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Original Article

Integrated statolith and genomic analysis reveals high connectivity in the nektonic squid *Illex argentinus*: implications for management of an international cephalopod fishery

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The neritic-oceanic squid *Illex argentinus* supports one of the largest fisheries in the Southwest Atlantic. It is characterized by extensive migrations across the Patagonian Shelf and complex population structure comprising distinct seasonal spawning groups. To address uncertainty as to the demographic independence of these groups that may compromise sustainable management, a multidisciplinary approach was applied integrating statolith ageing with genome-wide single-nucleotide polymorphism (SNP) analysis. To obtain complete coverage of the spawning groups, sampling was carried out at multiple times during the 2020 fishing season and covered a large proportion of the species' range across the Patagonian Shelf. Statolith and microstructure analysis revealed three distinct seasonal spawning groups of winter-, spring-, and summer-hatched individuals. Subgroups were identified within each seasonal group, with statolith microstructure indicating differences in environmental conditions during ontogeny. Analysis of >10 000 SNPs reported no evidence of neutral or non-neutral genetic structure among the various groups. These findings indicate that *I. argentinus* across the Patagonian Shelf belong to one genetic population and a collaborative management strategy involving international stakeholders is required. The connectivity among spawning groups may represent a "bet-hedging" mechanism important for population resilience.

Keywords: genomics, *Illex*, metapopulation, nektonic, squid, statolith, sustainability.

Introduction

Cephalopods have become an increasingly important fishery resource and are viewed and exploited as alternatives to many depleted traditional finfish fisheries (Caddy and Rodhouse, 1998). Fishing pressure on cephalopod populations has increased over the last 50 years as catches have levelled off to 3.6 million tonnes in 2018 from a peak of 4.9 million tonnes in 2014 (FAO, 2020). These species often occupy important roles as predators and prey in marine ecosystems (Xavier *et al.*, 2014). This makes identifying the population structure of these species crucial for both fishery management and ecosystem conservation. Nonetheless, our biological knowledge of some of the largest biomasses of exploited cephalopods in the world is limited as these species often exhibit high levels of phenotypic plasticity, short life cycles, and high growth rates (Rodhouse, 2001). These characteristics obfuscate the extent of population cohesion or independence when studied with only traditional approaches. Accordingly, there is the need to combine multiple stock identification methods (McKeown *et al.*, 2015, 2017). In-depth knowledge of the population structure of cephalopod populations will enable the establishment of baselines for groups of commercially exploited species, which may subsequently be used to adapt stock assessments (Arkhipkin *et al.*, 2015).

Nektonic cephalopods, some of which migrate over large oceanic distances and move between several oceanic ecosystems, present an additional challenge for aligning operational and biological management strategies in population management. As they pass through the exclusive economic zones (EEZs) of multiple states and international waters (Arkhipkin *et al.*, 2020, 2022), they may be exploited by multiple national fleets and at various life stages, making them "straddling stocks". The Argentine short-fin squid (*Illex argentinus*) is a neritic-oceanic species, distributed between 22 and 55°S along the Patagonian Shelf and slope (Rodhouse *et al.*, 2013). It supports one of the largest fisheries in the Southwest Atlantic; annual catches reached a peak of over 1 million tonnes in 2015 (FAO, 2020) and 16.4% of global squid catch in 2020 (FAO, 2022). Furthermore, as this species is targeted by multiple international stakeholders, namely Republic of China (Taiwan), Republic of Korea, Spain, Argentina, and People's Republic of China, it is of global importance (Arkhipkin *et al.*, 2015). This further complicates the management of the fishery as currently there is no cohesive management strategy in the region, with each coastal country setting independent conservation targets (Arkhipkin, 2015). The population structure of *I. argentinus* appears to be complex, as there are uncertainties regarding the total number of seasonal spawning groups, with up to four distinct groups hypothesized (Figure 1a) (Brunetti, 1988; Hatanaka, 1988). The winter-spawning group has been identified as the largest and spawning occurs in the austral winter (June–August;

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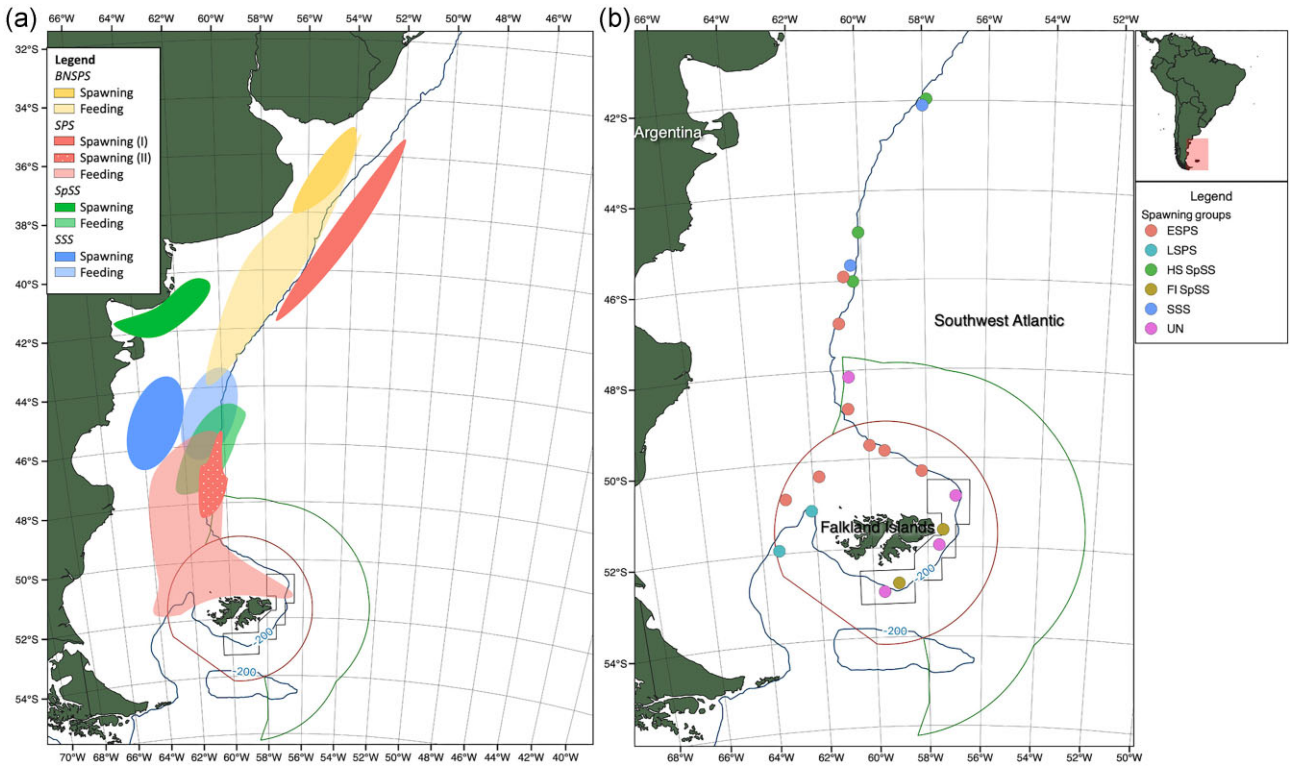

Figure 1. (a) Putative spawning and feeding areas of *I. argentinus* spawning groups. The two hypothesized spawning sites for the SPS group are depicted as (i) north of 35°S and (ii) between 45 and 48°S. (b) Sampling locations of *I. argentinus* and the occurrence of the spawning groups identified. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; FICZ, Falkland Islands Interim Conservation and Management Zone (red line); FOCZ, Falkland Islands Outer Conservation Zone (green line); and LOLBOX, Loligo Box (black line).

Rodhouse *et al.*, 2013). This group has been further divided into the Bonaerensis North Patagonian stock (BNPS), occurring north of 43°S, and the South Patagonian stock (SPS), occurring south of 44°S (Brunetti, 1988). The SPS group is the most abundant and individuals complete the largest-scale migrations, from 34–38 to 51°S to feed during the austral summer (November–March) and subsequently return north to spawn in early April (Arkhipkin, 1993). There are two hypotheses regarding the possible spawning site for this group: (i) north of 35°S in the south Brazil area of the shelf and shelf-break (Haimovici and Pérez, 1990; Arkhipkin, 2013); or (ii) between 45 and 48°S, on the Patagonian outer shelf (Haimovici *et al.*, 1998; Torres Alberto *et al.*, 2020). Statolith microstructure investigations revealed that the SPS comprised two subgroups: (i) the shelf subgroup, maturing at smaller sizes, with higher growth rates; and (ii) the slope subgroup, maturing at larger sizes, with slower growth rates (Arkhipkin, 1993). The slope SPS subgroup may be further divided into the early-maturing South Patagonian Stock (ESPS) and late-maturing South Patagonian Stock (LSPS) groups (Falkland Islands Government, 2021). The ESPS group has been primarily observed in the north and northeastern areas of the Falkland Islands Conservation Zones (FICZ and FOCZ) in March and attains smaller sizes at maturity. Conversely, the LSPS arrives in the FICZ in April, in the western areas, and attains larger sizes at maturity (Falkland Islands Government, 2021). The Falkland Islands Spring Spawning Stock (SpSS) group is hypothesized to spawn in coastal waters, such as the San Matias Gulf (41–42°S; Crespi-Abril *et al.*, 2008, 2013; Crespi-Abril and Barón, 2012), and may use the high-seas areas between 45 and 47°S during austral summer. The Summer Spawning Stock (SSS) group is believed to spawn on the shelf between 42 and 46°S from December to February, and occasionally migrate to the high seas (Crespi-Abril *et al.*, 2010). The definition of these spawning groups is often based on single techniques that focus on either phenotypes or genotypes with the primary focus on group phenotypes such as size and spatial distribution, whereas genotypic traits are often considered independently.

The identification of at least four seasonal groups highlights the uncertainty as to whether this species should be managed as a single stock or as a composite stock. Phenotypic markers may reveal differences among groups that reflect plastic responses to environmental heterogeneity within a single panmictic population (see Van Der Vyver *et al.*, 2016; McKeown *et al.*, 2019 for examples in squid). Therefore, phenotypic differences among groups may require their separate fisheries management (Kritzer and Liu, 2014). Genetic approaches represent the only method to confirm restricted interbreeding among groups; however, a lack of genetic differentiation may reflect resolution thresholds of the markers employed rather than actual connectivity. This is an important consideration here as previous genetic studies of *I. argentinus*, which reported a lack of genetic structure, employed small numbers of loci (Adcock *et al.*, 1999a, b). Furthermore, these studies did not investigate individual membership to the different spawning groups and/or their sampling was primarily focused on

latitudes south of 45°S, during the austral winter, targeting mainly the SSS and SPS groups (Roldán *et al.*, 2014). Recent advances in genomic methods, such as the development of restriction site-associated DNA sequencing (RADseq), are providing unprecedented insight into connectivity and local adaptation among marine populations with direct applications to harvest regulation and stock identification (Mullins *et al.*, 2018; McKeown *et al.*, 2020).

Another tool for the characterization of populations is the analysis of hard body structures. Statoliths are valuable sources of ecological and life history data for individuals and can be used as "black boxes" for numerous squid species (Arkhipkin, 2005). They are paired calcareous concretions located in the statocysts of the cephalic cartilage and are primarily used for balance (Arkhipkin and Bizikov, 2000). Statolith ageing and associated microstructure analysis make use of daily growth increments, which provide high-resolution life history data that allow for the distinction between groups based on their hatching dates and growth rates (Arkhipkin and Shcherbich, 2012; Petrić *et al.*, 2021).

The combined use of genetic and phenotypic methods can provide different insights into ontogenetic dispersal patterns, and the patterns and processes by which different groups may be demographically connected over varying spatial and temporal scales (McKeown *et al.*, 2015; 2017). Combining such methods offers considerable power to align fisheries management units with biological populations (Reiss *et al.*, 2009). Therefore, the combined use of these two powerful tools (statoliths and RADseq), also in combination with extensive spatial sampling throughout the *I. argentinus* range, allows the present study to investigate the extent of mixing of the seasonal groups of this species.

Following the contradicting evidence obtained from previous studies using either phenotypic characteristics or traditional genetic tools, the aim of this study was to combine information from phenotypic traits, in this case statoliths, and genetic variation assessed by genome-wide single-nucleotide polymorphism (SNP) analysis to explore population structure in *I. argentinus*. First, individual membership to spawning groups was estimated by back-calculation of hatching dates inferred from statoliths in order to determine how the spawning groups are distributed in space and time. Statolith microstructure was also examined in order to confirm the differentiation of subgroups within the seasonal spawning groups and subsequently inform the population genomic analysis. Second, genome-wide SNP analysis of individuals assigned to these spawning groups was used to investigate neutral and non-neutral genetic structuring among groups. The use of RADseq allowed for a higher resolution of population structure compared with more traditional population genomics studies. Consideration of all classically defined spawning groups was achieved by extensive spatial sampling across the Patagonian Shelf (42–52°S), and fortnightly sampling throughout the duration of the *I. argentinus* fishing season in the Southwest Atlantic. We first test the hypothesis that individuals collected at different times and locations have different hatching dates and different statolith microstructures and belong to different seasonal groups. Then, we investigate if there are genetic differences among these groups that may indicate some level of stock isolation. In contrast, absence of genetic structuring would indicate gene flow and interbreeding among spawning groups.

Material and methods

Sample collection

A total of 1878 specimens were collected on fortnightly basis from 15 January until 14 October 2020. Samples were obtained through a combination of routine deployments of staff on the Falkland Islands Fisheries Department (FIFD) Observer Programme, departmental research cruises onboard *F/V Castelo* and *F/V Argos Cies*, and random commercial catch supplied by vessels operating in the high seas. Squid were immediately frozen at sea and subsequently processed in the FIFD laboratory. Dorsal mantle length (DML) was measured to the nearest 0.5 cm, body weight was recorded to the nearest 0.1 g, and sex and maturity were assigned using the maturity scale by Lipinski (1979). Extracted statoliths were stored in 95% ethanol. Muscle tissue (1 × 2 cm piece) was cut from the mantle, approximately one-third away from the head, fixed in 96% ethanol in a glass vial, and stored at room temperature until DNA extraction. Implements used for the dissection of mantle tissue were washed first in water, then in 96% ethanol between individuals in order to avoid cross-contamination. To ensure high-quality DNA extraction, samples were slowly thawed on steel surfaces. Smaller individuals (<20 cm DML) were refrigerated at 4°C during processing to avoid tissue damage.

A sub-sample of 191 individuals was selected for further genomic and ageing analyses (Figure 1). Individuals were selected based on maturity, DML, sample location, and date with the aim of including as many of the spawning groups as possible. The selection of samples was focused on primarily females ($n = 189$) as they mature at a slower rate than males (Arkhipkin and Laptikhovsky, 1994). This allowed for a more accurate distinction between the spawning groups prior to ageing analysis.

Statolith preparation and ageing

One statolith from an individual squid was mounted concave side up using thermoplastic resin, CrystalBond™ 509 (AREMCO Products, Inc., USA). The statolith was subsequently ground and polished on both sides using P1200 and P2400 wet paper. To achieve maximum visibility of growth rings, statoliths were embedded in Canada Balsam™ mounting medium and covered with a cover glass slip. Statoliths were then dried in a temperature-controlled cabinet at 30°C for at least 1 week prior to reading. Statoliths were read using an Olympus BX51 compound microscope at ×500 magnification, with a phase-contrast Nomarski effect to improve readability as previously described in Arkhipkin and Shcherbich (2012). The total number of growth increments was counted from the natal ring to the edge of the dorsal dome. One growth increment was considered to represent 1 day, as per the "one growth increment—one day hypothesis" previously validated for the sister species *Illex illecebrosus* from the Northwest Atlantic (Dawe *et al.*, 1985; Hurley *et al.*, 1985). Therefore, the total number of increments counted per statolith was considered to be the age of an individual in days post-hatch.

The hatching date of each squid was back-calculated from the date of collection. Individuals were subsequently assigned to the seasonal spawning groups based on DML, hatch date, sample location, and statolith microstructure. Statolith microstructure was investigated by plotting the number of increments counted per 20 μm distance along the length of the statolith. Subsequently, a second-degree polynomial

locally weighted regression (loess) was performed with a span of 0.4 per spawning group in order to visualize the width of the growth increments.

Statistical analysis

Statistical analysis was performed using R 4.2.0 (R Core Team, 2022). The following packages were used: *tidyverse* (Wickham *et al.*, 2019) for compiling the dataset and visualization of data; *measurements* (Birk, 2019) for converting spatial coordinates into decimal degrees; and *lubridate* for the calculation of hatching dates (Grolemund and Wickham, 2011). Spatial data were visualized using *QGIS* 3.4.12 Madeira (QGIS Development Team, 2022).

Precision of ageing estimates was assessed by a random selection of 50 statoliths that were aged a second time by the primary reader (IC). The sub-sampled individuals were also aged by a second reader (AA) without any prior knowledge of the specimens in order to verify the age estimates. Average percent error [APE; Equation (1)] and average coefficient of variation [ACV; Equation (2)] were calculated for all three readings using the FSA package (0.9.3) in R (Ogle *et al.*, 2022) using the method described by Beamish and Fournier (1981) and Chang (1982), respectively.

$$\mathrm{APE} = 100 \times \frac{\sum_{j=1}^{n}\sum_{i=1}^{R}\frac{|x_{ij}-\bar{x}_j|}{\bar{x}_j}}{nR}, \quad (1)$$

where x_{ij} is the ith age estimate for the jth statolith, $\bar{x}_j$ is the mean age estimate for the jth statolith, R is the number of times that each statolith was aged, and n is the number of individuals in the sample.

$$\mathrm{ACV} = 100 \times \frac{\sum_{j=1}^{n}\frac{s_j}{\bar{x}_j}}{n}, \quad (2)$$

where s_j is the estimated standard deviation of R age estimates for the jth statolith.

Restriction site-associated DNA sequencing (RAD-seq) and bioinformatics

DNA was extracted following Winnepenninckx *et al.* (1993) using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, GmbH, Hilden, Germany) following the manufacturer's instructions. Genome-wide SNP analysis was performed using tuneable genotyping by sequencing (tGBS; Ott *et al.*, 2017) of a Bsp1286I digested library sequenced on an Illumina HiSeq X (Illumnina, Inc., San Diego, CA, USA). Sequenced reads were analysed using a custom Perl script (available at https://github.com/orgs/schnablelab), which assigned each read to a sample and removed barcode sequences. Seqclean (https://sourceforge.net/projects/seqclean) was used to remove adaptor sequences and chimeric reads harbouring internal restriction enzyme sites. Retained reads were subjected to quality trimming in two phases using the software Lucy2 (Li and Chou, 2004) in which bases with PHRED scores <15 (of 40) were removed. In the first phase, sequences were scanned at each end, whereas in the second phase, sequences were scanned using overlapping 10-bp windows. As there is no reference genome available for the *Illex* genus, *de novo* analysis was performed; sequence reads were aligned to one another and subsequently clustered to build loci. An SNP was called homozygous in an individual if at least 15 reads supported the genotype at the site and at least 90% of all reads covering that site shared the same nucleotide. A SNP was considered heterozygous in an individual if each of the two nucleotide variants was reported at least 10 times, and each allele was represented in >35% of the total reads. To reduce any bias that may be introduced by retaining low-frequency SNPs (Roesti *et al.*, 2012), the minimum allele frequency (MAF) was set at 5%.

Summary statistics and outlier detection

Allele frequencies and observed (H_O) and expected (H_E) heterozygosities were estimated using ARLEQUIN 3.4.2.2 (Excoffier *et al.*, 2005). ARLEQUIN was also used to test for departures from expectations of Hardy–Weinberg equilibrium (HWE). Genetic differentiation among samples was quantified by global and pairwise F_{ST} (Weir and Cockerham, 1984) with statistical significances evaluated in ARLEQUIN with 10000 permutations. The Bayesian clustering method in STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) was also employed to (i) identify the most probable number of genetically distinct groups (K) represented by the data; and (ii) estimate assignment probabilities (Q) for each individual (specifically their genomic components) to these groups. The analysis was performed with and without the LOCPRIOR model, in both cases assuming admixture. Simulations were run 10 times for each proposed value of K (1–5; higher values of K were tested in shorter pilot runs) to assess convergence. Each run had a burn-in of 100 000 Markov chain Monte Carlo (MCMC) samples followed by 1 000 000 MCMC repetitions. Models were assessed using L (K) (Pritchard *et al.*, 2000) and ΔK (Evanno *et al.*, 2005). Genetic structuring among the sampled individuals was further investigated by performing principal components analysis (PCA) on allele counts with the *adegenet* package in R (Jombart, 2008). Scaling was disabled as all alleles vary on a common scale and three principal components were retained.

The detection of loci potentially under selection was performed using the independent approaches in ARLEQUIN and BAYESCAN 2.1 (Foll and Gaggiotti, 2008). For the ARLEQUIN analysis, the hierarchical Fdist model was implemented following recommendations by Leone *et al.* (2019). SNPs with significantly higher F_{ST} values at $p < 0.001$ were considered positive outliers. For the BAYESCAN analysis, all parameters that can be modified in the software were left as default. The false discovery rate was set at 5% meaning that a marker with a q value <0.05 was considered an outlier. The BAYESCAN analysis was performed globally (*i.e.* across all samples) and between all pairs of samples as recommended by Vitalis *et al.* (2001). Functional significance of outlier loci was investigated by analysing the SNP-containing sequences using BLAST following Milano *et al.* (2011). The BAYESCAN method has been shown to have a low Type I error rate (Narum and Hess, 2011; De Mita *et al.*, 2013).

Results

Statolith ageing and spawning group identification

A total of 191 individuals were successfully aged, with a mean age of 178 days and a range of 118–230 days. Three independent statolith age estimates were successfully performed on a subsample of 50 individuals, with an APE of 2.54% and an ACV of 3.45%. The back-calculated hatching dates revealed that continuous hatching occurred throughout the year, with a distinct peak from early August until late October

Table 1. Summary of samples collected throughout 2020 and the spawning groups identified.

Spawning group	Sample location	*N*	DML range (cm)	Age range (days)
ESPS	FICZ/FOCZ	32	19–33	161–215
	HS	27	16–29	147–212
LSPS	FICZ/FOCZ	10	28–32	190–222
HS SpSS	HS	65	11–33	118–230
FI SpSS	LOLBOX	22	12–29	132–202
SSS	HS	19	10–31	122–198
Unassigned	FICZ/FOCZ	3	12–20	146–184
	LOLBOX	13	10–26	122–186

N, number of individuals sampled; DML, dorsal mantle length; ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; UN, Unassigned; FICZ, Falkland Islands Interim Conservation and Management Zone; FOCZ, Falkland Islands Outer Conservation Zone; LOLBOX, Loligo Box; and HS, High Seas.

(Figure 2; Table 1). The following seasonal spawning groups and associated subgroups were identified: (i) winter-hatched ESPS ($n = 59$) and LSPS ($n = 10$); (ii) spring-hatched High Seas SpSS (HS SpSS; $n = 65$) and Falkland Islands SpSS (FI SpSS; $n = 22$); and (iii) summer-hatched SSS ($n = 19$) and Unassigned (UN; $n = 16$).

Early-maturing South Patagonian Stock (ESPS)

ESPS individuals were located in the northern part of the FICZ or the High Seas south of 46°S (Figure 1). Hatching peaked between late July and early September; overall, individuals hatched continuously from the end of June until the beginning of October (Figure 2). Statolith microstructure presented narrow daily increments in the dark zone of the statolith, where more than five daily increments observed per 20 μm (Figure 3a; Figure 4). Individuals with medium DML (15 ≤ 25 cm) were generally older when compared with other spawning groups (Figure 5; Table 1).

Late-maturing South Patagonian Stock (LSPS)

For the LSPS group, individuals were found in the western part of the FICZ only (Figure 1). Hatching occurred between late August and early September (Table 1; Figure 2). Statolith microstructure was similar to that of ESPS individuals, with the notable difference the daily increments were narrower in the latter part of the dark zone of the statolith i.e. from 200 μm onwards the number of daily reached up to six increments per 20 μm (Figure 3b; Figure 4). Overall, individuals were older than the ESPS at similar DML (Table 1; Figure 5).

High Seas Spring Spawning Stock (HS SpSS)

HS SpSS individuals were found to be characterized by distribution in the high seas, mainly north of 46°S (Figure 1). Hatching of this group primarily occurred throughout November; however, some overlap with the ESPS group was noted as this group had the longest hatching period starting as early as the beginning of September until the end of December (Table 1; Figure 2). The HS SpSS presented wide daily increments in the statolith dark zone with just over five daily increments per 20 μm (Figure 3c, Figure 4). This spawning group contained the oldest individual sampled at a maximum age of 230 days (Table 1; Figure 5).

Falkland Islands Spring Spawning Stock (FI SpSS)

The FI SpSS spawning group was found to be distributed in areas south of 50°S, in the "Loligo Box" of the FICZ (Figure 1). Hatching dates ranged from the beginning of September until the end of October with a peak in the end of September, a considerable overlap with the HS SpSS group was evident (Figure 2). The statolith microstructure of this spawning group was distinct, due to the narrow daily increments observed in the dark zone with more than six per 20 μm (Figure 3d; Figure 4). Individuals were older than their HS SpSS counterparts with small DML (≤15 cm) (Table 1; Figure 5).

Summer Spawning Stock (SSS)

The SSS group was found in the high seas, primarily north of 46°S (Figure 1). Hatching dates ranged from the beginning of January until the end of March, with a peak in early January (Figure 2). The statolith microstructure revealed the widest daily increments in the statolith dark zone when compared with other spawning groups, with less than five daily increments per 20 μm (Figure 3e, Figure 4).

Unassigned (UN)

The Unassigned spawning group was primarily sampled within the FICZ, from 48 to 53°S with the largest number of individuals located in the "Loligo Box" (Figure 1). Hatching dates ranged from the late January until early April, with a peak in early March, there was considerable overlap with the SSS group (Figure 2). Examination of the statolith microstructure revealed narrowing daily increments over the distance between 200 and 300 μm with more than six increments per 20 μm (Figure 3f; Figure 4).

Genomic diversity

A total of 2 × 528 267 065 sequence reads were obtained with an average of 2 × 2 751 391 per individual (average read length = 144 bp; minimum read length = 30 bp; maximum read length = 213 bp). Following filtering of these sequences and trimming to 140 bp, a total of 298 756 SNPs were identified that were genotyped in at least 50% of individuals. Further filtering to include only SNPs genotyped in at least 90% and a minimum allele frequency of 5% resulted in 10 353 biallelic SNPs, which were used for downstream analysis.

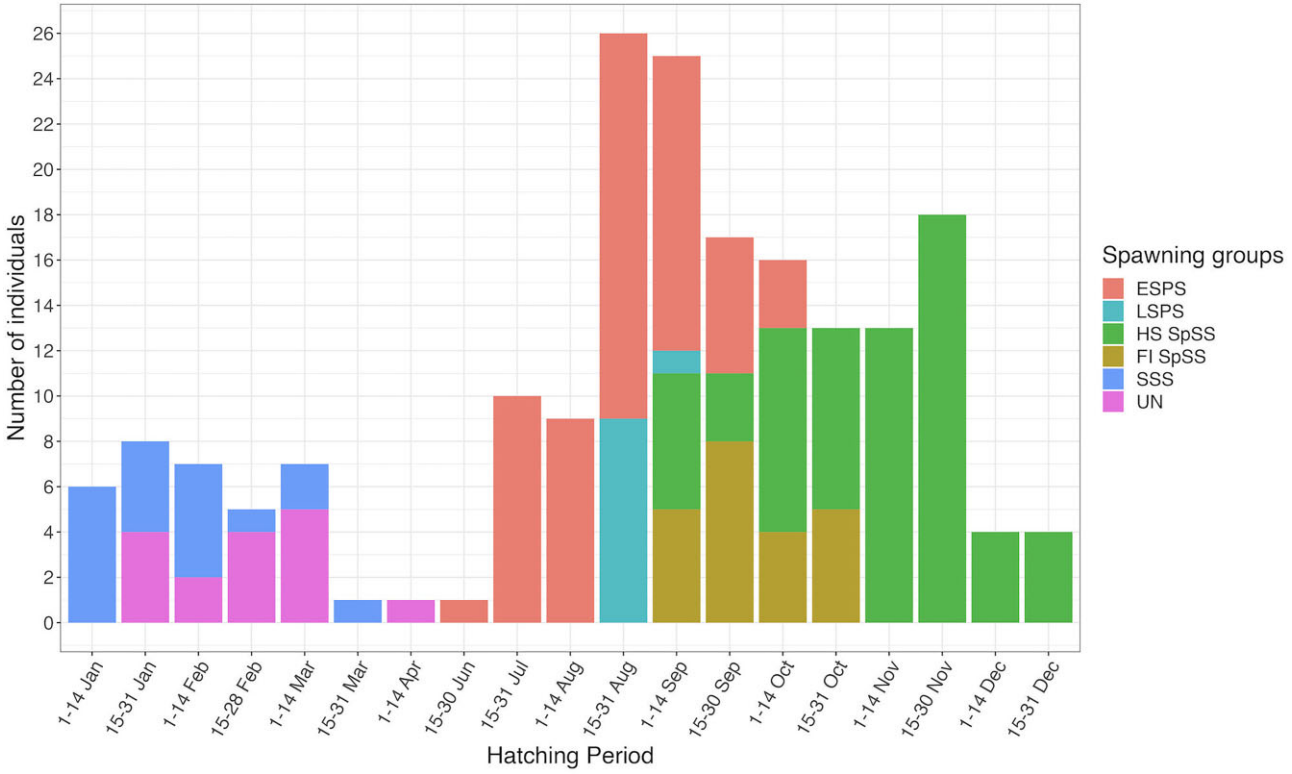

Figure 2. Hatching date distribution of spawning groups identified. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; and UN, Unassigned.

As each genotyped individual had been included in the statolith ageing, we initially grouped samples according to their membership to the six groups revealed in that analysis. These groups exhibited similar levels of multi-locus variability (Table 2). Each group exhibited a significant deviation from Hardy-Weinberg equilibrium due to a deficit of heterozygotes, evident in positive F_{IS} values in each case (Table 2). Across the groups the global F_{ST} was not significant ($F_{ST} = 0.0001$). In line with this, pairwise F_{ST} values were low and non-significant in most cases (Table 3). The only significant F_{ST} value was obtained in the comparison between the ESPS and FI SpSS samples. However, the corresponding p-value becomes non-significant after Bonferroni correction for multiple tests (Rice, 1989). Rearranging the samples according to other grouping schemes reported the same salient features of (i) negligible genetic differentiation between groups, and (ii) heterozygote deficits within groups. Bayesian clustering analysis in STRUCTURE provided unanimous support for $K = 1$, as estimated using L (K). In line with this, PCA reported considerable overlap among individuals from the assigned spawning groups (Figure 6).

The ARLEQUIN outlier analysis performed for different sample configurations typically identified less than five outlier SNPs in each case. The pattern of outliers was seemingly random. For example, the tests excluding either the HS SPSS or FI SpSS samples (both Spring spawners) recovered different (i.e. non-overlapping outliers). Analysis of the sequences of putative outliers provided no information as to functional significance, while individual-based clustering analysis of outlier genotypes did not reveal any structure. In line with the weak support for outliers, the corresponding BAYESCAN analyses did not identify any loci that deviated from neutral expectations. As there was no overlap in SNPs identified across the various analyses it was not possible to identify a robust suite of consensus outliers.

Discussion

An understanding of population structure in relation to management units and their continual alignment within a responsive management approach is necessary to ensure fishery sustainability and conservation of biodiversity (Reiss *et al.*, 2009; Kerr *et al.*, 2017). Given the complexity of the processes that shape stock structure, fisheries managers are increasingly combining information obtained from different stock identification methods. This is the first study to combine statoliths with genome-wide SNP analysis in a squid species. Specifically, statolith ageing and microstructure analysis revealed that individuals of *I. argentinus* collected at different times and locations can be assigned to three seasonal groups, each with respective subgroups: (i) winter-hatched (ESPS and LSPS); (ii) spring-hatched (HS SpSS and FI SpSS); and (iii) summer-hatched (SSS and UN). While these groups had been previously described in other studies (Brunetti, 1988; Crespi-Abril and Barón, 2012; Arkhipkin *et al.*, 2022), the larger spatial scale of sampling used here provided a greater insight into their spatial dynamics. Genome-wide SNP analysis of individuals assigned to spawning groups identified >10 000 SNPs, which permitted testing of our hypothesis that the different spawning groups may be derived from a single genetically cohesive population. This hypothesis of high connectivity was supported by F_{ST} and individual clustering analyses, which

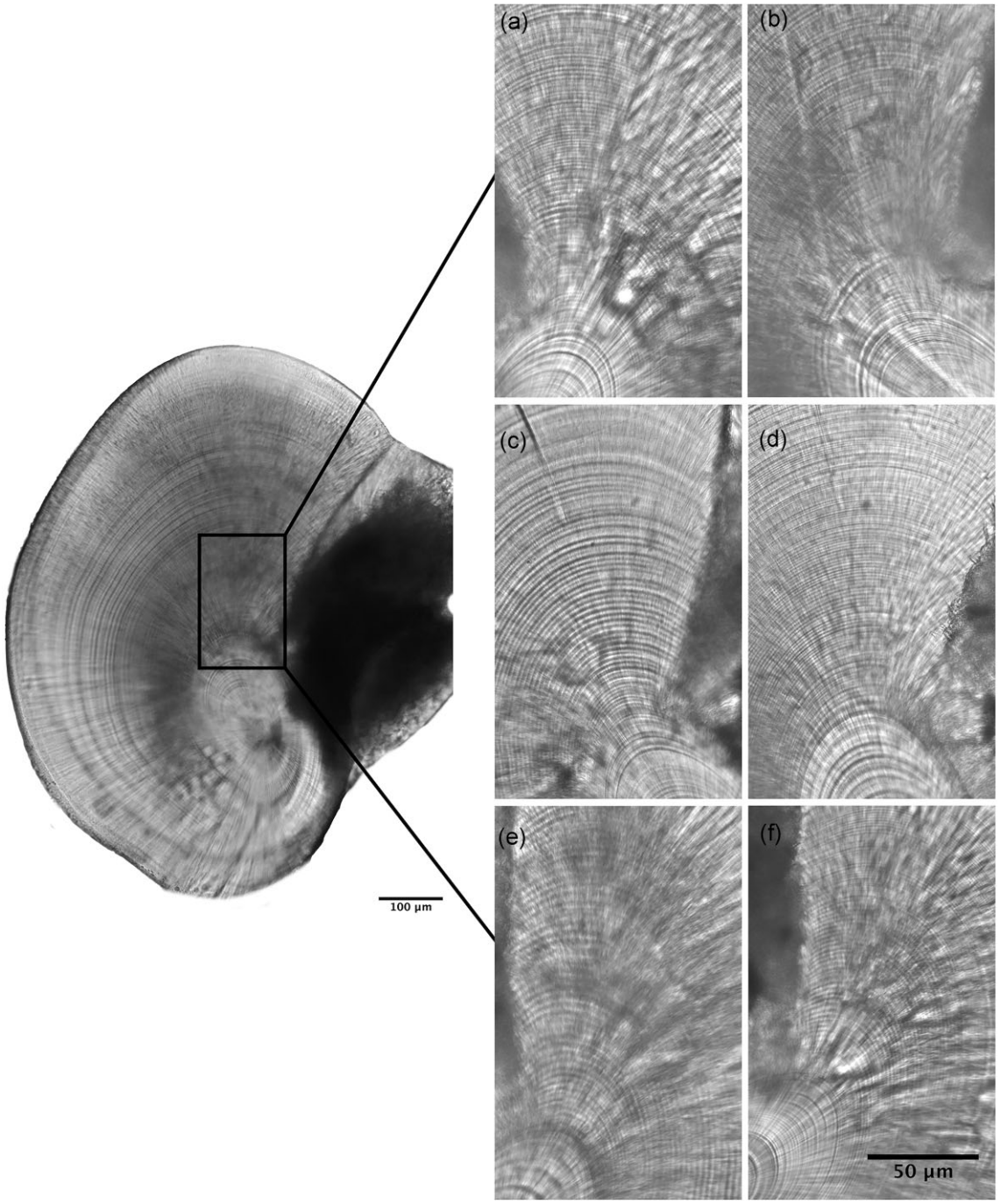

Figure 3. Statolith microstructure of the identified spawning groups: (a) ESPS; (b) LSPS; (c) HS SpSS; (d) FI SpSS; (e) SSS; and (f) UN. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; and UN, Unassigned.

reported a lack of genetic structure among spawning groups while outlier tests provided no evidence of non-neutral structuring. The statolith ageing analysis confirmed year-round hatching throughout the species range, highlighting the ecological plasticity of this species. Statolith microstructure also supported the identification of subgroups (i.e. LSPS, FI SpSS, UN) within the seasonal groups as differences in increment width revealed possible variation in the environmental conditions individuals experienced during ontogeny. Overall, the *I. argentinus* population on the Patagonian Shelf was characterized using phenotypic and genotypic markers to reveal dispersal among seasonal spawning groups against a background of high gene flow.

Ageing estimates in the present study were consistent with previous studies on *I. argentinus* (Bainy and Haimovici, 2012). Additionally, the low APE and ACV values obtained from the ageing validation in the current study indicated a high level of reproducibility.T herefore, the methods used for statolith microstructure analysis were confirmed. The continuous hatching identified in the present study is consistent with a previous study where statolith age processing was performed in real time, over the course of a fishing season, which showed

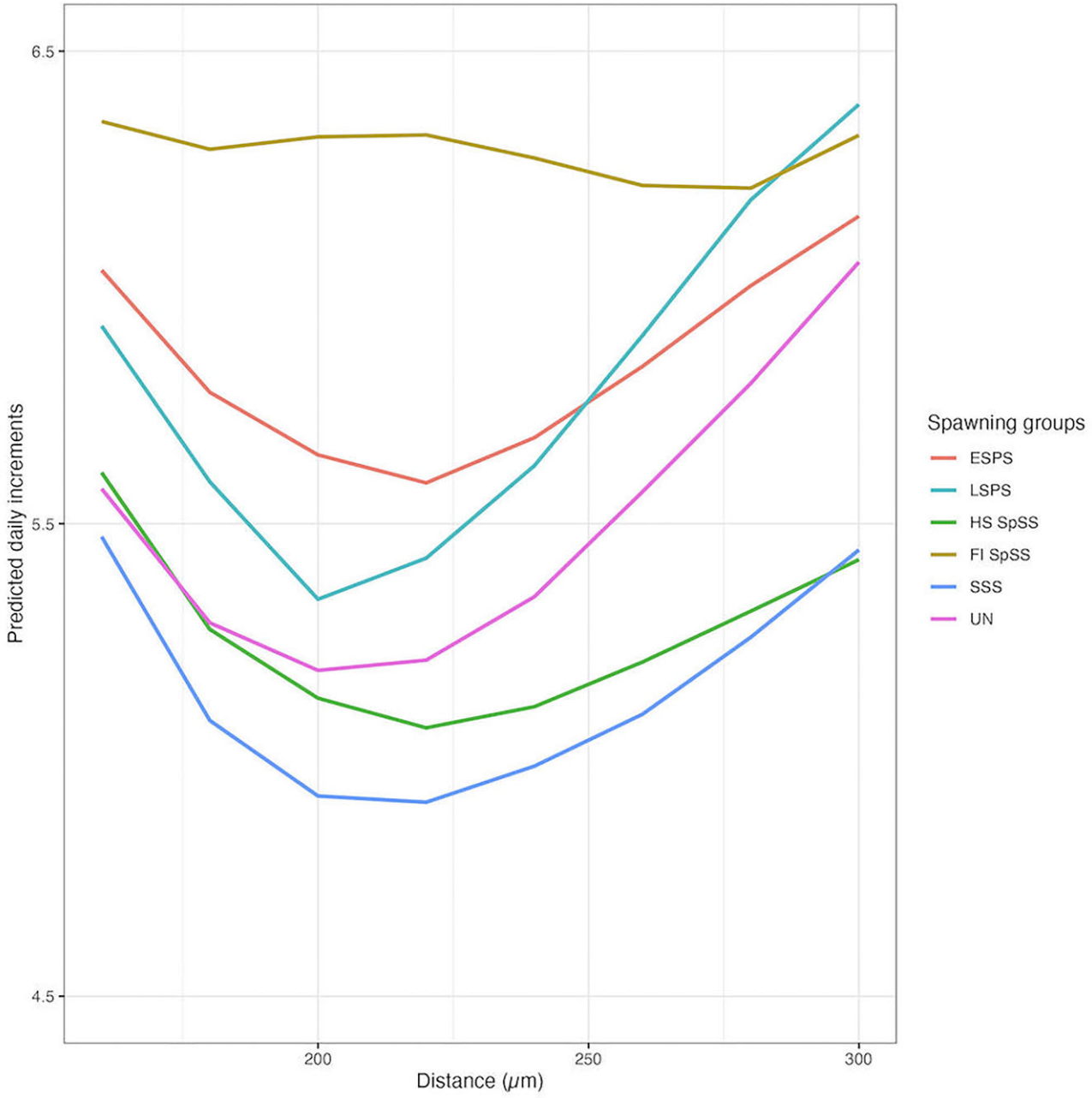

Figure 4. Predicted number of daily growth increments using loess smoothing (span = 0.4) in a subset of the dark zone of the statolith as a function of distance for the spawning groups identified. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; and UN, Unassigned.

that there were several "waves of abundance" of squid passing through the fishing grounds from 52 to 42°S (Arkhipkin, 1993). The seasonal spawning groups assigned here are consistent with the structure previously established for *I. argentinus* (Arkhipkin *et al.*, 2022). Similar seasonal structure has been observed in other ommastrephids, such as *I. illecebrosus* (Jones and Hendrickson, 2022) and *I. coindetti* (Arkhipkin *et al.*, 2000; Petrić *et al.*, 2021).

Statolith microstructure differed between the seasonal groups identified by the present study. This may be due to the different ambient temperatures individuals experience during their life span. Statolith growth has been positively associated with ambient temperature in loliginid species (Durholtz and Lipinski, 2000). Furthermore, laboratory experiments have revealed that squid exposed to lower ambient temperatures (11°C) compared to their warmer group counterparts (20°C) exhibited narrower growth increments in the statolith (Villanueva, 2000). Therefore, as the statolith increments are deposited throughout ontogeny (Rodhouse and Hatfield, 1990), increment width may also be an indicator of the ambient temperature of the water masses inhabited by individuals throughout ontogeny (Arkhipkin, 2005). The differences in the growth increment width of different seasonal groups observed in the present study suggest that individuals from the

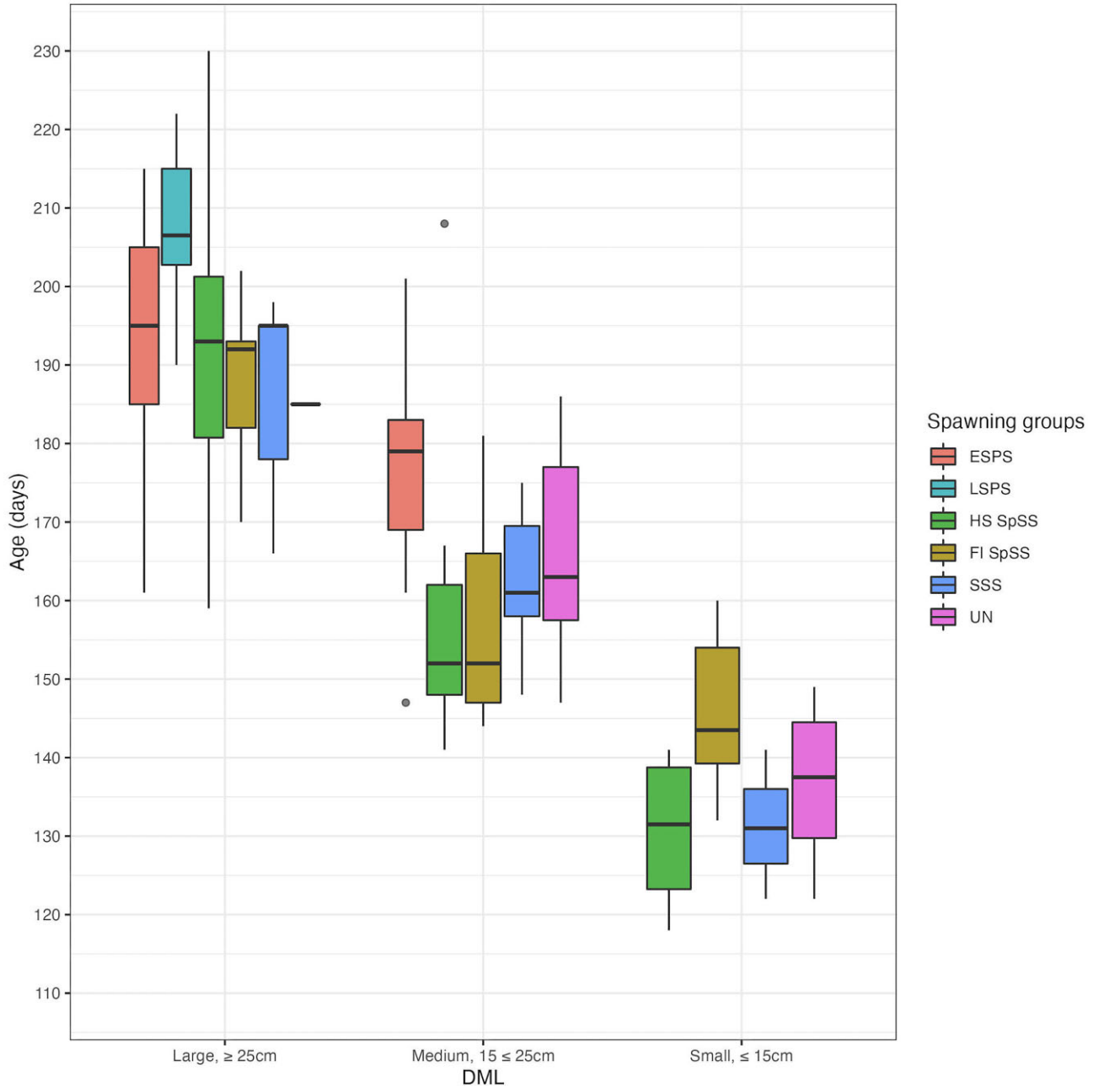

Figure 5. Age range per spawning group as a function of dorsal mantle length. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; UN, Unassigned; and DML, dorsal mantle length.

Table 2. Summary indices of genetic variation for the assigned spawning groups delineated by statolith ageing.

Spawning group	Individuals genotyped	Poly loci	H_O (*SD*)	H_E (*SD*)	F_{IS}
ESPS	59	10 349	0.170 (0.109)	0.207 (0.122)	0.178
LSPS	10	8 534	0.208 (0.143)	0.251 (0.134)	0.175
HS SpSS	65	10 350	0.172 (0.109)	0.206 (0.122)	0.167
FI SpSS	22	10 052	0.194 (0.129)	0.218 (0.131)	0.116
SSS	19	9 805	0.181 (0.122)	0.219 (0.131)	0.180
UN	16	9 628	0.194 (0.131)	0.227 (0.133)	0.153

Genetic variation is described using the number of polymorphic loci, observed and expected heterozygosity (H_O and H_E, respectively), with their associated standard deviations. Deviations from Hardy-Weinberg equilibrium expectations were tested using F_{IS}, which were significant in each case. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; and UN, Unassigned.

Table 3. Pairwise F_{ST} based on all loci (Weir and Cockerham, 1984).

Spawning group	ESPS	LSPS	HS SpSS	FI SpSS	SSS	UN
ESPS	–					
LSPS	0.004	–				
HS SpSS	0.001	0.004	–			
FI SpSS	**0.003**	0.006	0.003	–		
SSS	0.003	0.005	0.003	0.004	–	
UN	0.002	0.005	0.003	0.004	0.004	–

Significance was assessed after 10000 permutations with significant values denoted in bold. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; and UN, Unassigned.

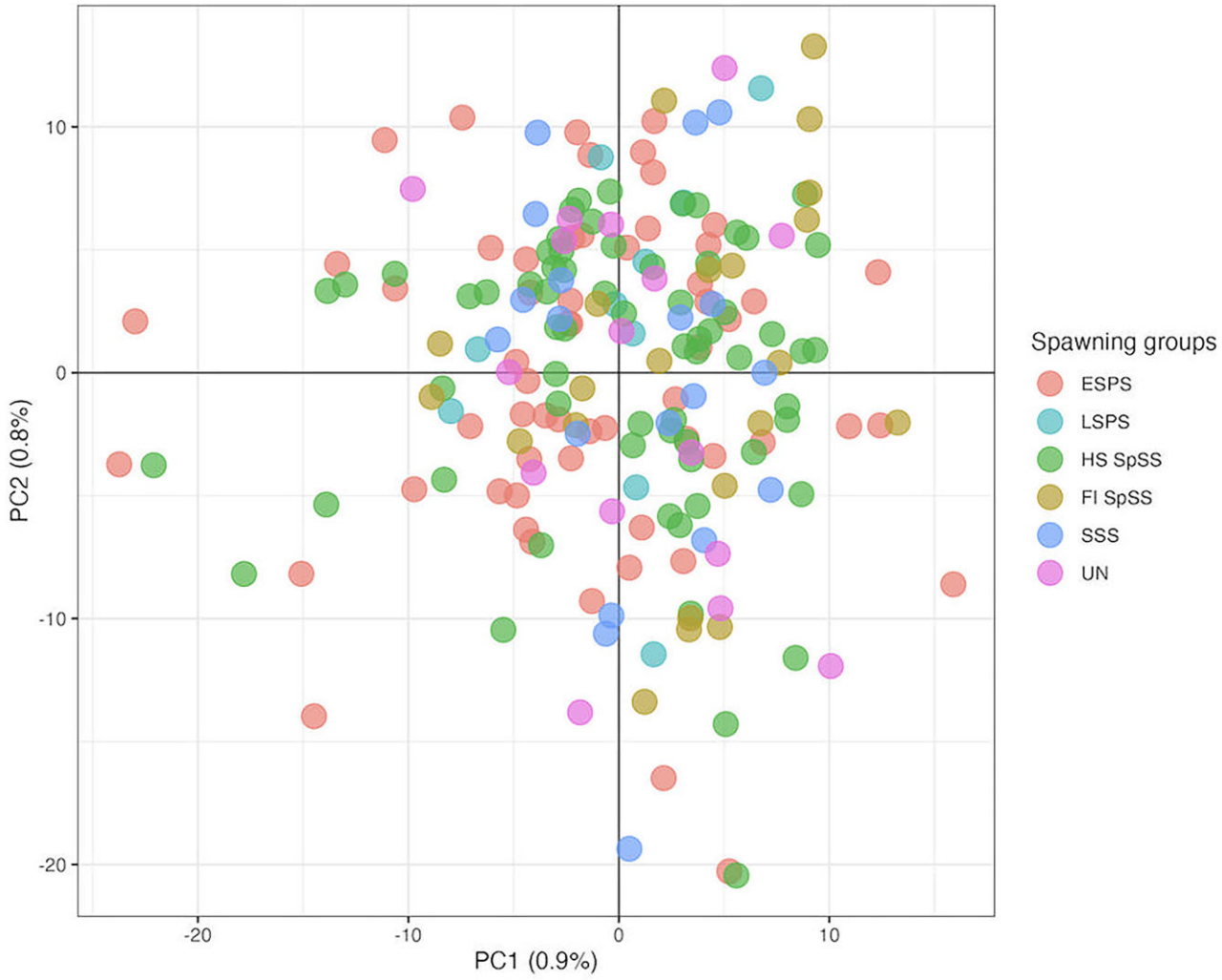

Figure 6. Principal component scatter plot with individuals denoted by spawning group assignment. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; and UN, Unassigned.

assigned spawning groups are not only separated in time (due to different hatching dates) but also in space, due to different migration/dispersal paths. This is clearly visible in the SSS and UN groups, where individuals all hatched during austral summer. However, the statoliths of the UN group had narrower increments in the latter part of the statolith dark zone, implying that individuals experienced slower growth due to presumably colder ambient temperatures compared with the SSS group, where increments were wider. The observed difference in increment width could be attributed to differences in water masses and migration of individuals through them. The sampling locations of the SSS group were north of 46°S, where sea surface temperatures can be as high as ∼15°C. Conversely, the UN group was found as far south as 52°S, where sea surface temperatures may be as low as ∼11°C in the same time period. Long-term temporal trends in temperature are unlikely to be explanatory of the differences observed in the present study as all individuals were sampled in the same year, thus all have experienced only seasonal variation.

Previous genetic studies of *I. argentinus* in the region have reported no population genetic structure (Adcock *et al.*, 1999a, b). However, such studies may have been limited in terms of resolution owing to the small number of loci analysed (fewer than nine microsatellite loci) and their more limited sampling, which was primarily focused on latitudes south of 45°S, during the austral winter and therefore would have mainly comprised the SSS and SPS groups (Roldán *et al.*, 2014). The present study addressed these concerns by the discovery and genotyping of >10 000 SNPs, and wider spatial sampling over the Patagonian Shelf ranging from 42 to 52°S. Furthermore, by integrating ageing data, we were able to partition samples *a priori,* according to spawning group. Overall, the data supported a lack of genetic differentiation among groups and individual assignment tests with and without *a priori* groupings provided no evidence of clustering of individuals. However, it is of note that the present study sampled predominantly females. Therefore, it is possible that the findings would differ if more males were included. Nevertheless, as no segregation by sex has been observed in *Illex coindetii* (Gonzalez and Guerra, 1996), it is possible that a similar behaviour occurs in *I. argentinus*. Therefore, it is unlikely that the sex ratio of the sample used in the present study had an inordinate effect on the findings. An allozyme study by Carvalho *et al.* (1992) reported significant differences among samples in contrast to the lack of structure reported here and previous microsatellite-based studies. The most likely explanation for this discrepancy is that the allozyme loci may have been shaped by environmental selection occurring against a background of high gene flow. In this case, an important consideration is the extent to which the allozyme patterns reflect locus-specific selection or locally adapted demes. Numerous genomic studies have reported signals of adaptive divergence, in the form of outlier loci, even when neutral markers suggest panmixia (Bradbury *et al.*, 2013; Bekkevold *et al.*, 2015; McKeown *et al.*, 2020). However, this does not seem to be the case for *I. argentinus*, as no outlier loci were detected in the present study despite extensive global and pairwise testing among ecologically and spatially defined groups. While cryptic local adaptation should not be discounted, as genome scans may often have limited power (Bourret *et al.*, 2014), the patterns suggest that the intergroup divergence reported by Carvalho *et al.* (1992) may reflect locus-specific selection rather than locally adapted demes and aligns with neutral patterns in supporting a genetically cohesive population. Overall, the findings of the present study are based on over 10 000 SNPs obtained from 191 individuals. It may be possible that some differentiation between the groups remains unidentified due to the limited sample sizes; however, the combination of the large number of markers and the mixing that occurs between the spawning groups during the adult phase means this is unlikely.

The lack of genetic structure reported for *I. argentinus* fits with the general pattern of geographically extensive gene flow reported for other squid species such as *Loligo forbesi* (Shaw *et al.*, 1999), *D. opalescens* (Reichow and Smith, 2001), *L. reynaudi* (Shaw *et al.*, 2010), *Doryteuthis pealeii* (Shaw *et al.*, 2010), and *D. gahi* (McKeown *et al.*, 2019). Spatial genetic structuring in cephalopods seems to occur where there is some form of oceanographic/physical barrier to dispersal (Sandoval-Castellanos *et al.*, 2007; Staaf *et al.*, 2010; McKeown *et al.*, 2019). An important consideration here is that high levels of evolutionary significant gene flow may obscure contemporary dispersal restrictions. However, restricted dispersal seems unlikely here for such a highly migratory species. *I. argentinus* individuals undertake a feeding and spawning migration across several large marine ecosystems in the Southwest Atlantic (Arkhipkin, 2013) and spawning has not been observed in *I. argentinus*. However, in *I. illecebrosus*, egg masses are spawned on the surface and subsequently sink, until neutral buoyancy is attained, and the masses float midwater (Rodhouse *et al.*, 2013). Therefore, it is possible that a similar mechanism in *I. argentinus* increases the dispersal ability of the species. As there is uncertainty regarding the exact spawning locations of the groups of interest, there may be a small number of migrants, which are sufficient to reduce genetic divergence between groups (Allendorf *et al.*, 2010). Furthermore, the most numerous spawning group (SPS) completes a 2000 km round trip from juveniles to spawning adults. The present study identified groups with spring (FI SpSS) and summer (UN) hatching dates located as far south as 52°S, in the FICZ. To the best of our knowledge, this is the first study to report these groups, which further underscores the dispersal ability of this species. This finding supports the theory put forward by Parfeniuk *et al.* (1992) that suggested the presence of a juvenile southward transport from the Brazil–Falkland Confluence (~38°S), for spring-hatched individuals, to the southern part of the Argentine Basin and their subsequent active migration through the eastern branch of the Falkland Current to the southern part of the Patagonian Shelf. Restricted gene flow in the face of such dispersal could be maintained if there was some level of philopatry, but this has not been reported in any squid species to date.

All samples were found to exhibit significant deficits of heterozygotes, which may indicate variability in recruitment success across all seasonal spawning groups identified in the present study. Cheng *et al.* (2020) reported no differentiation between samples collected at peak spawning times, but a low level of patchy differentiation among samples on more local scales, which they attributed to intra-annual pulses of recruitment. We propose that the heterozygote deficits reported in the present study are driven by similar processes of recruitment variability, where ephemeral genetic differences are generated among groups, followed by mixing at later life history stages. Heterozygote deficits among adult samples of this species have previously been described by Adcock *et al.* (1999b), with the authors excluding inbreeding or mixing of genetically distinct populations as causes. Interestingly, both Carvalho *et al.* (1992) and Adcock *et al.* (1999b) reported significant genetic differentiation between temporal samples despite a lack of spatial differentiation within an annual cohort. Therefore, temporal recruitment variability may play a role in generating allele frequency differences within a single population. Similar patterns have also been reported in Garoia *et al.* (2004), which the authors linked to spawning at different times. Individuals from the SPS group of *I. argentinus* have previously been shown to undertake the northward spawning migration in pulses of abundance (Arkhipkin, 1993). Therefore, it is possible that the offspring of the first arrivals to spawn would be exposed to different environmental conditions compared with the offspring of the last arrivals to spawn, leading to the within-group differences observed here. Environmental conditions in the hatching grounds have previously been shown to have a significant effect on the abundance and recruitment success of the SPS group in the sub-

sequent fishing season (Waluda *et al.* 1999; Chemshirova *et al.*, 2021). As the effects of such processes are predicted to be diminished by ontogenetic dispersal (Planes and Lenfant, 2002), the genetic patchiness observed here must be considered a conservative reflection of the extent of recruitment heterogeneity.

Genetic variability is recognized as fundamental for sustainable yields and adaptability of populations (Kenchington *et al.*, 2003). Levels of intrasample genetic diversity were similar across all samples providing no evidence of reduced genetic variation among any spawning group. Levels of SNP variability were also higher than those reported by Cheng *et al.* (2020) in the commercially harvested *D. opalescens*. Previous microsatellite-based studies of *I. argentinus* have also showed the species to retain high levels of intrasample diversity across a period of intense harvesting pressure (Adcock *et al.*, 1999b). Although the population variability at microsatellites and SNPs is not readily comparable due to different mutation rates of the different types of markers, the data support the view that despite harvesting intensity, and previously mentioned recruitment variability, if current stock sizes are maintained the genetic drift is not sufficient to reduce genetic variation.

The combination of statolith ageing and genomics in the present study is unique and revealed the lack of genetic structuring in *I. argentinus* in a more conclusive manner. Reliance on sampling location alone for distinction between groups may be misleading, particularly for a species with a migration rate of >20 km per day (Arkhipkin, 1993), and may result in multiple samples of the same spawning group throughout ontogeny, but from seemingly different locations. This perceived mismatch between ecological groupings and a single genetic population has wider implications, particularly with respect to management strategies of the *I. argentinus* fishery. First, the observed structuring in space and time of the assigned spawning groups implies that a "bet-hedging" mechanism may be in place to protect the population from collapse (Caddy, 1983). Therefore, the overexploitation of one of these groups, would reduce the capacity of the entire population to recover, thus consistent monitoring of all spawning groups would be required to ensure the full operational capacity of the fishery. Second, the lack of genetic structuring in *I. argentinus* confirms that the fishery should be considered a straddling stock, as individuals migrate through several EEZs and international waters. Nonetheless, no regional organization for management of this fishery exists in the Southwest Atlantic currently; instead, each coastal state implements individual management measures (Arkhipkin *et al.*, 2022). As this fishery is of a global interest due to the international stakeholders involved in harvesting and trade, the findings of this study underscore the need for a cohesive sustainable strategy in management that would ensure the stability of international trade and relations (Ospina-Alvarez *et al.*, 2022).

Future studies should consider resolving the provenance of the spawning groups assigned in the present study. This would reveal the extent of the mixing of the spawning groups in the early stages of their ontogeny. Furthermore, identifying the ontogenetic migrations of the spawning groups using time-resolved elemental chronologies may elucidate any further associations with environmental factors.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Author contributions

Irina Chemshirova: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Writing—original draft; Writing—review & editing. **Alexander Arkhipkin:** Conceptualization, Supervision, Resources, Funding Acquisition, Methodology, Writing—review & editing. **Paul W. Shaw**: Resources; **Niall J. McKeown**: Investigation, Data Curation, Formal Analysis, Supervision, Writing—original draft; Writing—review & editing.

References

Adcock, G. J., Carvalho, G. R., Rodhouse, P. G., and Shaw, P. W. 1999a. Highly polymorphic microsatellite loci of the heavily fished squid genus *Illex* (Ommastrephidae). Molecular Ecology, 8: 165–167.

Adcock, G. J., Shaw, P. W., Rodhouse, P. G., and Carvalho, G. R. 1999b. Microsatellite analysis of genetic diversity in the squid *Illex argentinus* during a period of intensive fishing. Marine Ecology Progress Series, 187: 171–178.

Allendorf, F. W., Hohenlohe, P. A., and Luikart, G. 2010. Genomics and the future of conservation genetics. Nature Reviews Genetics, 11: 697–709.

Arkhipkin, A. 1993. Age, growth, stock structure and migratory rate of pre-spawning short-finned squid *Illex argentinus* based on statolith ageing investigations. Fisheries Research, 16: 313–338.

Arkhipkin, A. 2005. Statoliths as "black boxes" (life recorders) in squid. Marine and Freshwater Research, 56: 573–583.

Arkhipkin, A. 2013. Squid as nutrient vectors linking Southwest Atlantic marine ecosystems. Deep Sea Research Part II: Topical Studies in Oceanography, 95: 7–20.

Arkhipkin, A. I., Nigmatullin, C.h.M., Parkyn, D. C., Winter, A., and Csirke, J. 2022. High seas fisheries: the Achilles' heel of major straddling squid resources. Reviews in Fish Biology and Fisheries, 33: 1–22.

Arkhipkin, A., and Bizikov, V. 2000. Role of the statolith in functioning of the acceleration receptor system in squids and sepioids. Journal of Zoology, 250: 31–55.

Arkhipkin, A., Hendrickson, L. C., Payá, I., Pierce, G. J., Roa-Ureta, R. H., Robin, J. P., and Winter, A. 2020. Stock assessment and manage-

ment of cephalopods: advances and challenges for short-lived fishery resources. ICES Journal of Marine Science, 78: 1–17.
Arkhipkin, A., Jereb, P., and Ragonese, S. 2000. Growth and maturation in two successive seasonal groups of the short-tinned squid, *Illex coindetii* from the Strait of Sicily (central Mediterranean). ICES Journal of Marine Science, 57: 31–41.
Arkhipkin, A., and Laptikhovsky, V. 1994. Seasonal and interannual variability in growth and maturation of winter-spawning *Illex argentinus* (Cephalopoda, Ommastrephidae) in the Southwest Atlantic. Aquatic Living Resources, 7: 221–232.
Arkhipkin, A., Rodhouse, P. G., Pierce, G. J., Sauer, W., Sakai, M., Allcock, L., Arguelles, J. *et al.* 2015. World squid fisheries. Reviews in Fisheries Science & Aquaculture, 23: 92–252.
Arkhipkin, A., and Shcherbich, Z. 2012. Thirty years' progress in age determination of squid using statoliths. Journal of the Marine Biological Association of the United Kingdom, 92: 1389–1398.
Bainy, M. C. R. S., and Haimovici, M. 2012. Seasonality in growth and hatching of the Argentine short-finned squid *Illex argentinus* (Cephalopoda: ommastrephidae) inferred from aging on statoliths in southern Brazil. Journal of Shellfish Research, 31: 135–143.
Beamish, R. J., and Fournier, D. A. 1981. A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences, 38: 982–983.
Bekkevold, D., Helyar, S. J., Limborg, M. T., Nielsen, E. E., Hemmer-Hansen, J., Clausen, L. A., and Carvalho, G. R. 2015. Gene-associated markers can assign origin in a weakly structured fish, Atlantic herring. ICES Journal of Marine Science, 72: 1790–1801.
Birk, M. A. 2019. Measurements: tools for units of measurement. https://cran.r-project.org/package=measurements (last accessed 08 August 2023).
Bourret, V., Dionne, M., and Bernatchez, L. 2014. Detecting genotypic changes associated with selective mortality at sea in Atlantic salmon: polygenic multilocus analysis surpasses genome scan. Molecular Ecology, 23: 4444–4457.
Bradbury, I. R., Hubert, S., Higgins, B., Bowman, S., Borza, T., Paterson, I. G., Snelgrove, P. V. R. *et al.* 2013. Genomic islands of divergence and their consequences for the resolution of spatial structure in an exploited marine fish. Evolutionary Applications, 6: 450–461.
Brunetti, N. E. 1988. Contribución al Conocimiento biológico-pesquero del calamar argentino (Cephalopoda: Ommastrephidae: *Illex argentinus*). PhD thesis, Universidad Nacional de La Plata, Buenos Aires.
Caddy, J. F. 1983. The cephalopods: factors relevant to their population dynamics and to the assessment of management of stocks. *In* Advances in Assessment of World Cephalopod Resources. FAO, Rome. pp. 416–452.
Caddy, J., and Rodhouse, P. G. 1998. Cephalopod and groundfish landings: evidence for ecological change in global fisheries? Reviews in Fish Biology and Fisheries, 8: 431–444.
Carvalho, G. R., Thompson, A., and Stoner, A. L. 1992. Genetic diversity and population differentiation of the shortfin squid *Illex argentinus* in the south-west Atlantic. Journal of Experimental Marine Biology and Ecology, 158: 105–121.
Chang, W. Y. B. 1982. A statistical method for evaluating the reproducibility of age determination. Canadian Journal of Fisheries and Aquatic Sciences, 39: 1208–1210.
Chemshirova, I., Hoving, H.-J., and Arkhipkin, A. 2021. Temperature effects on size, maturity, and abundance of the squid *Illex argentinus* (Cephalopoda, Ommastrephidae) on the Patagonian Shelf. Estuarine, Coastal and Shelf Science, 255: 107343.
Cheng, S. H., Gold, M., Rodriguez, N., and Barber, P. H. 2021. Genome-wide snps reveal complex fine scale population structure in the California market squid fishery (*Doryteuthis opalescens*). Conservation Genetics, 22: 97–110.
Crespi-Abril, A. C., and Barón, P. J. 2012. Revision of the population structuring of *Illex argentinus* (Castellanos, 1960) and a new interpretation based on modelling the spatio-temporal environmental suitability for spawning and nursery. Fisheries Oceanography, 21: 199–214.
Crespi-Abril, A. C., Morsan, E. M., and Barn, P. J. 2010. Analysis of the ontogenetic variation in body and beak shape of the *Illex argentinus* inner shelf spawning groups by geometric morphometrics. Journal of the Marine Biological Association of the United Kingdom, 90: 547–553.
Crespi-Abril, A. C., Morsan, E. M., and Baron, P. D. 2008. Contribution to understanding the population structure and maturation of *Illex argentinus* (Castellanos, 1960): the case of the inner-shelf spawning groups in San Matias Gulf (Patagonia, Argentina). Journal of Shellfish Research, 27: 1225–1231.
Crespi-Abril, A. C., Morsan, E. M., Williams, G. N., and Gagliardini, D. A. 2013. Spatial distribution of *Illex argentinus* in San Matias Gulf (northern Patagonia, Argentina) in relation to environmental variables: a contribution to the new interpretation of the population structuring. Journal of Sea Research, 77: 22–31.
Dawe, E. G., O'Dor, R. K., Odense, P. H., and Hurley, G. V. 1985. Validation and application of an agein techinique for short-finned squid (*Illex illecebrosus*). Journal of Northwest Atlantic Fishery Science, 6: 107–116.
De Mita, S., Thuillet, A.-C., Gay, L., Ahmadi, N., Manel, S., Ronfort, J., and Vigouroux, Y. 2013. Detecting selection along environmental gradients: analysis of eight methods and their effectiveness for outbreeding and selfing populations. Molecular Ecology, 22: 1383–1399.
Durholtz, M. D., and Lipinski, M. R. 2000. Influence of temperature on the microstructure of statoliths of the thumbstall squid *Lolliguncula brevis*. Marine Biology, 136: 1029–1037.
Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology, 14: 2611–2620.
Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary Bioinformatics, 1: 1–47.
Falkland Islands Government. 2021. Fisheries Department Fisheries Statistics, 2020. FIG Fisheries Department, Stanley.
FAO. 2020. The State of World Fisheries and Aquaculture 2020. FAO, Rome.
FAO. 2022. The State of World Fisheries and Aquaculture 2022. FAO, Rome.
Foll, M., and Gaggiotti, O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a bayesian perspective. Genetics, 180: 977–993.
Garoia, F., Guarniero, I., Ramšak, A., Ungaro, N., Landi, M., Piccinetti, C., Mannini, P. *et al.* 2004. Microsatellite DNA variation reveals high gene flow and panmictic populations in the Adriatic shared stocks of the European squid and cuttlefish (Cephalopoda). Heredity, 93: 166–174.
Gonzalez, A. F., and Guerra, A., 1996. Reproductive biology of the short-finned squid *Illex coindetii* (Cephalopoda, Ommastrephidae) of the Northeastern Atlantic. Sarsia, 81: 107–118.
Grolemund, G., and Wickham, H. 2011. Dates and times made easy with lubridate. Journal of Statistical Software, 40: 1–25.
Haimovici, M., Brunetti, N. E., Rodhouse, P. G., Csirke, J., and Leta, R. H. 1998. Chapter 3: *lllex argentinus*. *In* Squid Recruitment Dynamics. The Genus Illex as a Model. The Commercial Illex Species. Influences on Variability. FAO, Rome. pp. 27–58.
Haimovici, M., and Pérez, J. A. 1990. Distribución y maduración sexual del calamar argentino, *Illex argentinus* (Castellanos, 1960) (Cephalopoda: ommastrephidae), en el sur de Brasil. Scientia Marina, 54: 179–185.
Hatanaka, H. 1988. Feeding migration of short-finned squid *Illex argentinus* in the waters off Argentina. Nippon Suisan Gakkaishi, 54: 1343–1349.

Hurley, G. V., Odense, P. H., O'Dor, R. K., and Dawe, E. G. 1985. Strontium labelling for verifying daily growth increments in the statolith of the short-finned squid (*Illex illecebrosus*). Canadian Journal of Fisheries and Aquatic Sciences, 42: 380–383.

Jombart, T. 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24: 1403–1405.

Jones, J., and Hendrickson, L. 2022. Report of the Illex 2021 Research Track Assessment Working Group. NOAA, Silver Spring, MD.

Kenchington, E., Heino, M., and Nielsen, E. E. 2003. Managing marine genetic diversity: time for action? ICES Journal of Marine Science, 60: 1172–1176.

Kerr, L. A., Hintzen, N. T., Cadrin, S. X., Clausen, L. W., Dickey-Collas, M., Goethel, D. R., Hatfield, E. M. C. *et al.* 2017. Lessons learned from practical approaches to reconcile mismatches between biological population structure and stock units of marine fish. ICES Journal of Marine Science, 74: 1708–1722.

Kritzer, J. P., and Liu, O. R. 2014. Fishery management strategies for addressing complex spatial structure in marine fish stocks. *In* Stock Identification Methods, pp. 29–57. Elsevier, Amsterdam.

Leone, A., Álvarez, P., García, D., Saborido-Rey, F., and Rodriguez-Ezpeleta, N. 2019. Genome-wide SNP based population structure in European hake reveals the need for harmonizing biological and management units. ICES Journal of Marine Science, 76: 2260–2266.

Li, S., and Chou, H. H. 2004. LUCY2: an interactive DNA sequence quality trimming and vector removal tool. Bioinformatics, 20: 2865–2866.

Lipinski, M. 1979. Universal Maturity Scale for the Commercially-Important Squids (Cephalopoda: Teuthoidea). The Results of Maturity Classification of the Illex illecebrosus (LeSueur. 1821) Populations for the Years 1973–1977. Sea Fisheries Institute, Gdynia.

McKeown, N. J., Arkhipkin, A. I., and Shaw, P. W. 2017. Regional genetic population structure and fine scale genetic cohesion in the southern blue whiting *Micromesistius australis*. Fisheries Research, 185: 176–184.

McKeown, N. J., Arkhipkin, A. I., and Shaw, P. W. 2019. Genetic analysis reveals historical and contemporary population dynamics in the longfin squid *Doryteuthis gahi*: implications for cephalopod management and conservation. ICES Journal of Marine Science, 76: 1019–1027.

McKeown, N. J., Carpi, P., Silva, J. F., Healey, A. J., Shaw, P. W., and van der Kooij, J. 2020. Genetic population structure and tools for the management of European sprat (*Sprattus sprattus*). ICES Journal of Marine Science, 77: 2134–2143.

McKeown, N. J., Robin, J.-P., and Shaw, P. W. 2015. Species-specific PCR-RFLP for identification of early life history stages of squid and other applications to fisheries research. Fisheries Research, 167: 207–209.

Milano, I., Babbucci, M., Panitz, F., Ogden, R., Nielsen, R. O., Taylor, M. I, Helyar, S. J. *et al.* 2011. Novel tools for conservation genomics: comparing two high-throughput approaches for SNP discovery in the transcriptome of the European hake. PLoS One, 6: e28008.

Mullins, R. B., McKeown, N. J., Sauer, W. H., and Shaw, P. W. 2018. Genomic analysis reveals multiple mismatches between biological and management units in yellowfin tuna (*Thunnus albacares*). ICES Journal of Marine Science, 75: 2145–2152.

Narum, S. R., and Hess, J. E. 2011. Comparison of F_{ST} outlier tests for SNP loci under selection. Molecular Ecology Resources, 11: 184–194.

Ogle, D., Doll, J., Wheeler, P., and Dinno, A. 2022. FSA: fisheries stock analysis. R package version 0.9. 1. Ph. D. thesis, Francis Marion University, Florence, SC.

Ospina-Alvarez, A., de Juan, S., Pita, P., Ainsworth, G. B., Matos, F. L., Pita, C. and Villasante, S. 2022. A network analysis of global cephalopod trade. Scientific Reports, 12: 322.

Ott, A., Liu, S., Schnable, J. C., Yeh, C. T., Wang, K. S., and Schnable, P. S. 2017. tGBS® genotyping-by-sequencing enables reliable genotyping of heterozygous loci. Nucleic Acids Research, 45: e178.

Parfeniuk, A. V., Froerman, Y. M., and Golub, A. N. 1992. Particularidades de la distribución de los juveniles del calamar (*Illex argentinus*) en el área de la Depresión Argentina. Frente Maritimo, 12: 105–111.

Petrić, M., Škeljo, F., and Šifner, S. K. 2021. Age, growth and maturation of *Illex coindetii* (Cephalopoda: ommastrephidae) in the eastern Adriatic Sea. Regional Studies in Marine Science, 47: 101935.

Planes, S., and Lenfant, P. 2002. Temporal change in the genetic structure between and within cohorts of a marine fish, *Diplodus sargus*, induced by a large variance in individual reproductive success. Molecular Ecology, 11: 1515–1524.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945–959.

QGIS Development Team. 2022. QGIS geographic information system.. *In* Open Source Geospatial Foundation Project.

R Core Team. 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Reichow, D., and Smith, M. J. 2001. Microsatellites reveal high levels of gene flow among populations of the California squid *Loligo opalescens*. Molecular Ecology, 10: 1101–1109.

Reiss, H., Hoarau, G., Dickey-Collas, M., and Wolff, W. J. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish and Fisheries, 10: 361–395.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution; International Journal of Organic Evolution, 43: 223–225.

Rodhouse, P. G. 2001. Managing and forecasting squid fisheries in variable environments. Fisheries Research, 54: 3–8.

Rodhouse, P. G., Arkhipkin, A., Laptikhovsky, V., Nigmatullin, C. M., and Waluda, C. M. 2013. Chapter 4: *Illex argentinus*, Argentine shortfin squid. *In* Advances in Squid Biology, Ecology and Fisheries Part II: Oegopsid Squids, Fish, Fishing and Fisheries, pp. 109–148. Nova Science Publishers, New York, NY

Rodhouse, P. G., and Hatfield, E. M. C. 1990. Age determination in squid using statolith growth increments. Fisheries Research, 8: 323–334.

Roesti, M., Salzburger, W., and Berner, D. 2012. Uninformative polymorphisms bias genome scans for signatures of selection. BMC Evolutionary Biology, 12: 1–7.

Roldán, M. I., Planella, L., Heras, S., and Fernández, M. V. 2014. Genetic analyses of two spawning stocks of the short-finned squid (*Illex argentinus*) using nuclear and mitochondrial data. Comptes Rendus Biologies, 337: 503–512.

Sandoval-Castellanos, E., Uribe-Alcocer, M., and Díaz-Jaimes, P. 2007. Population genetic structure of jumbo squid (*Dosidicus gigas*) evaluated by RAPD analysis. Fisheries Research, 83: 113–118.

Shaw, P. W., Hendrickson, L., McKeown, N. J., Stonier, T., Naud, M. J., and Sauer, W. 2010. Discrete spawning aggregations of loliginid squid do not represent genetically distinct populations. Marine Ecology Progress Series, 408: 117–127.

Shaw, P. W., Pierce, G. J., and Boyle, P. R. 1999. Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. Molecular Ecology, 8: 407–417.

Staaf, D. J., Ruiz-Cooley, R. I., Elliger, C., Lebaric, Z., Campos, B., Markaida, U. and William, G. 2010. Ommastrephid squids *Sthenoteuthis oualaniensis* and *Dosidicus gigas* in the eastern Pacific show convergent biogeographic breaks but contrasting population structures. Marine Ecology Progress Series, 418: 165–178.

Torres Alberto, M. L., Bodnariuk, N., Ivanovic, M., Saraceno, M., and Acha, E. M. 2020. Dynamics of the Confluence of Malvinas and Brazil currents, and a southern Patagonian spawning ground, explain recruitment fluctuations of the main stock of *Illex argentinus*. Fisheries Oceanography, 30: 127–141.

Van Der Vyver, J. S. F., Sauer, W. H. H., McKeown, N. J., Yemane, D., Shaw, P. W., and Lipinski, M. R. 2016. Phenotypic divergence despite high gene flow in chokka squid *Loligo reynaudii* (Cephalopoda: loliginidae): implications for fishery management. Journal of the Marine Biological Association of the United Kingdom, 96: 1507–1525.

Villanueva, R. 2000. Effect of temperature on statolith growth of the European squid *Loligo vulgaris* during early life. Marine Biology, 136: 449–460.

Vitalis, R., Dawson, K., and Boursot, P. 2001. Interpretation of variation across marker loci as evidence of selection. Genetics, 158: 1811–1823.

Waluda, C. M., Trathan, P. N., and Rodhouse, P. G. 1999. Influence of oceanographic variability on recruitment in the *Illex argentinus* (Cephalopoda: ommastrephidae) fishery in the South Atlantic. Marine Ecology Progress Series, 183: 159–167.

Weir, B, and Cockerham, C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, 1: 1358–1370.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G. *et al.* 2019. Welcome to the Tidyverse. Journal of Open Source Software, 4: 1686.

Winnepenninckx, B., Backeljau, T., and De Wacher, R. 1993. Extractions of high molecular weight DNA from mollusks. Trends in Genetics, 9: 407.

Xavier, J., Walker, K., Elliot, G., Cherel, Y., and Thompson, D. 2014. Cephalopod fauna of South Pacific waters: new information from breeding New Zealand wandering albatrosses. Marine Ecology Progress Series, 513: 131–142.

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