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Otolith microchemistry suggests local populations of Antarctic silverfish *Pleuragramma antarctica* (Boulenger, 1902) around Antarctica are exposed to similar environmental conditions at early life stages

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Abstract: The Antarctic silverfish *Pleuragramma antarctica* is a key species in the Southern Ocean ecosystem. Here we characterized the otolith microchemistry of 163 adult Antarctic silverfish collected from three areas located thousands of kilometers apart from each other: Cape Hallett, Adelie Land, and Joinsville Island. Otoliths were analyzed for chemical composition of both the edge (reflecting the exposure of individuals to environmental conditions at the site where they were sampled) and the core (reflecting exposure to environmental conditions during early life periods after the egg fertilization). The homogeneity or heterogeneity of the otolith core chemical composition indicates the possible exposure to similar or different environmental conditions at early life stages, respectively, and could provide insights regarding the number of natal origins of the three investigated populations. This information can be key for planning sound management strategies. We found that the chemistry deposited along otolith edges was heterogeneous between samples collected at Joinsville Island and those collected at the other two sampling areas. In contrast, the chemistry of otolith cores was homogenous. Our study suggests that adult Antarctic silverfish inhabiting areas very distant from each other have been exposed to similar environmental conditions at early life stages. This finding has a number of potential implications for the understanding of the demographic processes driving the survival and growth of early life stages, and for the structuring of silverfish population(s) in Antarctic waters.

Key words: otoliths, Antarctic silverfish, early life stages, natal origins

Conflicts of interest: The authors declare no conflict of interest.

Introduction

The notothenioid Antarctic silverfish *Pleuragramma antarctica* (Boulenger, 1902) - formerly known as *P. antarcticum* - is a key forage species in the marine ecosystem of the Southern Ocean. It plays a pivotal role at mid-trophic level, feeding upon planktonic organisms and being preyed upon by fishes, seals, whales and penguins (La Mesa et al. 2004; Brooks et al. 2018b).

It has a circumpolar distribution range along the Antarctic continental shelf, including the southern Scotia Arc and neighboring islands (Duhamel et al. 2014), and it can be found at high abundances between the shelf break and the continental margin, from 0 to ~900 m depth (La Mesa and Eastman 2012). Unusually for a notothenioid, it is a holopelagic species, spending its whole life in the water column, from egg to adult (Vacchi et al. 2004).

The Antarctic silverfish eggs are pelagic and positively buoyant (Evans et al. 2012). Embryo development occurs with eggs floating in the platelet ice layer (Vacchi et al. 2004; Guidetti et al. 2015) consisting of various-sized flat plate-like crystals (up to above 10 cm in diameter) randomly oriented, and forming a quite thick layer (sometimes >2m) underneath the solid sea-ice.

Due to the presence of a rich ice-associated community (cryopelagic community) available as a food resource for larvae (Giraldo et al. 2011), and to the crystal lattice structure providing shelter from predators, the platelet ice is a suitable brooding microhabitat (Guidetti et al. 2015). After hatching and throughout the fish life cycle, individuals move offshore and young stages, facilitated by the upper water column circulation, disperse over a wide area of the continental shelf (La Mesa et al. 2010). Post-larvae and juveniles recruit to the adult population 3–5 years after hatching. Reproduction is hypothesized to occur at the end of the winter with adults migrating shoreward and spawning in the vicinity of the ice-shelf (Vacchi et al. 2012). The observation of acoustic reflections consistent with adult silverfish in Terra Nova Bay in mid-September by the use of an upward-looking moored echo sounder (O'Driscoll et al. 2018) supports the hypothesis of mass migration of the silverfish to coastal spawning sites in winter.

Although spawning events have never been directly observed, the location of multiple spawning grounds along the coasts of Antarctica was inferred based on information on actual and potential nursery areas for early larvae (Ghigliotti et al. 2017, Figure 1). Newly hatched larvae have been collected in the Bay of Whales (Eastern Ross Sea), strongly suggesting the presence of a nursery area for the Antarctic silverfish there (Brooks et al. 2018b). However, the area of Terra Nova Bay is the only area known to date where egg development and hatching have been documented (Guidetti et al. 2015 and references therein; Ghigliotti et al. 2017).

Based on these observations, it has been hypothesized that interactions between life history and circulation associated with glacial trough systems drive the species' circumpolar distribution over the continental shelf (Ashford et al. 2017). Specifically, it has been hypothesized that, after hatching, larvae encounter an outflow trough circulation advecting them offshore towards the shelf-break. Then, mixing with trough inflow facilitates the return of a portion of these individuals back inshore, toward the inner shelf and the spawning area as adults. Fish reaching the continental shelf-break become exposed to currents along the continental slope, which transport them to trough systems downstream (Ashford et al. 2017; Brooks et al. 2018b). This theory seems to be supported by recent evidence suggesting the lack of genetic differentiation between locations connected by the Antarctic Slope Front Current (ASF). This is indicative of high levels of gene flow, while this flow is significantly reduced at the South Orkney Islands and the western Antarctic Peninsula where the ASF is absent (Caccavo et al. 2018).

However, genetic patterns reflect the spatial scale at which populations can be differentiated into discrete units due to the process of genetic drift and highlight population processes on a multi-generational timescale (Leis et al. 2011). A lack of genetic differentiation may result from an array of situations ranging from fairly total demographic independence among large-sized populations to the existence of a unique panmictic population (see Gagnaire et al. 2015 for a detailed discussion about this issue). Therefore, inferences of genetic homogeneity should not be directly interpreted as implying the existence of a single ecological unit for management purposes (Caccavo et al. 2019).

In this context, analysis of the elemental composition of otoliths has been widely proven to be a powerful tool to elucidate patterns of demographic connectivity and the number of natal origins of fish (Calò et al. 2013). This approach can delineate non-connected fish populations within the overall distribution of a species (Campana 2005). The method is based on the premise that as otoliths grow, they record information about the fish life history. The concentration of some trace elements (e.g. Mg, Ba and Sr) within the calcareous otolith matrix is, in fact, influenced by site-specific environmental factors (i.e. water chemistry, temperature, salinity etc.). As the carbonate in otoliths is metabolically inert, i.e. not reworked or resorbed (Campana and Thorrold 2001), a site-specific signature is permanent and the otoliths retain a chronological record of the environment(s) experienced by the fish throughout life (Elsdon et al. 2008). The otolith core, the portion laid down during embryogenesis, stores information specifically related to natal origin. Therefore, core analyses can reveal, for example, whether fish populations inhabiting different areas are connected by sharing a common natal origin or if they are demographically segregated. Among notothenioid fish, core chemical composition has been used to investigate the population structure of Antarctic toothfish *Dissostichus mawsoni* (Ashford et al. 2012), Patagonian toothfish *Dissostichus*

eleginoides (Ashford et al. 2006), Scotia Sea icefish *Chaenocephalus aceratus* (Ashford et al. 2010), and Antarctic silverfish (Ferguson 2012; Caccavo et al. 2019). The potential of otolith microchemistry to characterize life history patterns for the Antarctic silverfish has already been already highlighted several decades ago (Radtke et al. 1993). Previous studies on the composition of Antarctic silverfish otolith core have attempted to resolve population structure for specific portions of species distribution, i.e. Weddell Sea (Caccavo et al. 2019) and Antarctic Peninsula (Ferguson 2012), while no studies have used this approach at a larger spatial scale.

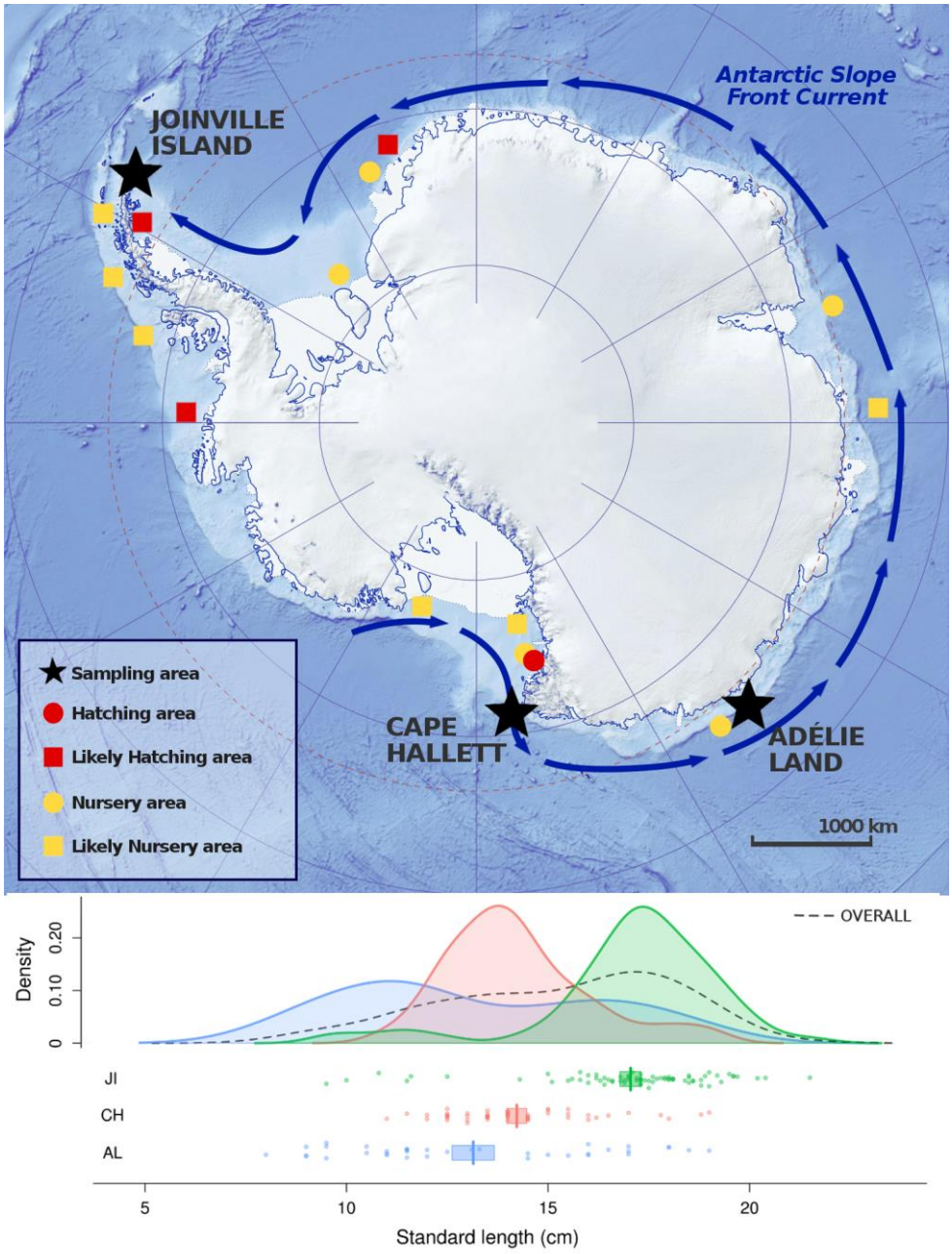
The aim of this work is to characterize the otolith microchemistry of three populations of Antarctic silverfish *Pleuragramma antarctica* located thousands of kilometers apart from each other, over a significant portion of the species' distribution. The homogeneity or heterogeneity of the otolith core fingerprint (i.e. chemical composition) indicates the possible exposure to similar or different environmental conditions at early life stages and could provide insights about connectivity or segregation of the three investigated populations.

Material and Methods

Sample collection

Adult Antarctic silverfish were collected at Cape Hallett (CH, Western Ross Sea), Adelie Land (AL), and Joinsville Island (JI, Antarctic Peninsula) during scientific cruises aboard the research vessels Tangaroa (New Zealand expedition, January-March 2004), L'Astrolabe (French expedition, January-March 2010), and Polarstern (German expedition, March-April 2012), respectively (Fig. 1). Sampling operations were conducted by bottom trawl aboard RV Tangaroa and RV Polarstern; an Isaacs-Kidd Midwater Trawl (IKMT) was used aboard L'Astrolabe.

Individuals were measured to estimate standard length (SL, to the nearest 0.1 cm) immediately after capture and then frozen at -20°C. A total of 163 individuals were collected in the three sampling areas: 37 from AL (SL = 13.15 ± 0.52 cm, mean ± se), 52 from CH (SL = 14.23 ± 0.25 cm) and 74 from JI (SL = 17.05 ± 0.26 cm) (Fig. 1).



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157 **Figure 1**. Sampling areas around Antarctica (Cape Hallett, Adelie Land and Joinsville Island).
158 Approximate position of actual and likely hatching and nursery areas from Ghigliotti et al. 2017.
159 The blue dashed line and arrows represent the Antarctic Slope Front Current. In the bottom panel
160 standard length (SL) of *P. antarctica* in the three sampling areas are shown: curves represent
161 density distribution of fish SL, vertical bars and rectangles represent mean ± SE of SL for the three
162 sampling areas.

163

164 *Otolith preparation and analysis*

In the laboratory, one sagittal otolith was removed from each specimen, cleaned of soft tissue using plastic dissecting pins, and mounted sulcus side up on a glass slide using crystal bond (Aremco Products, Inc.). Otoliths were polished with 40, 3 and 1 μm Imperial 3M lapping film to expose inner growth layers for analysis. We chose not to polish the otolith to the core and to leave material above it in order to ensure the core was not removed during pre-ablation procedures, which potentially allowed us to sample all the material associated with the core. After polishing with lapping film, otoliths were rinsed and sonicated for 10 minutes in ultra-pure water. Otoliths were dried and arranged onto new glass slides (6 otoliths per slide). All otoliths were randomly ordered to prevent sample batch bias.

All otoliths were analyzed using a Thermo X Series II inductively coupled plasma mass spectrometer (ICP-MS) coupled to a NewWave Research UP213 with aperture imaging laser ablation (LA) system. External calibration was performed with two Standard Reference Materials (SRM) from National Institute of Standards and Technology: NIST 610 and NIST 612. Calcium was used as an internal standard to account for variation in ablation and aerosol efficiency. All seven elements analyzed (7Li, 24Mg, 55Mn, 66Zn, 88Sr, 138Ba, 208Pb) were expressed as ratios relative to 44Ca.

Detection limits (LOD) were calculated from the concentration of analyte, yielding a signal equivalent to 3× the standard deviation of the blank signal for each of the elements.

Otoliths were analyzed for chemical composition of both the edge (reflecting exposure of the individual to environmental conditions at the site where the individual was sampled) and the core (in order to acquire information about natal origin).

Otoliths were placed in the ablation chamber and viewed remotely on the computer screen where the area for ablation was selected. The laser was focused on the sample surface and fired through the microscope objective lens using a spot size of 30 μm. Each run generally consisted of 62 s acquisition: (i) 25 s blank to correct for background, which was subtracted from each sample; (ii) 2 s of preablation to remove surface contamination (laser at 50% power); (iii) 10 s ablation (laser at 65% power, about 13 J cm^{-2}) resulting in a pit about 10 μm deep; and (iv) 25 s for washout. Helium gas was flushed into the ablation cell to reduce the deposition of ablated aerosols and to improve signal intensities. The ablated aerosol was then mixed with argon before entering the ICP torch.

Elemental analyses of otolith cores and edges produced concentrations that were greater than the limits of detection (LOD) in 96%, 100% and 99.9% of the samples respectively for Mg, Sr and Ba. Concentrations of other elements analyzed were predominantly <LOD; for this reason, these elements were excluded from subsequent analyses.

In the otolith margin, we ablated three horizontal pits that were considered in subsequent analysis in order to account for within-otolith variability (see Di Franco et al. 2011, 2014 for further details).
We used laser ablation to sample material associated with the core using three discrete vertical pits from the surface of the otolith through the visible core. Due to the exclusion of Mn from the analyses (because it was consistently below the LOD, in agreement with what reported in Caccavo et al. 2019) and due to the fact that the LOD of Mn in the present study was similar to or lower than those from other studies where a spike in Mn:Ca was adopted as an indicator of the core location (e.g. Di Franco et al. 2012, 2015), we hypothesized that, in the studied species, a spike in Mn:Ca could not be an effective 'core localizer'. We thus chose to consider for the core analyses all the three replicates sampled. In the present work, the core identifies the area laid down at egg fecundation and very early larval stages (as in Miller and Shanks 2004; Papetti et al. 2013).
Due to otolith breakage during polishing operations, chemical analysis was not possible for 5 otolith cores and 4 otolith edges.

Data analysis

We assessed the potential spatial variability (among the three sampling areas) in otolith chemical composition both for the core and the edge portions (i.e. the otolith portion laid down just before capture). Three-ways unbalanced permutational multivariate analysis of variance (PERMANOVA) were used, both on multivariate data (all elemental ratios together) and on each of the elemental ratios. Pairwise comparisons between sampling areas were run in case of significance of this factor. Two Multi-Dimensional Scaling (MDS) were used to graphically show differences in chemical composition of otolith edges and cores between sampling areas. Combined barplots-violinplots (R 'yarrr' package, (Phillips 2017)) were used to graphically examine differences in edge and core concentrations of Mg, Sr and Ba in the three sampling areas.
We used individual standard lengths to estimate relative fish age adopting the length-age curve developed by (Sutton and Horn 2011). Then, spawning year was back-calculated from sampling year by subtracting the estimated fish age for each individual.
For what concerns the edge, PERMANOVA design included: fish age (fixed, 13 levels), sampling area (fixed, 3 levels), otolith (random, nested in fish age and sampling area, up to 22 individuals for each combination of sampling area and fish age), for a total of 159 otoliths analyzed. Three replicate ablations for each otolith were collected: the assessment of intra-otolith variability is, in fact, instrumental to then assess the inter-otolith variability (Di Franco et al. 2011, 2014). Fish age was included in order to account for potential ontogenetic effect in elemental absorption (Tanner et al. 2011).

For the analyses of the otolith core, PERMANOVA design included the following factors: spawning year (fixed, 16 levels), sampling area (fixed, 3 levels) and otolith (random, nested in spawning year and sampling area, up to 21 individuals for each combination of sampling area and spawning year), for a total of 158 otoliths analyzed. Spawning year has been included in order to account for potential temporal variability within each sampling area.

For each factor included in the experimental design, effect sizes were determined as the percentage of ‘‘components of variation’’ a term that includes variation due to both fixed and random effects (Anderson et al. 2008; Wernberg and Vanderklift 2010).

In order to assess sample size adequacy (in our case the minimum number of otoliths required) for properly discriminating sampling areas, we estimated multivariate pseudo-standard error (MultSE) (Anderson and Santana-Garcon 2015), a direct analogue to the univariate standard error considered as a useful quantity for assessing sample-size adequacy with multivariate data implemented in dissimilarity-based multivariate analyses.

All statistical analyses were run using the PRIMER 6 software package (Clarke and Gorley 2006) with the PERMANOVA+ add-on (Anderson et al. 2008), while all graphical outputs were produced with R 3.6.3 (R Core Team, 2020).

Results

For the otolith edge, both factors 'Fish Age' and 'Sampling area' were significant (Tab. 1), while their interaction was not. *A posteriori* comparisons between pairs of sampling areas showed no significant differences between AL and CH, while both significantly differed from JI (Fig. 2). A significant variability was observed for the factor 'Otolith'. The factors with the highest relative contribution to the component of variation were 'Sampling area' and 'Otolith', that together accounted for >65% of total variation.

Table 1. PERMANOVA on data of multivariate chemical composition of otolith edges. Ag: fish age; Sa: sampling area, Ot: otolith. ** Term has one or more empty cells. The effect size was calculated as the relative contribution (in %) of each source to the components of variation.

Source	df	SS	MS	Pseudo-F	P(perm)	Effect size (%)
Ag	12	1.4068	0.11723	5.6461	0.0001	15.53
Sa	2	1.1123	0.55614	26.922	0.0001	32.16

AgxSa**	15	0.442	2.9467E-2	1.4265	0.1135	4.68
Ot (SaxSp)	127	2.6216	2.0643E-2	8.4536	0.0001	33.98
Res	313	0.76432	2.4419E-3			13.64
Total	469	6.347				

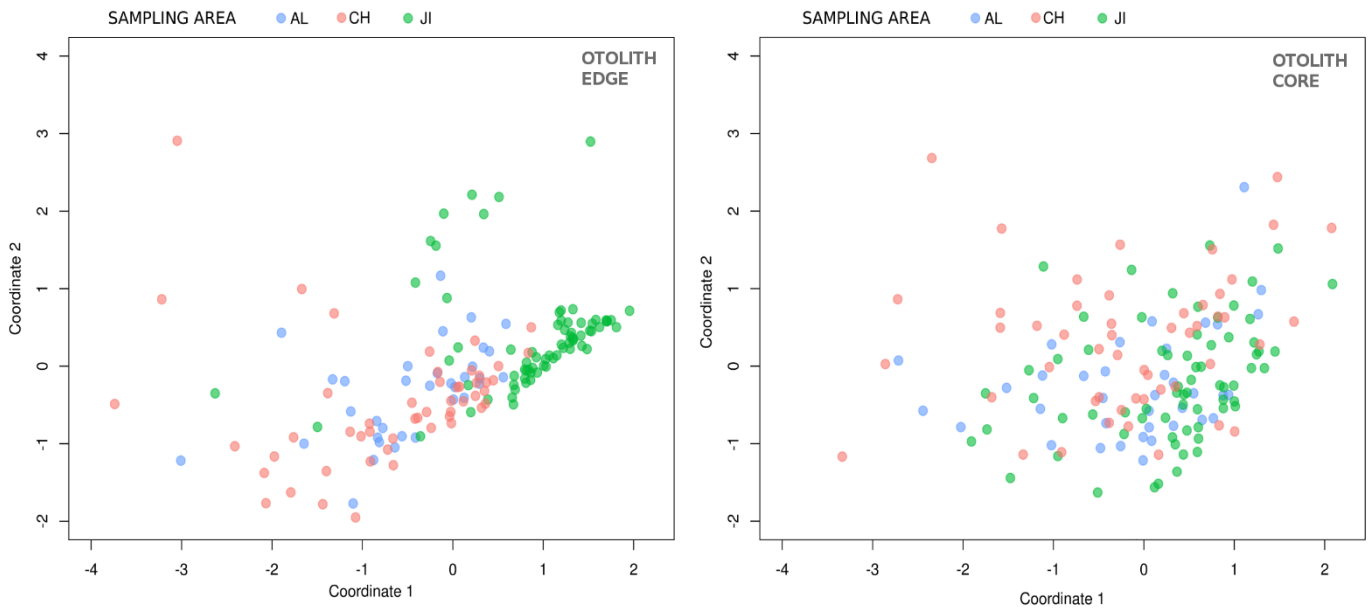

Figure 2. MDS of chemical composition of edge (left panel) and core (right panel) portion of otoliths. Different colors represent the three sampling areas. AL= Adélie Land (blue dots), CH= Cape Hallett (Western Ross Sea; red dots), JI= Joinsville Island (Antarctic Peninsula; green dots). Dots represent individual otoliths/fish (multiple replicates per otolith edge and core were averaged).

The same pattern was highlighted for each of the three elemental ratios in otolith edge (Online resources 1-3). A significant variability was observed for the factors 'Fish Age', 'Sampling Area' and 'Otolith'. For each of the three elemental ratios the lowest concentration was recorded in the samples from JI (Antarctic Peninsula) (Fig. 3).

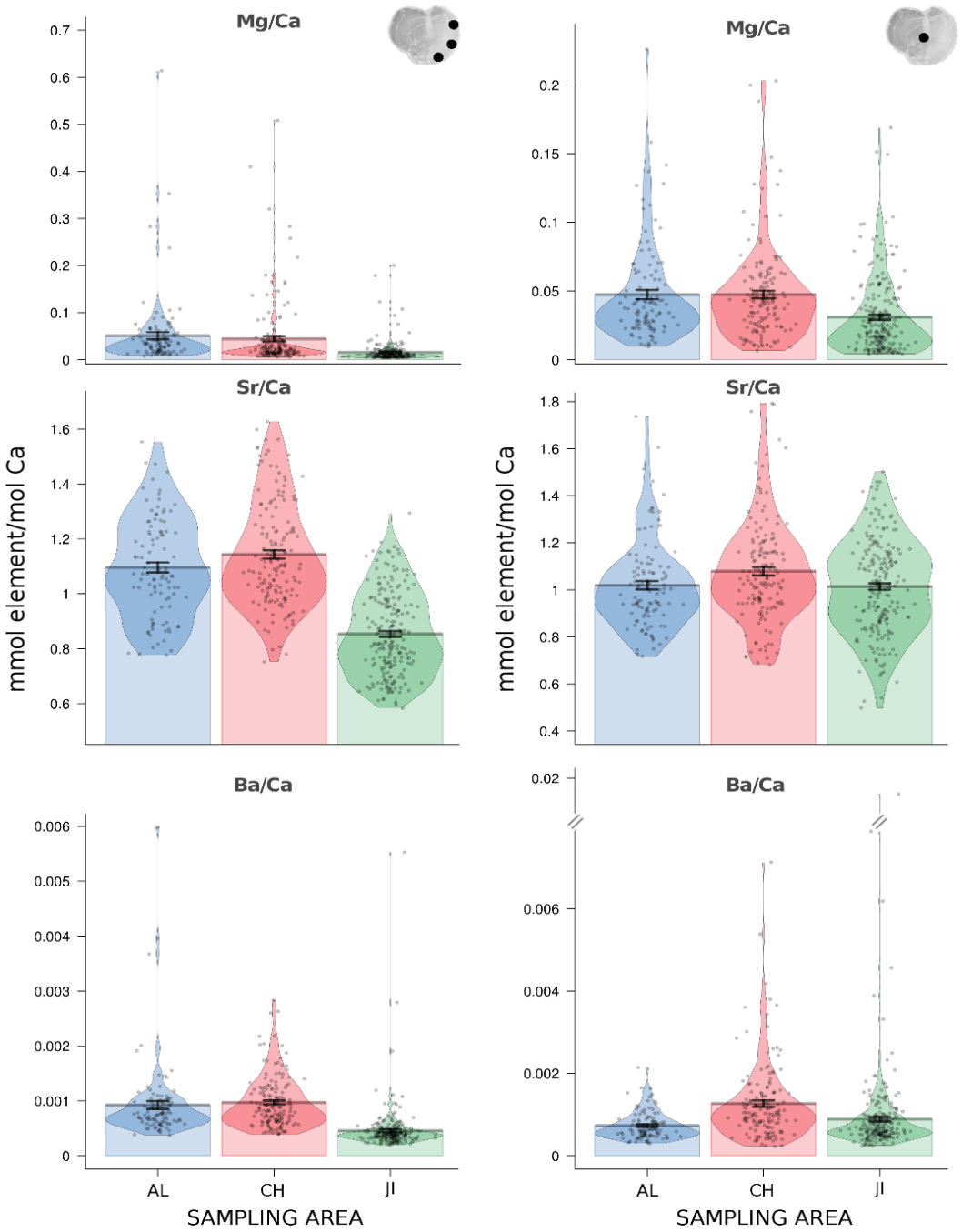

Figure 3. Combined barplot-violinplot showing element/calcium ratios in the otolith edge portion (left panels) and core (right panels) for the three sampling areas. AL= Adélie Land, CH= Cape Hallett (Western Ross Sea), JI= Joinsville Island (Antarctic Peninsula). Black horizontal and vertical bars indicate average values (± standard error); violins indicate density distribution of values (i.e. dots, horizontal jittering added to dots to improve figure clarity). Otolith miniatures show the approximate position of sampling ablations on edge and core (3 vertically coincident ablations).

The chemical composition of the Antarctic silverfish otolith cores was homogenous among sampling areas (Fig. 2) and spawning years (i.e., no significant main effects of factors 'sampling area' and 'spawning year', nor of their interaction, were detected; Tab. 2). A significant variability was only detected for the factor 'Otolith', which explains most of the total variation (>70%) in chemical composition of otolith cores (Tab. 2).

Table 2. PERMANOVA on data of multivariate chemical composition of otolith cores. Sp: spawning year; Sa: sampling area, Ot: otolith. ** Term has one or more empty cells. The effect size was calculated as the relative contribution (in %) of each source to the components of variation. Negative components of variation were set to 0 (Graham and Edwards 2001).

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Effect size (%)**
Sp	15	0.59449	3.9632E-2	1.4966	0.1089	4.00
Sa	2	1.1584E-2	5.7922E-3	0.2154	0.8454	0.00
SpxSa**	13	0.40623	3.1249E-2	1.1631	0.3193	4.47
Ot (SaxSp)	128	3.4515	2.6965E-2	12.568	0.0001	72.62
Res	319	0.68443	2.1456E-3			18.91
Total	477	5.1482				

No significant effect of factors 'spawning year' and 'sampling area', nor of their interaction, were detected in terms of core composition, even taking into account each of the three elemental ratios here considered separately (Online resources 4-6). A significant variability for the factor 'Otolith' was recorded for each of the three elemental ratio (Online resources 4-6). The highest percentage of total variation was explained by the factor 'Otolith' for Sr/Ca and Ba/Ca and by Residual for Mg/Ca. The absence of variability in core chemical composition between the 3 populations investigated in this study is not related to a lack of statistical power. The estimated multivariate pseudo-standard error (MultSE), in fact, showed that for both otolith edges and cores between 15 and 20 otoliths were sufficient to detect differences between sampling areas (i.e. levelling-off MultSE around these sample sizes, Fig. 4), a number far lower than the minimum otolith sample size per group considered in this study (37 for cores and 35 for edges).

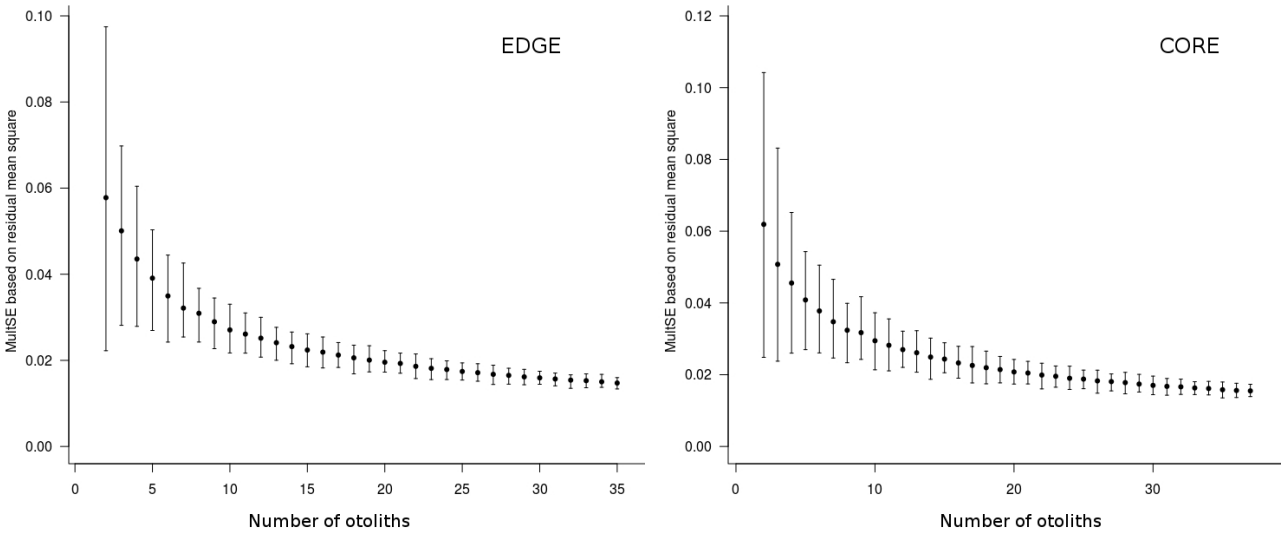

Figure 4. Multivariate pseudo-standard error (MultSE) for edges (left panel) and cores (right panel), calculated from the residual mean square of a one-way PERMANOVA model as a function of sample size on the basis of Euclidean dissimilarities.

This finding shows that our sample size (number of otoliths analyzed for each population) is approximately twice the lower bound required to detect existing difference between sampling areas.

Discussion

The main output of this study, in a nutshell, is that the otolith fingerprint (i.e. chemical composition) of the Antarctic silverfish sampled in three areas around the Antarctic continent is i) substantially homogeneous in the cores and ii) significantly heterogeneous along the edges. This result enables us to draw some hypotheses that will be here discussed in the light of the available literature.

Previous studies on the otolith microchemistry of multiple species highlighted that the elements displaying spatial structuring around the Southern Ocean and the Antarctic continental shelf are Mg:Ca, Sr:Ca and Ba:Ca (Ashford et al. 2010, 2012; Zhu et al. 2018; Caccavo et al. 2019). Conversely, Mn:Ca was nearly absent at southern latitudes higher than the Antarctic Circumpolar Current (ACC) (Caccavo et al. 2019). This is in line with our findings (Mg:Ca, Sr:Ca and Ba:Ca are the 3 ratios above LOD and that we retained for analyses).

We found that chemistry deposited prior to capture along otolith edges was heterogeneous between samples collected at Joinsville Island and samples collected at the other two sampling areas (Cape

Hallett and Adelie Land). Such a pattern could be attributable to the environmental heterogeneity of the areas where the individuals have spent their last periods as adults. In contrast, the chemistry in otolith cores, deposited during early life periods after egg fertilization, was homogenous.

Based on the homogeneity in elemental composition of the Antarctic silverfish otolith cores among the 3 sampling areas, we can consider two explanatory scenarios: 1) a single natal origin/area replenishes the three local populations; 2) multiple natal origins/areas replenish the three local populations, but these origins are not distinguishable based on otolith microchemistry because fish are exposed to similar environmental conditions at early life stages.

Scenario 1 would be consistent with current evidence of a single documented hatching of silverfish (Terra Nova Bay), and with the genetic homogeneity of silverfish over a large portion of its distribution range around Antarctica (Zane et al. 2006; Caccavo et al. 2018). Based on this scenario, local sub-populations would belong to a single widely-distributed population. However, this scenario is in disagreement with the likely presence of multiple hatching areas as reconstructed by Ghigliotti et al. (2017). On the other hand, what was reported by Ghigliotti et al. (2017) could support scenario 2 suggesting that environmentally homogenous spawning/hatching habitats might imply the impossibility of discriminating between multiple natal origins. At the only spawning/hatching area known to date in Terra Nova Bay, the eggs develop and hatch in the platelet ice layer, a peculiar physicochemical environment (Guidetti et al. 2015) whoseenvironmental homogeneity would not allow to chemically discriminate the cores of otoliths coming from different areas.

In the Weddell Sea, Caccavo et al. 2019 detected some heterogeneity in core composition of samples collected over a smaller spatial scale compared to that we have investigated. We should consider that the samples were collected in different years from those analyzed in the present study and temporal variability in connectivity patterns cannot be excluded. Furthermore, Caccavo et al. 2019 analyzed a portion of the otolith (a grid raster type 150 × 200 μm) that corresponds to the first austral summer of growth, while we investigated a smaller portion (30 μm spot) that likely corresponds to the first month of life (based on distancing between daily growth rings measured by (La Mesa et al. 2015)).

Caccavo et al. 2019 also detected significant differences in core composition between groups of individuals belonging to large and small length modes, suggesting that immature and mature individuals were exposed to different environmental conditions during early life. This could suggest different natal origins for these two groups. However, it should be taken into account that environmental conditions vary over time as well, and differences in core chemistry such those that Caccavo et al. 2019 found between length modes may simply reflect variations across inter-annual

366 time scales within a single natal trough. In this perspective, we tried as far as possible to control for
367 temporal variability by including time-related factors (i.e. spawning years) in our analyses.
368 Considering the possible existence of multiple hatching areas (Ghigliotti et al. 2017) and evidence
369 from otolith microchemistry suggesting that juvenile Antarctic silverfish inhabit different areas
370 during their first months after the hatching (Caccavo et al. 2019), our findings are likely better
371 explained by scenario 2.
372 Our findings regarding sample-size adequacy (our sample size being far larger than the threshold
373 required to detect existing differences between sampling areas) indicate that the homogeneity in
374 otolith core microchemistry is genuine and not related to a lack of statistical power. This result is
375 further confirmed by the significant difference detected in the chemical composition of otolith edge,
376 highlighting that individuals collected at distant areas have heterogeneous elemental fingerprints.
377 Ashford et al. 2017 hypothesized that, based on the physical-biological population framework they
378 developed, when advection is predicted to reach a trough occupied by a neighboring population,
379 mixing can be detected as a bimodal distribution in the nucleus chemistry. On the other hand, in
380 situations where simulations predict scarce advection, population separation can be tested by
381 differentiation in the core chemistry characterized by underlying unimodal distributions. We did not
382 find any bimodal distribution in the core chemistry that could suggest an absence of major
383 advective processes. In addition, we need to consider that, for each population, our distribution
384 includes observations coming from individuals spawned in different years.
385 Previous work on the otolith chemical composition of Antarctic silverfish suggests that differences
386 in Ba:Ca are thought to reflect ambient levels of dissolved Ba (Ashford et al. 2005). In contrast,
387 Mg:Ca and Sr:Ca are directly influenced by physiology: Sr:Ca is thought to reflect growth
388 (Campana 1999), mediated by ambient temperature (higher Sr:Ca ratios are interpreted as
389 representing lower water temperatures following Radke et al. 1992) and food availability, whereas
390 Mg:Ca is associated with physiological processes related to fish activity such as reproduction and
391 movement, again influenced by spatially variable properties of the ambient water (Ashford et al.
392 2005, 2010; Caccavo et al. 2019). This being so, the homogeneity detected in core chemical
393 composition between samples collected at the three sampling areas could be consistent with the
394 hypothesis that early life stages, potentially originating from different spawning/hatching areas,
395 were exposed to similar environmental conditions. In fish, environmental conditions experienced in
396 early life stages are known to affect early survival and growth and can therefore be an important
397 determinant of individual life histories (Vindenes et al. 2016). Our findings could thus suggest that
398 individuals collected at different sampling areas could have experienced similar growth rates and
399 physiological processes at early life stages. This would suggest that environmental drivers probably

do not play a role in determining potential spatial variability in individual fitness at early life stages and should not have a major impact on population replenishment (e.g. no carry-over effects). Life history traits, including individual growth, fitness, development and survival, are vulnerable to environmental alterations and may be affected directly or indirectly by changes in temperature, salinity, prey availability and composition, competition and predation (see Mintenbeck and Torres 2017 for a review of the limited information available for the Atlantic silverfish). These traits can have a major impact on recruitment, year-class strength and, finally, on population size (Stige et al. 2019). However, growth and survival at early stages also depend on ecological processes such as predation and competition that could have differed between the areas we investigated and that would not be captured by the otolith chemical composition. Future studies specifically focusing on these aspects are required to shed light on potential variability in early life processes throughout the silverfish distribution range) and assess their demographic effects.

Considering the ecological relevance of Antarctic silverfish in the Southern Ocean ecosystem, identifying spawning areas and natal origins, and gaining insights into demographic connectivity, population(s) structure and dynamics can be key to better understanding the potential impact of climate change on this species and planning sound management strategies to enhance its resilience, finally supporting Southern Ocean ecosystem health. Climate change and its effects on Antarctic ecosystems represent a current and serious concern for scientists and policy makers (Brooks et al. 2018a). Ocean temperatures, currents and weather patterns are dramatically changing, and the northwest coast of the Antarctic Peninsula is one of the fastest-warming places on Earth — summer mean temperatures are on average 3 °C higher than they were in 1950. Diminishing sea ice due to temperature rise would imply fewer algae, krill and Antarctic silverfish and so a substantial degradation of the Southern Ocean ecosystem (Brooks et al. 2018a). Recent reviews on the Antarctic silverfish reproduction and life history (Ghigliotti et al. 2017), and its spatio-temporal population structure and dynamics (Ashford et al. 2017), have provided insights regarding the occurrence of multiple nursery grounds around Antarctica and the species' population structure and connectivity. However, to date, conclusive evidence relative to the number and locations of spawning areas, and the ecological connectivity between local populations, is still lacking.

Our work did not provide a conclusive answer concerning the number of natal origins of silverfish in Antarctica, but highlighted that individuals inhabiting as adults areas that are very distant from each other have been exposed to similar environmental conditions at early life stages. This has a number of potential implications for the understanding of the demographic processes driving the survival and growth of early life stages, and more generally the structure of the silverfish population(s) in Antarctica.

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Figure 1

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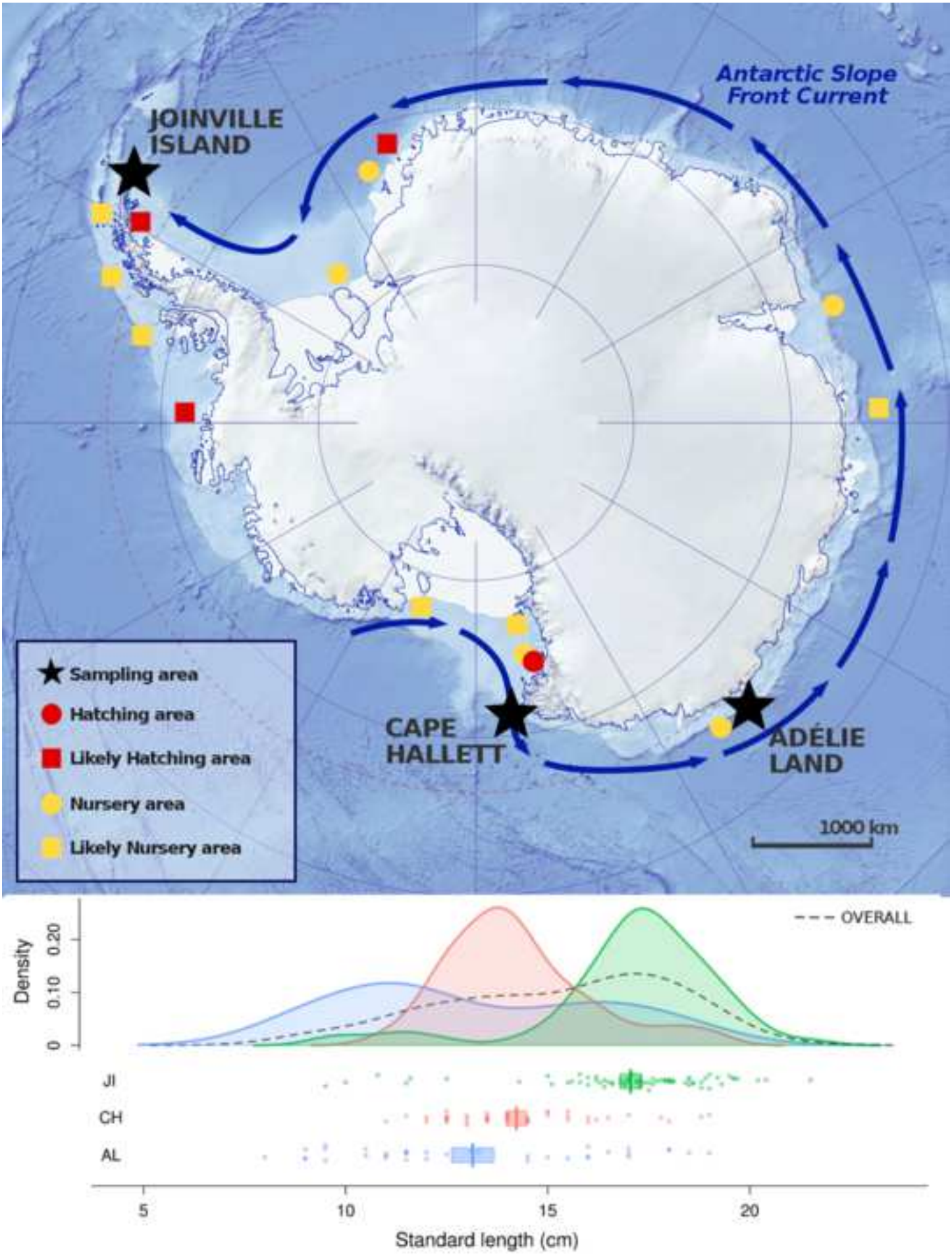

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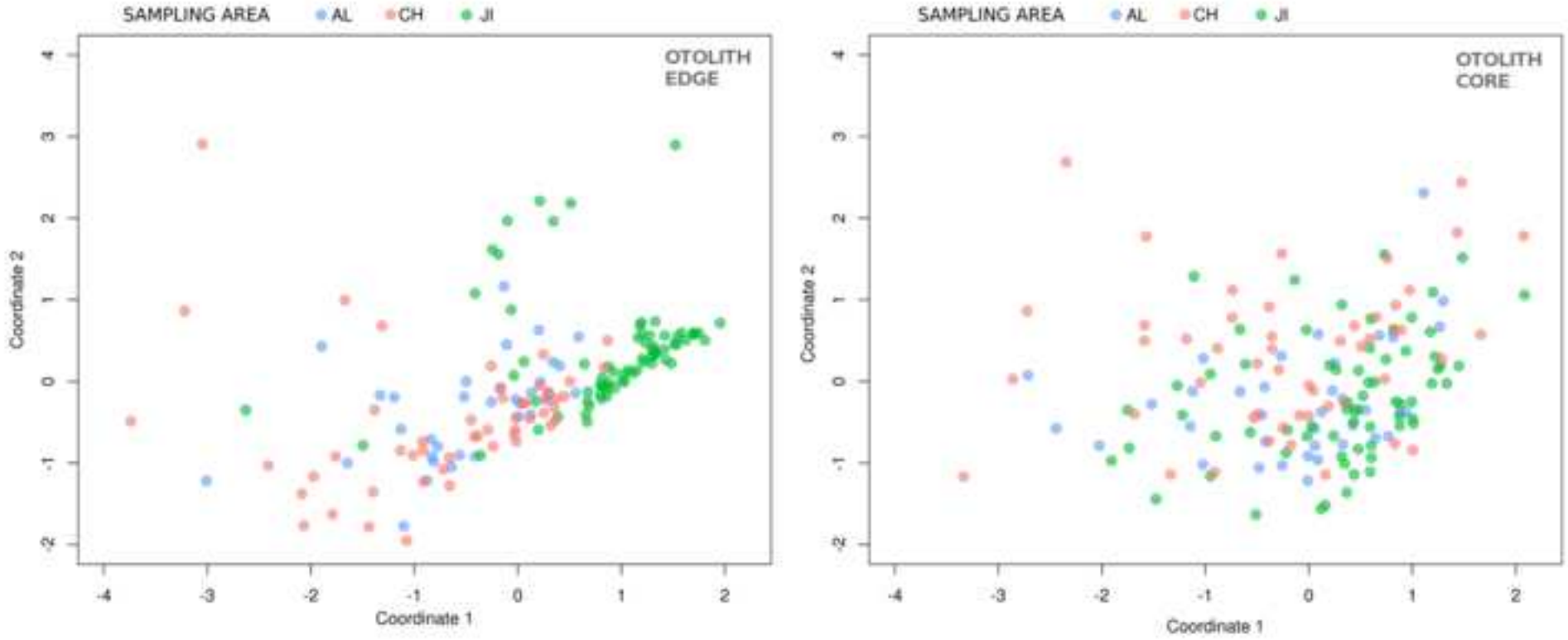

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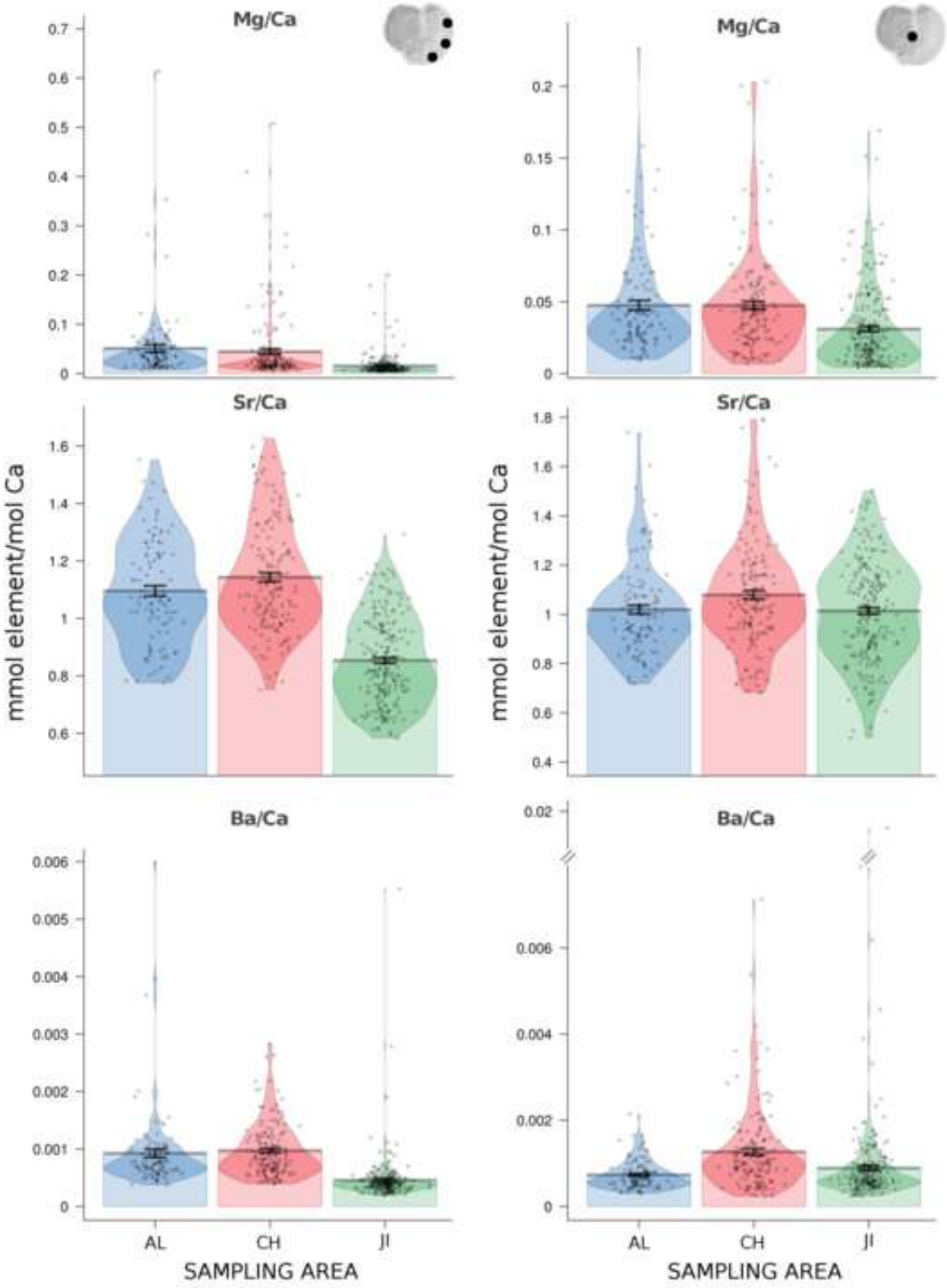

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