000 to 410 000 tons over the period 1950–2015 (de Moor *et al.*, 2017). Although sardine was initially only exploited off the west coast, fishing off the south coast was initiated in the late 1980s and significant catches have been taken in that region, particularly during the mid-2000s (Figure 1). Sardine is also caught off the east coast by a small beach seine fishery (annual catches have never exceeded 700 t) that targets this species during the annual migration of sardine from the Agulhas Bank up the east coast in austral winter that is locally known as the “KwaZulu-Natal sardine run” (van der Lingen *et al.*, 2010a).

Understanding the population structure and identifying stocks (or management units) of exploited species are crucial for developing appropriate management strategies, and disregarding population structure, should it exist, could lead to changes in genetic diversity and biological attributes including productivity, which may result in overfishing and local depletion of less productive stocks (Begg *et al.*, 1999; Ying *et al.*, 2011; Goethel and Berger, 2017). Whereas historical management of the small pelagic fisheries assumed a single sardine population, the existence of semi-discrete western and southern stocks (de Moor and Butterworth, 2015), as well as an eastern sardine stock (Fréon *et al.*, 2010), off South Africa has recently been hypothesized based on several lines of evidence. These have included non-continuous distribution patterns (Coetzee *et al.*, 2008), separated spawning areas and different although overlapping spawning periods on the west, south, and east coasts (van der Lingen *et al.*, 2015; van der Lingen and McGrath, 2017), and analyses of spatial variability in meristic and morphometric characteristics (van der Lingen *et al.*, 2010b; Idris *et al.*, 2016; Groenewald *et al.*, 2019), parasite loads (van der Lingen *et al.*, 2015; Weston *et al.*, 2015), metallic elemental (Uren *et al.*, 2020) and halogenated natural product (Wu *et al.*, 2020) compositions, and genetic characteristics (Teske *et al.*, 2018). Importantly, Lagrangian individual-based models of the transport/retention of early life history stages of sardine coupled with three-dimensional hydrodynamic model representations of the South African west and south coasts have indicated that all sardine eggs spawned on the west coast (i.e. west of Cape Agulhas; 20°E) are either retained on the west coast or advected offshore

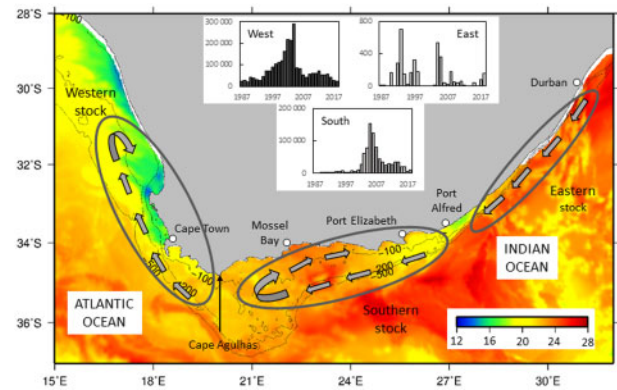


Figure 1. Satellite-derived (GHRSSST Level 4 OSTIA Global Foundation Sea Surface Temperature Analysis; UK Met Office, 2005) Sea surface temperature around South Africa on 1 January 2017 demonstrating the marked oceanographic variability in the study area and with the 100, 200, and 500 m depth contours and places named in the text shown. Grey ellipses indicate the approximate distribution areas of the putative western, southern, and eastern sardine stocks and the grey arrows within each ellipse indicate hypothesized transport of early life history stages of each stock. Histograms show annual sardine catches by a purse seine fishery off the west and south coasts, and by a beach seine fishery off the east coast, 1987–2018 (note that the y-axis scales differ between histograms).

while none is transported to the south coast, whereas relatively few (<20%) of eggs spawned on the south coasts are transported to the west coast (Miller *et al.*, 2006; McGrath *et al.*, 2020). Management of the South African purse seine fishery for sardine now takes account of this hypothesized population structure, with development of a two-stock assessment model that assumes some mixing (primarily eggs and larvae from the south to the west, and recruits and some older fish from the west to the south) between the stocks (de Moor *et al.*, 2017) and of an operational management procedure that provides for explicit spatial management under certain circumstances.

Despite these advances, however, differences in ecological characteristics between the putative subpopulations of South African sardine, and of their early life history stages in particular, are yet to be fully understood. Describing basic habitat use during early life is important for understanding population dynamics in small pelagic fishes, given that variability in early stage survival can be a significant driver of recruitment and hence population variability in these species (Politikos *et al.*, 2018; Yatsu, 2019).

South African sardine adults are mainly distributed to the west of 20°E and to the east of 22°E, and the area 20–22°E is considered as the mixing area between the putative western and southern subpopulations (Coetzee *et al.*, 2008). Spawning by the putative western and southern subpopulations occurs year-round from mid-shelf to offshore but mainly from spring to summer off the west coast and from winter to spring off the south coast (Van der Lingen and Huggett, 2003; van der Lingen and McGrath, 2017). Sardine eggs are seldom observed in the 20–22°E mixing area (van der Lingen *et al.*, 2015). Sardine from the putative eastern subpopulation spawn along the east coast during their northward migration in winter (Coetzee *et al.*, 2010) and eggs are

found in that region from winter to spring (Connell, 2010). Larvae and juveniles recruit inshore in all regions; however, their nursery environments and growth patterns during early life stages are not well understood, especially for the putative southern and eastern subpopulations.

Inferences about the life history of small pelagic fishes can be drawn from studies of their otoliths, the chronological properties of which are unparalleled (Campana and Thorrold, 2001). The otoliths of larval and juvenile stages of many small pelagic fishes, including sardine off Namibia (Thomas, 1986) and South Africa (Waldron, 1998), show clear daily rings. As otolith radius is strongly correlated with body length in many species (e.g. Harvey, 2000), the width between the daily rings can be used as a proxy of daily growth, thereby revealing early life stage growth trajectories (Campana, 1990). Moreover, the chemical composition of otoliths reflects ambient water chemistry, and as otoliths are metabolically inert their composition is preserved even after the fish has died (Campana, 1999). Hence, the otoliths of fish from waters with different hydrochemical characteristics will differ, which has led to otolith elemental composition being used to investigate population structure in several exploited fish species [see Tanner *et al.* (2016) for a recent review]. Analysing the oxygen stable isotope ratio ($\delta^{18}\text{O}$) of fish otoliths may provide further detailed information of habitat usage, since $\delta^{18}\text{O}$ in otoliths is known to sensitively reflect the temperature and $\delta^{18}\text{O}$ of ambient water (e.g. Høie *et al.*, 2004). Although the temporal resolution of otolith $\delta^{18}\text{O}$ analysis is still poor compared to that for elemental concentrations, it has recently improved up to 10–30 days resolution due to the developments of micromilling and microvolume analysing techniques (Sakamoto *et al.*, 2019). As $\delta^{18}\text{O}$ quantitatively records ambient water temperature at the time of otolith deposition, it can be used to characterize the nursery environment the fish had utilized during early life history stages and hence may also be of use in population structure studies as was done for *S. sagax* off Australia by Edmonds and Fletcher (1997).

Here, we performed $\delta^{18}\text{O}$ and microstructure analyses on the otoliths of juvenile and adult South African sardine collected from around the coast, aiming (i) to test for spatial differences in nursery habitat temperatures and early life growth rates and (ii) to infer the mixing patterns between the putative subpopulations in older stages using observed differences as markers. If the nursery temperature and early growth of both adults and juveniles within a region are similar, and if there are spatial differences that reflect environmental gradients, these would suggest that migrations from one region to another are limited and hence provide further evidence for the existence of semi-discrete subpopulations. The distribution of seawater $\delta^{18}\text{O}$ around the coast was also investigated to aid interpretation of otolith $\delta^{18}\text{O}$ data.

Material and methods

Seawater sampling and $\delta^{18}\text{O}$ analysis

A total of 56 near-surface seawater samples for ambient $\delta^{18}\text{O}$ analysis were collected around the coast of South Africa during two research surveys and a nearshore sampling campaign conducted during June to August (austral winter) 2017 (Figure 2a). Samples were collected from seawater taken at 6 m depth using a continuous, underway fish egg sampler at inshore and offshore points of selected cross-shelf transects along the west and south coasts during the first survey (conducted by the Department of

Forestry, Fisheries and the Environment; DFFE). During the second survey (conducted by the Department of Environmental Affairs, DEA), samples were collected at 5 m depth using Niskin bottles attached to a Conductivity Temperature Depth profiler (CTD) along the south and east coasts. Near-shore (<20 m water depth) samples from the east coast only were collected with a plastic bucket immediately below the water surface from a ski boat. As the surface mixed layer depth on the Agulhas Bank and along the east coast is generally larger than 10 m (Swart and Largier, 1987; Coetzee *et al.*, 2010; Jackson *et al.*, 2012), the samples are likely to represent isotopic condition of the surface mixed layer. All samples were taken as duplicates and were preserved in sealed 10 ml glass vials to avoid evaporation and air transported to Japan where they were analysed at the National Institute of Technology, Ibaraki College, Hitachinaka, Japan. Two millilitres from each seawater sample was membrane-filtered (pore size: 0.45 μm , Toyo Roshi Kaisha, Ltd) prior to isotope analysis to reduce suspended particle loads and avoid blocking the sampling line, and samples were analysed using a wavelength-scanned cavity ring-down spectroscopy isotopic water analyser (L2130-i; Picarro Inc.) with analytical precision better than $\pm 0.05\text{‰}$ for $\delta^{18}\text{O}$. The isotope values are reported using delta notation relative to the Vienna Standard Mean Ocean Water (VSMOW).

Because the seawater sampling was only performed in winter, potential seasonal variations of seawater $\delta^{18}\text{O}$ were evaluated by analysing the climatological seasonality of salinity that is known to closely and linearly correlate with seawater $\delta^{18}\text{O}$ (LeGrande and Schmidt, 2006). To estimate the seasonal discrepancies in seawater $\delta^{18}\text{O}$, we used the decadal average, 0.25° gridded monthly salinity data from the World Ocean Atlas 2018 (Zweng *et al.*, 2018) to estimate the difference of 0–30 m mean salinity from winter (June–August) mean salinity for each month (i.e. monthly decadal mean – winter decadal mean) for each grid point in the region 28–37°S and 16–34°E, which covers the entire South African shelf (Supplementary Figure S2). As we could not establish region-specific salinity–seawater $\delta^{18}\text{O}$ relationships due to limited CTD data, we converted the salinity anomalies to seawater $\delta^{18}\text{O}$ anomalies in the VSMOW scale by multiplying by 0.51, the slope of the salinity–seawater $\delta^{18}\text{O}$ relationship proposed for the South Atlantic Ocean (LeGrande and Schmidt, 2006).

Otolith samples

A total of 367 juvenile and adult sardine were collected from 35 stations in coastal and shelf waters off the west, south, and east coasts of South Africa during the period 2015–2017 (Table SM1 and Figure 2b–d). Fish from the putative western and southern stocks were collected from mid-water trawl catches made during DFFE research surveys conducted to estimate pelagic fish recruitment strength in May–July (austral winter) and total biomass in October–December (austral spring/summer). Samples of 2–25 (mean 10 ± 6 SD) fish per station were collected from areas of high sardine density as observed during these surveys (see Supplementary Material and Figure 1a–c) and also opportunistically to ensure broad spatial coverage. Fish were categorized as juvenile or adult depending on their caudal length (CL), with fish <14 cm considered juvenile. Almost all (11 out of 13) samples of adults from the west and south coasts were collected during spring/summer 2016, with all winter-collected juveniles sampled in autumn/winter 2017 and almost all (8/10) summer-collected

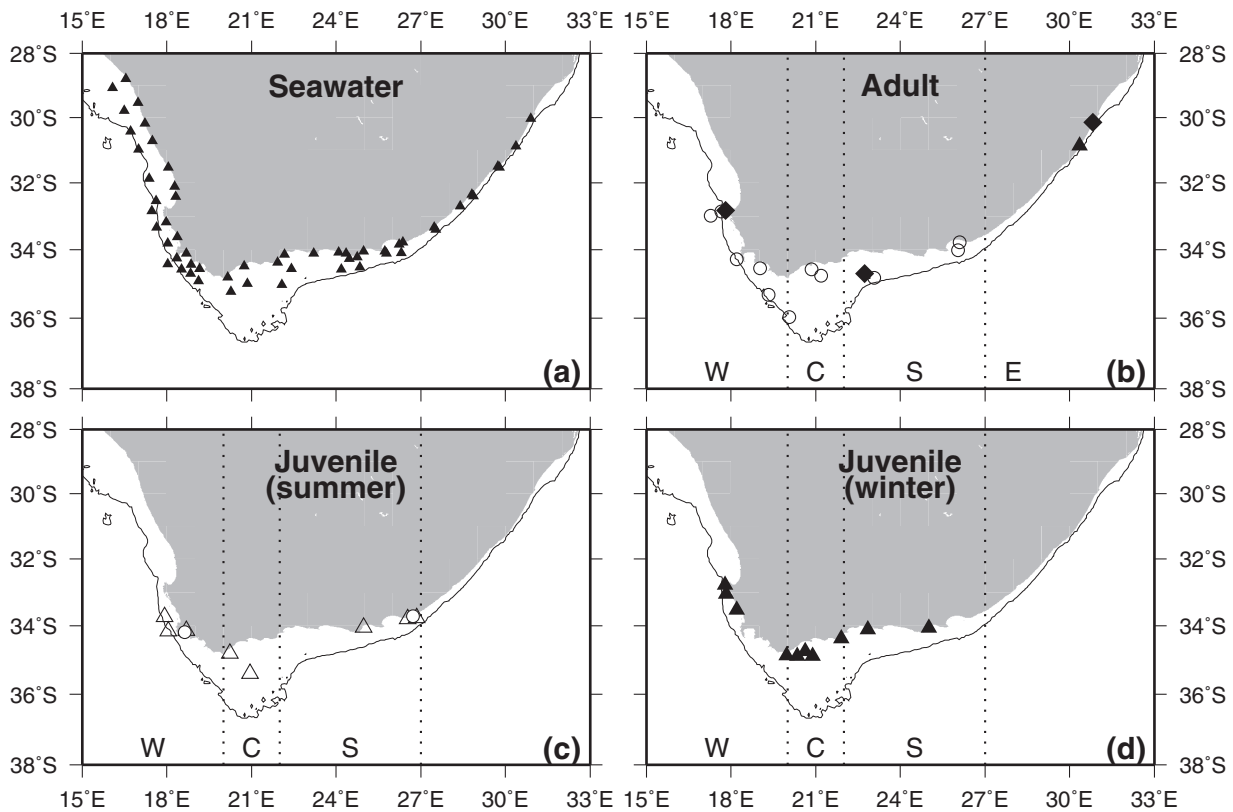


Figure 2. Sampling locations of (a) sub-surface seawater, (b) adult sardine, (c) juvenile sardine captured in summer, and (d) juvenile sardine captured in winter. The shape and filled/open represent the sampling year and season, respectively [diamond: 2015, circle: 2016, and triangle: 2017 and filled: winter (June to August) and open: summer (November to January)].

juveniles in spring/summer 2017. Only adult sardine from the putative eastern stock were collected from beach seine net catches taken during the winter sardine run events of 2015 and 2017, and one sample of adult fish was collected off each of the west and south coasts in winter 2015 (Figure 2). The mean CL for each station was 15.2–20.1 cm for adults and 7.9–10.5 cm for juveniles (see [Supplementary Materials](#) for further details). To facilitate spatial comparisons, stations were assigned to one of four regions (West, Central, South, and East; Figure 2) based on their location [West (<20°E), Central (20–22°E), South (22–27°E), and East (>27°E)]. Regions were longitudinally defined to represent the spatial distribution of the putative western (West), southern (South), and eastern (East) sardine stocks off South Africa ([van der Lingen et al., 2015](#)), with the Central region representing the mixing area between western and southern sardine stocks (as in [de Moor et al., 2017](#)).

Sardine samples were frozen shortly after capture and subsequently transported to DAFF laboratories in Cape Town, where they were later defrosted, and each fish had their CL recorded and their sagittal otoliths extracted. Otoliths were then air dried, placed in labelled vials, and transported to the Atmosphere and Ocean Research Institute (AORI) of the University of Tokyo, Japan, for microstructure and isotope analyses.

Otolith microstructure analysis

Otoliths were cleaned using a wooden toothpick and a thin paintbrush under 10–20× magnification, rinsed with Milli-Q water

and air dried for a few hours, embedded in Petropoxy 154 (Burnham Petrographics LLC) resin, and kept at 80°C for 12 h to cure. Embedded otoliths were then ground with sandpaper (no. 2000) and polished with an alumina suspension (BAIKOWSKI International Corporation) to reveal the daily rings and expose the otolith nucleus. The position and number of daily rings in otoliths were examined from the core as far as possible along the axis of the post-rostrum using an otolith measurement system (RATOC System Engineering Co. Ltd). Daily rings became indistinct after 100–150 counts from the core and before reaching the edge in most of the samples, except for juveniles captured in winter 2017 whose daily rings could be read from core to edge and which enabled estimation of their hatch dates. Hatch date data were, therefore, only available for the juveniles captured in winter 2017. The first daily ring was assumed to be formed after 3 days post-hatch (dph) based on the observation that the mean difference between sardine age and daily ring counts for 18 larvae reared in the laboratory from eggs collected at sea was 3.3 days ([Brownell, 1983](#); [Thomas, 1986](#)). Daily increment widths of each fish were averaged for consecutive 10-day intervals (except the first interval that was of 7 days only) for the first 100 dph (i.e. 3–10, 11–20, 21–30, ..., 91–100 dph), and the mean increment width for each interval for all individuals collected at each station was calculated for comparison between regions. Sardine body length at 60 dph was calculated using the observed otolith radius at this age and the allometric relationship between fork length and otolith radius of sardine *S. sagax* off Namibia [otolith radius

= $17.32 \times (\text{fork length})^{1.977}$; Thomas, 1986] for comparison with otolith isotope data (see below).

Otolith $\delta^{18}\text{O}$ analysis

For up to eight individual sardine per station, and a total of 243 individuals, the otolith portion that was formed during the first 60 days from hatch was milled out using a high-precision micro-milling system (Geomill 326, Izumo-web, Japan), and the resulting powder was collected in a stainless steel microcup. The $\delta^{18}\text{O}_{\text{otolith}}$ of powdered samples was measured using an isotope ratio mass spectrometer (Delta V plus, Thermo Fisher Scientific), equipped with an automated carbonate reaction device (GasBench II, Thermo Fisher Scientific), and installed at the AORI, the University of Tokyo, Tokyo. Detailed analytical conditions have been reported elsewhere (e.g. Shirai *et al.* 2018), with minor modification of using 4.5-ml glass vials (Breitenbach and Bernasconi, 2011). All isotope values are reported using delta notation relative to the Pee Dee Belemnite. No correction was applied for the acid fractionation factor between calcite and aragonite [phosphoric acid–calcium carbonate reaction temperature 72°C (Kim *et al.* 2007)]. Analytical precisions of $\delta^{18}\text{O}$ for international standards (NBS-19) were $\pm 0.10\text{--}0.16\text{‰}$ (1σ).

To exclude the effect of seawater $\delta^{18}\text{O}$ variation on otolith $\delta^{18}\text{O}$ and to convert the otolith $\delta^{18}\text{O}$ difference to a temperature scale, water temperature was calculated from otolith $\delta^{18}\text{O}$ based on the relationship between these two parameters established for Japanese sardine by Sakamoto *et al.* (2017), namely:

$$\delta_{\text{otolith}} - \delta_{\text{seawater}} = -0.18 \times T + 2.69,$$

in which seawater $\delta^{18}\text{O}$ estimated from longitude using a fitted quadratic function (see Results) was substituted. Because the difference between acid fractionation factors of calcite and aragonite is temperature dependent (Kim *et al.*, 2007), 0.09‰ was added to the otolith $\delta^{18}\text{O}$ before calculating water temperature to adjust for the different reaction temperature between this study and Sakamoto *et al.* (2017).

Statistical analysis

To assess differences between otolith $\delta^{18}\text{O}$ and growth increment widths of sardine from four regions along the South African coast, we used one-way ANOVA and the Tukey–Kramer test for the former and repeated-measures ANOVA (rm-ANOVA) and the Tukey–Kramer test for the latter, respectively. Statistical tests were performed separately for: (i) adult, (ii) summer-captured juvenile, and (iii) winter-captured juvenile sardine, using MATLAB and Statistics and Machine Learning Toolbox R2017a (The MathWorks Inc., Natick, MA, USA). Station mean values were treated as minimum data units in these analyses, hence stations that included data from only two individuals were considered unreliable and were excluded. In the analyses of growth increment widths, the data averaged for consecutive 10-day intervals from hatch to 60 dph (i.e. 3–10, 11–20, 21–30, 31–40, 41–50, 51–60 dph) were used because within this age range most individuals showed a near-linear increase of daily increment widths with age. To test whether the growth data meet the assumption of sphericity for rm-ANOVA, Mauchly's test for sphericity was performed beforehand. As the test suggested the violation of the sphericity in the adult and the summer-captured juvenile sample sets ($p = 0.02$ and 0.04 , respectively), epsilon corrections using the

Greenhouse–Geisser approximation were used to compute the p values for rm-ANOVA for those sample sets.

Results

Distribution of seawater $\delta^{18}\text{O}$

Near-surface seawater showed a strong longitudinal variation and an inshore–offshore difference in $\delta^{18}\text{O}$. Values were relatively low (around $+0.2\text{‰}$) off the west coast to the north of Cape Columbine. Seawater $\delta^{18}\text{O}$ increased with longitude until $\sim 23^\circ\text{E}$ (where the continental shelf narrows), then became relatively stable at $+0.5$ to $+0.6\text{‰}$ on the south and east coasts, and offshore stations generally showed higher values than nearby inshore stations along the entire coast (Figure 3a). A quadratic curve [$\delta^{18}\text{O} = -0.003 \times (\text{Longitude})^2 + 0.1578 \times (\text{Longitude}) - 1.5505$; $r^2 = 0.57$, $p < 0.001$, root mean square error = 0.09] was fitted (least squares method) to express the geographical variation of seawater $\delta^{18}\text{O}$ around the South African coast. The estimation of potential seasonal variations of 0–30 m mean seawater $\delta^{18}\text{O}$ based on salinity monthly climatology showed that the seasonal discrepancies from the values in winter was in the range of -0.17 to $+0.08\text{‰}$ off the South African coast (Supplementary Figure S3), which would only cause 1.0°C difference at most in subsequent temperature estimations.

Otolith $\delta^{18}\text{O}$ and daily increment width

Otolith $\delta^{18}\text{O}$ data were collected from 243 and daily increment width data were collected from 367 individual sardine. For adult fish, both otolith $\delta^{18}\text{O}$ during the 60 days from hatch and daily increment width until 100 dph showed clear spatial variation. Otolith $\delta^{18}\text{O}$ values were relatively higher (about $+0.8\text{‰}$) in the West, lower ($+0.3$ to $+0.4\text{‰}$) in the South, and intermediate in the Central and the East region (Figure 4a). One-way ANOVA and *post hoc* pairwise comparisons detected a significant difference between the West and the South regions (ANOVA: $F = 5.87$, $p = 0.01$, Tukey–Kramer test: difference = 0.51 , $p = 0.01$) but not between any other regions. In the South and the East regions, daily increment width peaked at $\sim 9\ \mu\text{m}$ over 51–70 dph, while in the West and the Central regions, it peaked at $\sim 7\ \mu\text{m}$ over 71–90 dph (Figure 4b). rm-ANOVA detected a significant interaction between age and region ($F = 7.36$, $p = 2.7\text{e-}5$) during 0–60 dph, and the *post hoc* Tukey–Kramer test found significant differences between the West and the South regions (difference = -2.50 , $p = 6.0\text{e-}6$), the West and the East regions (difference = -1.88 , $p = 2.6\text{e-}4$), the Central and the South regions (difference = -1.87 , $p = 2.6\text{e-}4$), and the Central and the East regions (difference = -1.25 , $p = 0.01$). Hence, during their early life adult sardine from the West and Central regions showed significantly slower initial growth that had a later peak than observed for adults from the South and the East regions (Figure 4b). Otolith $\delta^{18}\text{O}$ values and increment widths during < 60 dph of adult sardine within each region were remarkably similar, despite samples being collected in different seasons and years. Spatial differences in otolith $\delta^{18}\text{O}$ and increments were still significant when the data were merged into a West and Central region and a South and East region ($\delta^{18}\text{O}$: ANOVA, $F = 9.92$, $p = 0.008$, increment width: rm-ANOVA, $F = 16.75$, $p = 2.5\text{e-}10$).

Juvenile sardine captured in summer showed trends similar to those of the adults in both otolith $\delta^{18}\text{O}$ during the 60 days from hatch and in daily increment width during early life. Otolith $\delta^{18}\text{O}$ showed relatively higher values (about $+0.9\text{‰}$) in the West, lower

values (about $+0.4\text{‰}$) in the South, and intermediate values in the Central region (Figure 4c). No sardine juveniles were collected from the East. Otolith $\delta^{18}\text{O}$ values were significantly different between the West and the South regions (ANOVA: $F=12.345$, $p=0.01$, Tukey–Kramer test, difference = 0.50, $p=0.01$), but not between the West and Central or the Central and South regions. Daily increment width peaked at $\sim 8\ \mu\text{m}$ over 61–70 dph for fish from the South region, while for those from the West daily increment widths of $\sim 4\text{--}6\ \mu\text{m}$ were stable and continued through 31–100 dph with no marked peak (Figure 4d). The otolith growth trajectories of juvenile sardine from the Central region were similar to but slightly higher than those of fish from the West. rm-ANOVA detected a significant interaction between age and region ($F=5.28$, $p=0.02$) during 0–60 dph, and the *post hoc* Tukey–Kramer test found significant differences between the West and South (difference = -1.47 , $p=0.01$) and the Central and South regions (difference = -1.11 , $p<0.05$), but not between the West and the Central. The spatial differences in otolith $\delta^{18}\text{O}$ and increments were still significant when the data were merged within West and Central regions and compared with the South region ($\delta^{18}\text{O}$: ANOVA, $F=16.57$, $p=0.007$, increment width: rm-ANOVA, $F=8.78$, $p=3.2e-5$).

Otolith $\delta^{18}\text{O}$ and early growth of juvenile sardine captured in winter showed a different trend to those from juveniles captured in summer and adult fish. Otolith $\delta^{18}\text{O}$ values were highest in fish from the Central and South regions (at about $+0.8\text{‰}$) and lower in those from the West (at about $+0.6\text{‰}$; Figure 4e). No significant differences were detected between otolith $\delta^{18}\text{O}$ values of juvenile sardine from the West, Central, and South regions (ANOVA: $F=1.362$, $p=0.32$). rm-ANOVA detected no significant interaction ($F=0.977$, $p=0.48$) between age and region in daily increment widths for fish from the West, Central, and South regions, all of which peaked at $\sim 7\ \mu\text{m}$ over 81–90 dph (Figure 4f). The estimated hatch date distribution of juvenile sardine sampled in winter in 2017 was similar for fish from the West, Central, and South regions, with most individuals hatched between November 2016 and January 2017 (austral spring/summer; Figure 5).

Ambient water temperature and growth

The overall geographical trends for adults and juveniles captured in summer were still apparent when otolith $\delta^{18}\text{O}$ values were converted into water temperature and taking into account the observed spatial variation of seawater $\delta^{18}\text{O}$. Estimated temperature was highest in the South at $\sim 16^\circ\text{C}$, high in the East at $\sim 15^\circ\text{C}$, and low in the West and the Central regions at $10\text{--}14^\circ\text{C}$ (Figure 6a). The trend for juveniles captured in winter was different although otolith $\delta^{18}\text{O}$ values showed an increase with longitude from the West to the South region (Figure 4e), estimated temperatures did not decrease but were stable at $\sim 12^\circ\text{C}$, suggesting the increase in otolith $\delta^{18}\text{O}$ was due to the increase of seawater $\delta^{18}\text{O}$ at a constant water temperature.

The mean fork length at 60 dph and the mean ambient water temperature estimated for the period from hatch to 60 dph for each sample showed a significant positive correlation when all three sample sets (adults, and summer-caught and winter-caught juveniles) were combined ($r^2=0.64$, $p=4.0e-8$, Figure 6b), suggesting that higher water temperatures result in faster growth during the early life stages of the South African sardine. The distribution of mean values showed a separation at $\sim 14^\circ\text{C}$, with the

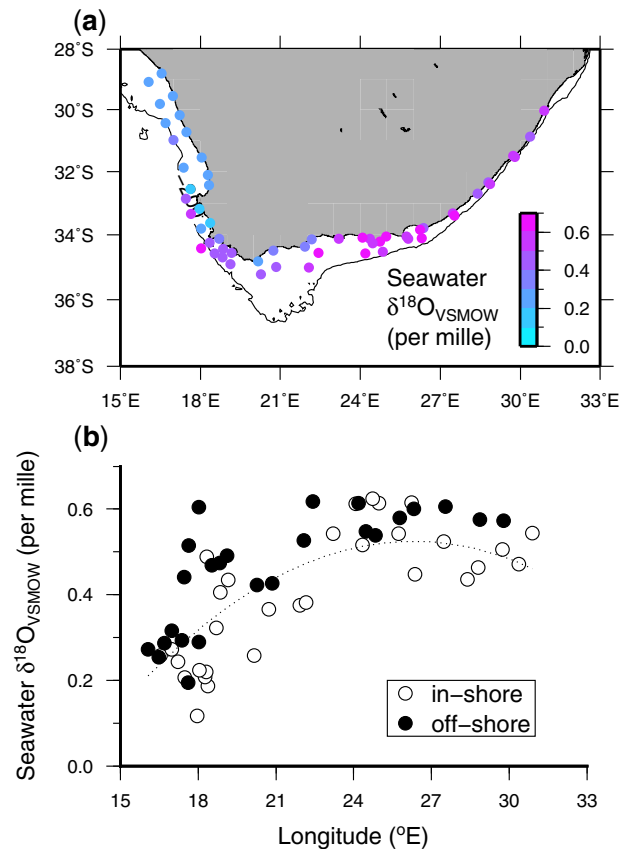


Figure 3. (a) Horizontal distribution and (b) longitudinal variation of near-surface seawater $\delta^{18}\text{O}$. The dotted line in (b) shows the fitted quadratic curve [$\delta^{18}\text{O} = -0.003 \times (\text{Longitude})^2 + 0.1578 \times (\text{Longitude}) - 1.5505$; $r^2 = 0.57$, $p < 0.001$, root mean square error = 0.09].

higher temperature group including all samples from the East region, most from the South, one from the Central and none from the West region, whereas the lower temperature group included all of the samples from the West region, all but one from the Central, and two from winter-captured juvenile samples in the South region (Figure 6b). This separation indicated the existence of two categories of sardine growth and nursery habitat off South Africa, slower growth in cooler waters west of 22°E and faster growth in warmer waters east of 22°E .

Discussion

This study investigated nursery environments and early life growth of sardine around the coast of South Africa by conducting the first otolith $\delta^{18}\text{O}$ analysis of this species in this region and also by using otolith microstructure analysis. Our results revealed that the nursery temperature for the first 2 months of life estimated from otolith $\delta^{18}\text{O}$ and otolith increment widths of adults and summer-captured juveniles showed significant and consistent spatial differences. Sardine showed faster growth in warmer temperatures off the south and the east coasts and slower growth in cooler temperatures off the west coast, likely reflecting the different oceanographic characteristics of western boundary current and coastal upwelling systems, respectively. The coherent trends observed in both juveniles captured in summer, and adults captured in both summer and winter suggest that adults tend to

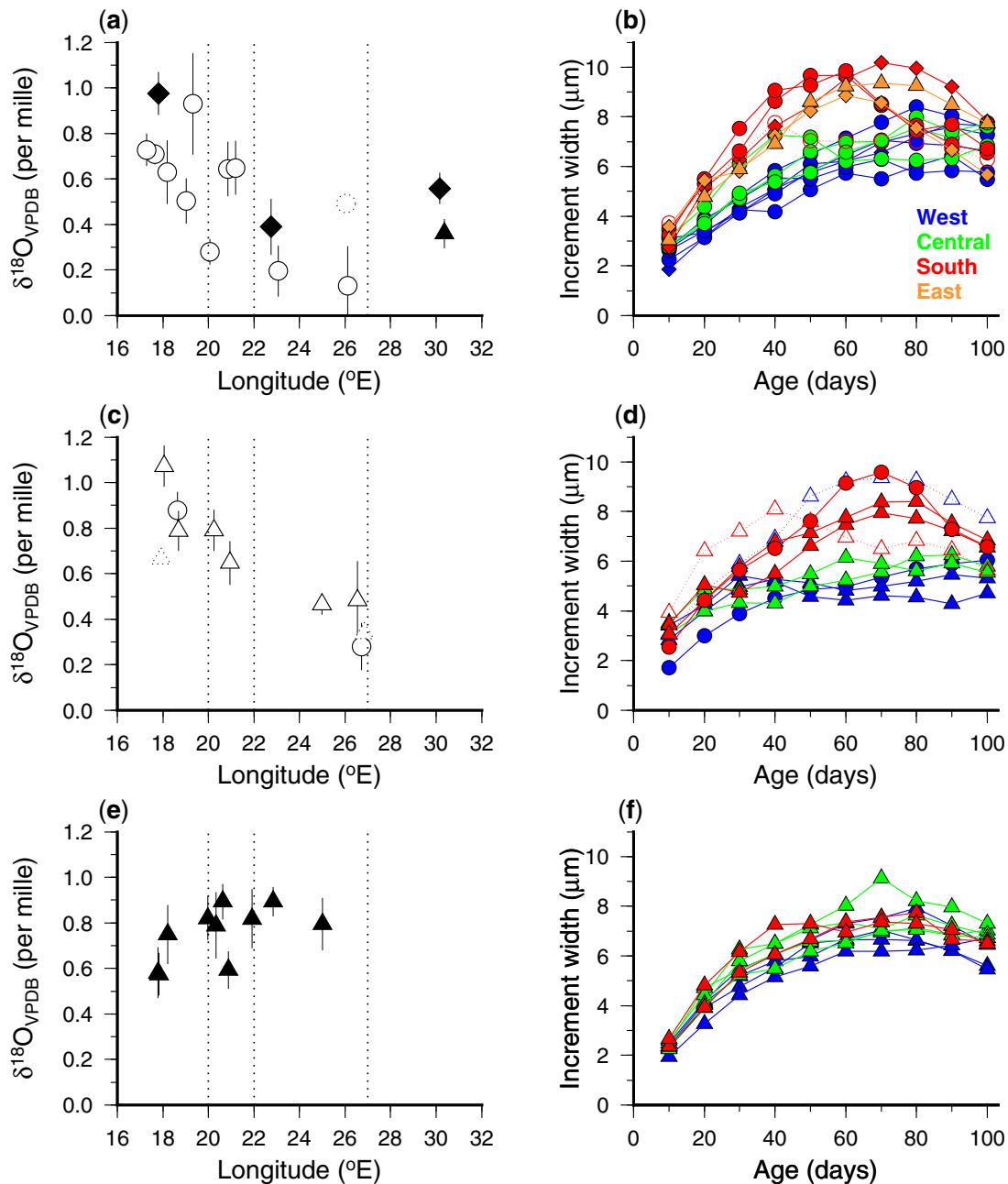


Figure 4. Geographical variations of otolith $\delta^{18}\text{O}$ and daily increment width for (a and b) adults, (c and d) juveniles captured in summer, and (e and f) juveniles captured in winter. In (a), (c), and (e), station means and standard errors are shown, and the shapes and filled/open represent the sampling year and season, respectively [diamond: 2015, circle: 2016, and triangle: 2017 and filled: winter (June to August) and open: summer (November to January)]. Edge-dotted plots are the stations that included only two individuals. In (b), (d), and (f), the shapes represent the sampling year and the colours shows the region (blue: West, green: Central, red: South, and orange: East) to which the station belongs. The open plots are the stations that included only two individuals.

remain in the same regions as their young and that migrations of fish from one region to another are relatively limited. Hence, these results support the existence of semi-discrete sardine subpopulations that utilize different nursery habitats around the South African coast. However, the inter-annual and seasonal coverages for these samples are not large, and the geographical trends in otolith $\delta^{18}\text{O}$ and growth described above were absent in the

winter-captured juveniles, suggesting that further efforts are needed to understand the whole movement patterns and mixing between subpopulations of sardine off South Africa.

We have two hypotheses for the cause of the observed seasonal differences in spatial gradients of juvenile sardine otolith $\delta^{18}\text{O}$ and early growth rate. One is the seasonality of coastal upwelling. Juveniles captured in summer 2016 and 2017 showed clear

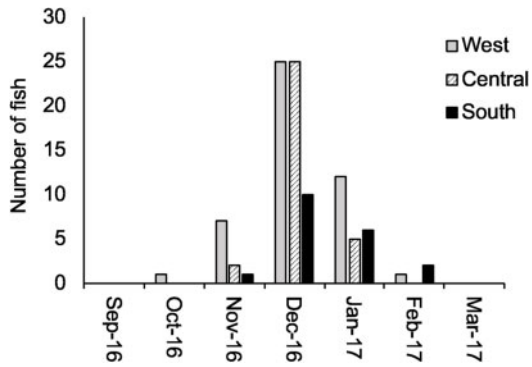


Figure 5. Distribution of estimated hatch month of juveniles captured in June to July 2017 based on the number of daily increments. Individuals from different stations are pooled within each region.

differences in otolith $\delta^{18}\text{O}$ and daily increment widths between the different areas, whereas juveniles captured off the south coast in winter 2017 showed similar temperatures to those captured off the west coast (Figure 6). In summer, when the winter-captured juveniles hatched, wind-driven coastal upwelling does occur along the south coast, in addition to the strong upwelling off the west coast at this time (Lamont *et al.*, 2018). In addition, a quasi-permanent ridge of cool water extends along the 100 m isobath on the eastern Agulhas Bank between 22 and 24°E due to shelf-edge upwelling during spring and summer (Swart and Largier, 1987). The disappearance of the temperature gradient in the summer-hatched (winter-captured) cohort suggests that sardine off the south coast may have utilized these upwelled waters that resulted in their high otolith $\delta^{18}\text{O}$ values. Such individuals with high otolith $\delta^{18}\text{O}$ and slow growth, however, were rarely found in the adults captured off the south coast (Figure 4a and b). This may be because summer-hatched cohorts do not contribute substantially to recruitment in the southern subpopulation, as the spawning by these fish peaks in winter to spring, hence, it can be concluded that the majority of individuals in the southern subpopulation grows faster in warmer waters than those in the western subpopulation. Alternatively, juvenile sardine collected off the south coast in winter 2017 may in fact have been spawned off the west coast in the preceding summer and subsequently migrated south and eastward to the join the southern subpopulation. This may be a more likely explanation as there is clear evidence from survey-derived length frequency distributions of recruits having moved from the west to the south coast in some years. In addition, hatch dates and the otolith characteristics of winter-collected juveniles from the South were not different to those from the West or Central regions (Figures 4e and f and 5). The sardine two-stock assessment model assumes mixing between western and southern subpopulations via a year-varying proportion of western recruits and some older fish that move permanently to the southern subpopulation (de Moor *et al.*, 2017).

Although Cape Agulhas (20°E) has been considered to approximate the boundary between western and the southern sardine subpopulations off South Africa, our results suggest that it may occur several degrees east of Cape Agulhas, which can be important when setting the boundary of management units. Based on data collected during hydroacoustic and trawl surveys conducted over the period 1984–2007, Coetzee *et al.* (2008) showed that

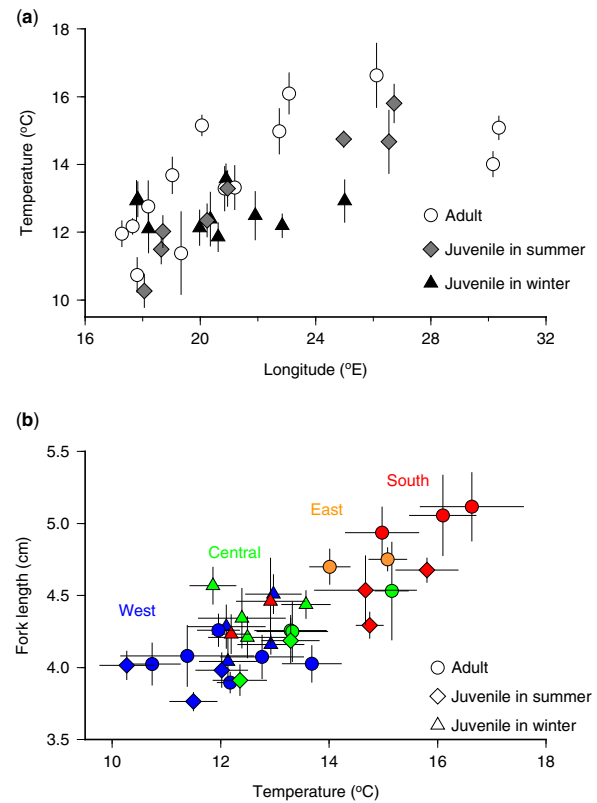


Figure 6. (a) Longitudinal variation of estimated temperature and (b) relationship between estimated temperature and fork length at 60 dph. Station means and standard errors are shown. Stations including less than three individuals were excluded. The colours in (b) represent the areas of capture.

sardine adults are mainly distributed to the west of 20°E and to the east of 22°E, and considered the area 20–22°E, the Central region in this study, as the mixing area between the putative western and southern subpopulations. However, the nursery temperature and early life growth of sardine from the Central region were similar to those of fish from the West in all sample sets we analysed but were significantly different to those of fish from the South for adult and summer-captured juvenile sardine. Therefore, based on their ecology, sardine in the Central region appear to be more likely to belong to the western subpopulation and thus may have to be treated as such in fishery management.

Further investigation is required to clarify whether fish that participate in the sardine run along the east coast comprise a third subpopulation. The run “most likely corresponds to a seasonal spawning migration of a genetically distinct subpopulation of sardine responding to a strong instinct of natal homing” (Fréon *et al.*, 2010). Sardine catches off the south coast typically peak in winter (Beckley and van der Lingen, 1999), indicating that not all sardine in that region participate in the run, and estimates of the biomass of sardine in the run from three surveys (conducted in 1986, 1987, and 2005) were all ~30 000 t, despite overall population size ranging from 0.3 to 3 million t between the two time periods (Coetzee *et al.*, 2010). However, otolith $\delta^{18}\text{O}$ and growth rates of early life stages did not show marked differences between adult sardine from the south and east coasts. This appears to contradict the hypothesis that relatively lower

$\delta^{18}\text{O}$ values (indicative of higher temperatures) would be observed in sardine from the east coast, where the dominance of the Agulhas Current is particularly strong because of the very narrow continental shelf in that region. Even if individuals in the sardine run had originated from spawning off the east coast, they appear to have been rapidly transported to and grown in cooler areas off the south coast, such as the area around Port Alfred where strong upwelling is frequently observed (Lutjeharms *et al.*, 2000). Sardine larvae are commonly encountered off the east coast in spring (September/October) but juveniles are infrequently recorded there (see van der Lingen *et al.*, 2010a and references therein), hence, it appears likely that the east coast (in particular the KZN north coast, i.e. east of 30°E) is not an important sardine nursery area and that early life history stages are transported southwards to recruit on the south coast, where they likely mix with members of the southern subpopulation. Future studies that provide $\delta^{18}\text{O}$ analyses at a higher resolution using microvolume carbonate isotope analytical system MICAL3c (Ishimura *et al.*, 2004, 2008; Sakamoto *et al.*, 2019) may allow a more accurate view of the life history of fish that participate in the sardine run.

A clear, positive relationship between water temperature and growth during the first 2 months post-hatch is observed for sardine sampled around the coast of South Africa (Figure 6b). This is consistent with previous observations that sardine larvae captured at higher temperatures off Namibia and the South African west coast had faster recent (over the 5 days before capture) growth rates compared to those captured at lower temperatures (Thomas, 1986). Laboratory experiments for European sardine *Sardina pilchardus* have shown that under good feeding conditions larval growth increased with increasing temperature, but that this was achieved at the expense of a significant increase in the number of foraging events (Garrido *et al.*, 2016), which indicates that fast growth in warm waters requires a sufficient food supply. Recently, Costalago *et al.* (2020) compared otolith microstructure and nucleic acid ratios of larval (5–25 mm TL) Cape anchovy (*Engraulis encrasicolus*) sampled off the South African west (33–34.5°S) and southeast (26–26.5°E) coasts. Those authors reported higher individual growth rates at any given age, and higher RNA: DNA values indicative of higher instantaneous growth rates, for anchovy larvae on the southeast coast than on the west coast. Both our results and those of Costalago *et al.* (2020) indicate that food does not limit early growth of sardine and anchovy on the South African south and east coasts.

Small-scale coastal upwelling at capes and large-scale shelf-edge upwelling drive productivity off the south coast, whereas large-scale coastal upwelling and large-scale wind-stress-curl-driven upwelling are the primary drivers off the west coast (Kirkman *et al.*, 2016). Additionally, the two regions show different seasonality in primary production, with *Chl a* levels highest in spring-summer off the west coast and in autumn-winter off the south coast (Demarcq *et al.*, 2007). Sardine spawning peaks between spring and summer off the west coast and during winter to spring off the south coast, indicating that the putative sardine subpopulations off the west and the south coast have different strategies to utilize different ocean enrichment processes. This suggests that factors driving sardine biomass fluctuations may be different between these different regions, which will be the scope of future studies. In upwelling systems, retention in stable and productive coastal areas is considered key for larval survival of small pelagic fishes (Agostini and Bakun, 2002). Off the south coast, however, temporal and spatial match-mismatch between

larvae and their prey may be more important because the enrichment processes do not persist as long as coastal upwelling off the west coast. As match-mismatch has been considered a key process determining survival of Japanese sardine *Sardinops melanostictus* in warm current systems in the western North Pacific (Nishikawa and Yasuda, 2008; Kodama *et al.*, 2018), this may also be the case for South African sardine off the south coast.

It should be noted that the estimation of nursery temperature based on otolith $\delta^{18}\text{O}$ includes some potential errors. First, the seawater $\delta^{18}\text{O}$ value used for temperature estimation was predicted from the longitude at catch. This implicitly assumes that the fish had not moved substantially from where they were located during their first 2 months of life, which is not necessarily the case especially for adults. Second, the equation between otolith $\delta^{18}\text{O}$ and ambient water temperature, which was used to convert otolith $\delta^{18}\text{O}$ to temperature, was derived for Japanese sardine (Sakamoto *et al.*, 2017) and was not calibrated for sardine around South Africa. It has been suggested that the intercept of the otolith $\delta^{18}\text{O}$ ambient water temperature relationship can vary even between closely related species while the slope is similar among a wide variety of fish species (Storm-Suke *et al.*, 2007). Thus the estimated temperature may have consistently shifted to some extent (estimated temperatures are ~2–4°C lower than observed sea surface temperatures for each of the four regions). Nonetheless, the original otolith $\delta^{18}\text{O}$ before temperature conversion was significantly higher in sardine from the West region than in fish from the South in both adults and summer-captured juveniles. As it would be difficult to explain this difference due to spatial variation in seawater $\delta^{18}\text{O}$ because it has the opposite trend (being lower in the West) with limited seasonal variations, the otolith $\delta^{18}\text{O}$ solely suggests that the sardine nursery environment is cooler in the West than in the South or East regions. In addition, the potential variation in the intercept of the fractionation equation does not affect the relative difference of nursery temperature. Hence, although the estimated temperature may not be completely accurate, it does not weaken the conclusion that the nursery temperatures differ by several degrees between the West and the South regions.

Overall, the application of otolith $\delta^{18}\text{O}$ and otolith microstructure analyses has suggested the existence of sardine subpopulations off the west and the south coasts of South Africa that grow in different nursery environments and at different rates, with possibly a third subpopulation off the east coast. This pattern in South African sardine may reflect general features of the ecology of *Sardinops* sp. in upwelling and western boundary current regions around the world, hence comparisons of these data with those from sardine in other systems would be of particular interest. For example, the biomass of Japanese sardine in the Kuroshio Current system tends to increase in relatively cooler periods while that of Pacific sardine in the Californian Current system tends to increase in warmer periods (e.g. Chavez *et al.*, 2003); hence, analysing the responses of South African sardine subpopulations to environmental variations may provide further insights into the contrasting patterns observed in the North Pacific. As the combination of the otolith $\delta^{18}\text{O}$ and otolith microstructure analyses could help to understand population structures and also provide insights into drivers of population dynamics, the methods applied here will likely be useful where stock structure has been an issue for fisheries management elsewhere in the world.

Supplementary data

Supplementary material is available at the ICESJMS online version of the manuscript.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

TS and CDvdL designed and conceived the study. CDvdL, YG and JP collected fish, otolith, and seawater samples. TS performed otolith microstructure analysis. TS, KS, and TI performed isotope analyses. TS and CDvdL wrote the manuscript. All authors contributed to manuscript editing.

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