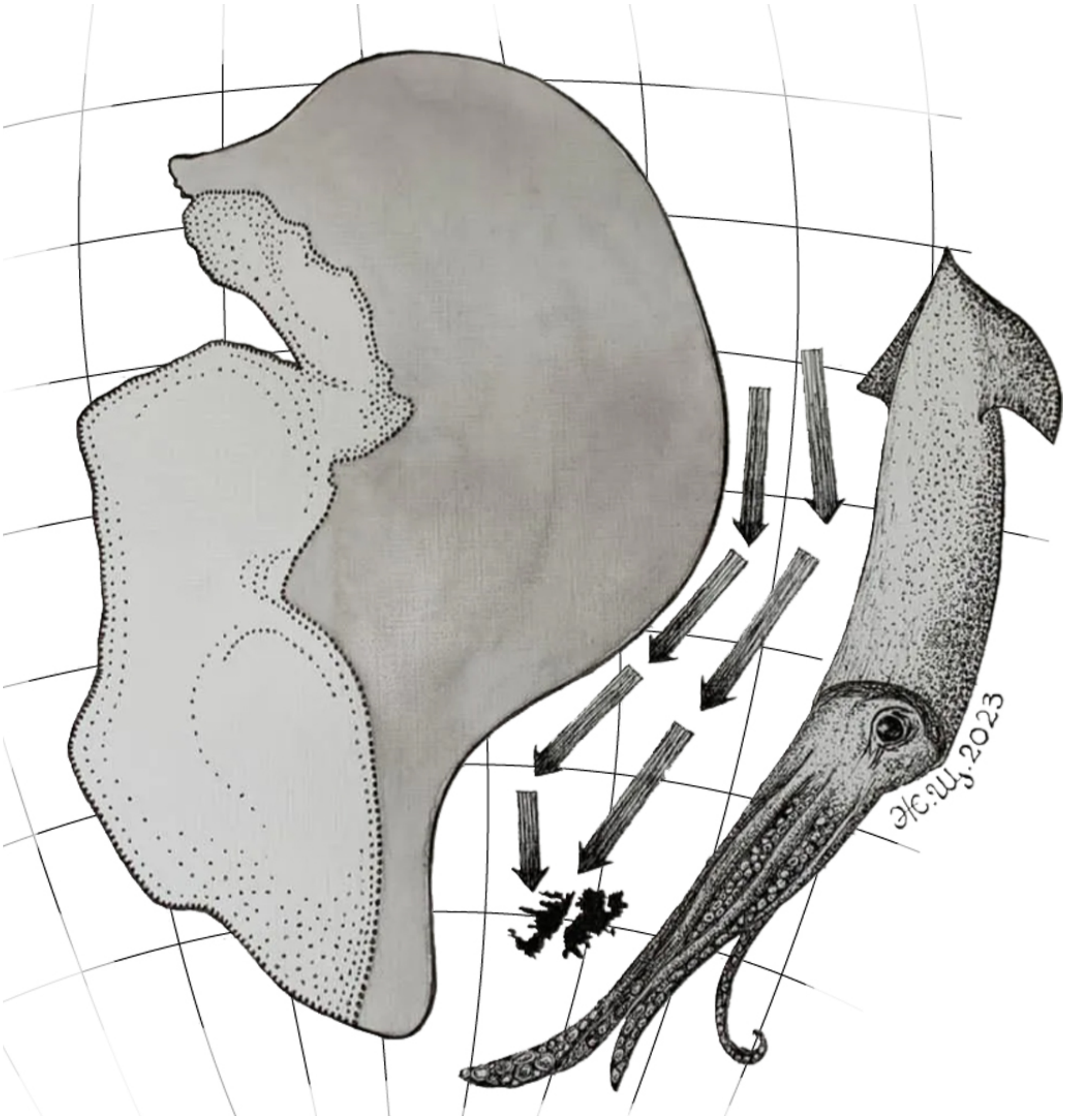

Population structure of the Argentine shortfin squid *Illex argentinus* (Castellanos 1960, Family Ommastrephidae)

IRINA CHEMSHIROVA

Population structure of the Argentine shortfin squid *Illex argentinus* (Castellanos 1960, Family Ommastrephidae)

Dissertation

In fulfilment of the requirements for the degree of “Doctor rerum naturalium”

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submitted by

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List of Abbreviations

ACF	autocorrelation function
ACV	average coefficient of variation
AIC	Akaike's information criterion
ANOVA	analysis of variance
APE	average precent error
App	Appendix
BC	Brazil Current
BFC	Brazil-Falkland Confluence
BNPS	Bonaerensis North Patagonian Stock
CPS	counts per second
CPUE	catch per unit effort
DML	dorsal mantle length
EEZ	Exclusive Economic Zone
ESPS	Early-maturing South Patagonian Stock
FC	Falkland Current
FI SpSS	Falkland Islands Spring Spawning Stock
FICZ	Falkland Islands Interim Conservation and Management Zone
FIFD	Falkland Islands Fisheries Department
FOCZ	Falkland Islands Outer Conservation Zone
GLM	genralised linear model
HS SpSS	High Seas Spring Spawning Stock
HWE	Hardy-Weinberg Equilibrium
ITAG	invertebrate eco-sensor tag
LA-ICP-MS	laser ablation-inductively coupled plasma-mass spectrometry
LOD	limit of detection
LOLBOX	Loligo Box
LSPS	Late-maturing South Patagonian Stock
LV	latent variable
MAF	minimum allele frequency
MCMC	Markov Chain Monte Carlo
MI	Migration Inshore
MO	Migration Offshore
NIST-SRM	National Institute of Standards and Technologies- Standard Reference Material
PCA	principal components analysis
ppm	parts per million
RADseq	restriction site-associated DNA sequencing
SD	standard deviation
SNP	single nucleotide polymorphisms
SPS	South Patagonian Stock
SpSS	Spring Spawning Stock
SSS	Summer Spawning Stock
tGBS	tunable genotyping by sequencing
UN	Unassigned

Summary

Cephalopod fisheries have increased in importance over the last decade, partly due to reduced yields of finfish fisheries. The Argentine shortfin squid *Illex argentinus* is the target of one of the largest fisheries in the Southwest Atlantic. This species is characterised by large annual population fluctuations and a complex population structure that consists of up to four seasonal spawning groups. *Illex argentinus* has a wide geographic distribution range (22˚S to 55˚S) and individuals undertake long-range ontogenetic migrations. This species is exploited by numerous coastal states and on the high seas where harvesting is unregulated, making it a "straddling stock". All these factors and characteristics combined make *I. argentinus* particularly vulnerable to exploitation. Therefore, the overall goal of this thesis is to disentangle how environmental factors affect key population characteristics and investigate the population structure of *I. argentinus* to inform future management of the fishery.

The **first chapter** of this dissertation investigated the response of *I. argentinus* population- and individual-level characteristics to changing environmental conditions, namely temperature. This study used a 20-year time series of fisheries data and monthly sea surface temperature data that correspond to the life cycle of the winter-spawning group. One of the objectives was to determine whether higher temperatures in the hatching grounds lead to smaller but more numerous mature adults. Additionally, these data were used to determine if abundance of the winter-spawning group in Falkland Islands waters was positively correlated with temperature on the hatching grounds. Individuals in the Falkland Islands Conservation Zones (FICZ and FOCZ) were found to be smaller in size and a lower proportion of females were mature, when the temperatures in the hatching grounds exceeded 17.5˚C. Furthermore, temperatures above 21.5˚C in the hatching grounds led to a higher abundance of *I. argentinus* during the fishing season in Falkland Islands waters. These findings elucidate the effect that changing environmental conditions have on vital fishery resources and provide a baseline for future forecasting of the *I. argentinus* fishery within the FICZ and FOCZ.

The **second chapter** investigated the connectivity between the seasonal spawning groups of *I. argentinus*, particularly whether seasonal spawning groups identified through statolith ageing and microstructure represented a single interbreeding genetic metapopulation. This was achieved by a genome-wide single nucleotide polymorphism (SNP) analysis to determine the extent of the genetic connectivity of the seasonal spawning groups identified from statolith ageing analysis. Three seasonal spawning groups, each with subgroups, were identified: winter-hatched, spring-hatched and summer-hatched individuals. No neutral or non-neutral genetic

structuring was evident following analysis of over 10 000 SNPs. These findings highlight the need for future fisheries management measures to consider the entire population over the species' entire distribution range, which encompasses several Exclusive Economic Zones, as opposed to focusing on single groups. By using population genomics and statolith analyses this study revealed the extent of genotypic similarities between seasonal groups of *I. argentinus*. These seasonal groups may exhibit different phenotypes due to the heterogeneity in spawning times.

The third and **final chapter** of this dissertation aimed to reveal differences in the early ontogeny of *I. argentinus* spawning groups. Statolith ageing and microstructure analyses were combined with elemental signatures from the statolith core of individuals to i) test whether different spawning groups had different single elemental signatures and ii) relate these findings to environmental conditions of the putative hatching grounds of each seasonal group. Multi-elemental signatures were also applied with the aim of identifying a unique signature for each spawning group. Single-elemental signatures of ^{88}Sr, ^{24}Mg, ^{137}Ba, and ^{55}Mn confirmed the seasonal differences between the spawning groups. However, no distinct multi-elemental signatures (^{88}Sr, ^{24}Mg, ^{137}Ba, ^{55}Mn, ^{7}Li, ^{57}Fe) were found for the spawning groups investigated. Ordination of latent variables extracted from the multi-elemental model revealed two axes of unexplained covariation. Therefore, fine-scale structuring within seasonal groups in terms of putative hatching location, could not be ruled out completely. These findings confirm the high level of spatial and temporal population connectivity, and plasticity of *I. argentinus* in the Southwest Atlantic.

Zusammenfassung

(*Summary translated by Rebecca Piontek*)

Die Fischerei von Cephalopoda hat in den letzten zehn Jahren an Bedeutung zugenommen, teilweise aufgrund der geringeren Erträge der auf Knochenfische spezialisierten Fischerei. Der argentinische Kurzflossenkalmar *Illex argentinus* ist das Ziel einer der größten Fischereien im Südwestatlantik. Diese Art zeichnet sich durch große jährliche Schwankungen der Biomasse und eine komplexe Populationsstruktur aus, die aus bis zu vier saisonalen Kohorten (Laichgruppen) besteht. I. argentinus hat ein grossflächiges geografisches Verbreitungsgebiet (22˚S bis 55˚S) und Individuen überwinden lange Distanzen im Zuge ontogenetischer Migration. Diese Art wird sowohl von zahlreichen Küstenstaaten als auch auf hoher See durch unregulierter Fischerei ausgebeutet, was sie zu einer „gebietsübergreifenden Spezies“ macht. All diese Faktoren und Eigenschaften zusammen machen *I. argentinus* besonders anfällig für Überfischung. Daher ist das übergeordnete Ziel dieser Arbeit zu untersuchen, wie Umweltfaktoren wichtige Populationsmerkmale beeinflussen, und die Populationsstruktur von *I. argentinus* zu untersuchen, um das zukünftige Management der Fischerei zu unterstützen.

Das erste Kapitel dieser Dissertation untersuchte die Reaktion von Populations- und Individualmerkmalen von *I. argentinus* auf sich ändernde Umweltbedingungen in Form von Temperaturschwankungen. Diese Studie verwendete eine 20-jährige Messreihe von Fischereidaten und monatlichen Meeresoberflächentemperaturdaten, die dem Lebenszyklus der im Winter laichenden Gruppe entsprachen. Eines der Ziele war es festzustellen, ob höhere Temperaturen in den Brutplätzen zu kleineren, aber zahlreicheren erwachsenen Tieren führen. Darüber hinaus wurden diese Daten verwendet, um zu bestimmen, ob die Häufigkeit der im Winter laichenden Gruppe in den Gewässern der Falklandinseln positiv mit der Temperatur an den Brutplätzen korrelierte. Es wurde festgestellt, dass Individuen in den Schutzzonen der Falklandinseln (FICZ und FOCZ) kleiner und ein geringerer Anteil der weiblichen Tiere ausgewachsen waren, wenn die Temperaturen in den Brutgebieten 17,5 °C überschritten. Darüber hinaus führten Temperaturen über 21,5 °C in den Brutgebieten zu einer höheren Häufigkeit von *I. argentinus* während der Fangsaison in den Gewässern der Falklandinseln. Diese Ergebnisse Auswirkungen, die sich ändernde Umweltbedingungen auf lebenswichtige Fischereiressourcen haben, und liefern eine Grundlage für zukünftige Prognosen der *I. argentinus*-Fischerei innerhalb der FICZ und FOCZ.

Das zweite Kapitel untersuchte die Verbindung zwischen den saisonalen Kohorten von *I. argentinus*, insbesondere, ob saisonale Kohorten, die durch Altersbestimmung („lesen“ von

Statolithen) und die Analyse von Statolith-Mikrostrukturen identifiziert wurden, eine einzige sich kreuzende genetische Metapopulation darstellten. Dies wurde durch eine genomweite Single Nucleotide Polymorphism (SNP)-Analyse erreicht, um das Ausmaß der genetischen Konnektivität der saisonalen Kohorten zu bestimmen, die aus der Statolith-Alterungsanalyse identifiziert wurden. Drei saisonale Kohorten mit jeweiligen Untergruppen wurden identifiziert: im Winter, im Frühjahr und im Sommer geschlüpfte Individuen. Nach der Analyse von über 10.000 SNPs war keine neutrale oder nicht neutrale genetische Strukturierung ersichtlich. Diese Ergebnisse unterstreichen die Notwendigkeit zukünftiger Fischereimanagementmaßnahmen, welde die gesamte Population über das gesamte Verbreitungsgebiet der Art berücksichtigen, und mehrere speperate Wirtschaftszonen umfasst, anstatt sich auf einzelne Gruppen zu konzentrieren. Durch die Verwendung von Populationsgenomik und Statolith-Analysen zeigte diese Studie das Ausmaß der genotypischen Ähnlichkeiten zwischen saisonalen Gruppen von *I. argentinus*. Diese saisonalen Gruppen können aufgrund der Heterogenität der Laichzeiten unterschiedliche Phänotypen aufweisen.

Das dritte und letzte Kapitel dieser Dissertation zielte darauf ab, Unterschiede in der frühen Ontogenese von *I. argentinus*-Kohorten aufzuzeigen. Statolith- und Mikrostrukturanalysen wurden mit elementaren Signaturen aus dem Statolithen-Kern von Individuen kombiniert, um i) zu testen, ob verschiedene Kohorten unterschiedliche elementare Signaturen hatten, und ii) diese Ergebnisse mit den Umweltbedingungen der mutmaßlichen Brutplätze jeder saisonalen Gruppe in Beziehung zu setzen. Es wurden auch nach Signaturen von Elementkombinationen gesucht mit dem Ziel, eine eindeutige Signatur für jede Kohorte zu identifizieren. Signaturen einzelner Elemente (^{88}Sr, ^{24}Mg, ^{137}Ba, ^{55}Mn) bestätigten die saisonalen Unterschiede zwischen den Kohorten. Für die untersuchten Kohorten wurden jedoch keine eindeutigen Multi-Element-Signaturen gefunden. Die Multi-Element-Analyse (^{88}Sr, ^{24}Mg, ^{137}Ba, ^{55}Mn, ^{7}Li, ^{57}Fe) zeigte jedoch zwei Achsen nicht näher zu bestimmender Kovarianten in den Ordinaten der latenten Variabeln. Eine feinräumige Strukturierung innerhalb saisonaler Kohorten in Bezug auf mutmaßliche Schlupforte konnte daher nicht vollständig ausgeschlossen werden. Diese Ergebnisse bestätigen das hohe Maß an räumlicher und zeitlicher Populationskonnektivität und Plastizität von *I. argentinus* im Südwestatlantik.

General Introduction

The world's oceans are under increasing pressure (Halpern et al., 2015; Jouffray et al., 2020). Subsequently, the future of fisheries, which provide valuable source of protein for humans, is uncertain (Costello et al., 2020). Factors such as climate change (Lotze et al., 2019) and overfishing (Jackson, 2001) may act in synergy, contributing to the decline of the ecosystems that support wild-capture fisheries. The global depletion of finfish stocks (FAO, 2022) may have provided an opportunity for the development of invertebrate fisheries (Anderson et al., 2011). Cephalopods, in particular, may have benefitted from reduced competition and predation (Caddy and Rodhouse, 1998; Doubleday and Connell, 2018).

Cephalopods and fisheries

Cephalopods have grown in importance as a fishery resource in the last half of the 20th century. This is exemplified by cephalopod landings that peaked in 2014 at 4.9 million tonnes and remained stable in the past two decades (FAO, 2022). Their significance will continue to grow as the world population increases beyond 8 billion people and market demand increases (Arkhipkin et al., 2015b; Mouritsen and Styrbæk, 2021). Squid have an essential role in ecosystem functioning, as they frequently act as links between trophic levels (de la Chesnais et al., 2019; Xavier et al., 2018). Furthermore, species that undertake feeding migrations also act as "biological pumps" that link distant large marine ecosystems (Arkhipkin, 2013). The ecosystem service provision of cephalopods worldwide is estimated to be between 15 and 20% (Hunsicker et al., 2010). This estimate included cephalopods as a fishery resource and a supporting mechanism in the form of prey for other commercial species (Hunsicker et al., 2010).

Approximately 30-40 squid species are considered to hold commercial value; however, the majority of the world squid landings can be attributed to few squid species (Arkhipkin et al., 2015b). Squid life history characteristics such as rapid growth, short life span, and high degree of phenotypic plasticity can pose a challenge for sustainable cephalopod fisheries management (Anderson and Rodhouse, 2001; Arkhipkin et al., 2021). Nektonic squid are at a higher risk of over-exploitation as they are migratory, thus may pass through several Exclusive Economic Zones (EEZ) and international waters, where they may be exploited with varying degrees of management and various life stages (Arkhipkin et al., 2022). Ommastrephid squid fisheries are characterised by "boom and bust" cycles (Rodhouse, 2008). Much of this fluctuation is attributed to variation in environmental conditions, as these species are generally associated with boundary current systems, which in turn are defined by highly dynamic environmental conditions (Boyle and Rodhouse, 2005). Large climatic events such as El Niño, may also trigger major changes in life history strategies and species distribution (Hoving et al., 2013). Therefore, to ensure the sustainable management of cephalopod fisheries, it is essential to consolidate our understanding of species' responses to different environmental conditions and their population structure. This knowledge may subsequently inform effective management strategies and promote the long-term sustainability of these valuable fisheries. Southwest Atlantic cephalopod fisheries are of a particular importance, as the highest production volume of cephalopods in the last 20 years was attributed to East Asia and South America (FAO, 2020). The Argentine shortfin squid (*Illex argentinus;* Castellanos, 1960) fishery in the waters of Argentina and the Falkland Islands significantly contributes to this production (Ospina-Alvarez et al., 2022).

Illex argentinus fishery

Illex argentinus is the target of the largest ommastrephid fishery in the Southwest Atlantic, with annual world catch attaining over 1 million tonnes in 2015 (FAO, 2019). However, as with other ommastrephids, catches can vary greatly, as seen in 2009 when the global catch for this species was as low as 260 thousand tonnes (FAO, 2012). *Illex argentinus* was primarily caught as minor bycatch in the hake fishery operating in the Argentine EEZ until the late 1970s (Brunetti, 1990). Subsequently, fishing fleets started actively targeting *I. argentinus* aggregations, with catches up to 122, 000 tonnes recorded in 1979 (Csirke, 1987). Large-scale fishing operations began in the 1980s, in the area just outside the Argentine EEZ from 41˚S to 47˚S and the Falkland Islands (Brunetti, 1990). Up to 120 jigging vessels and 90 factory trawlers operated per year (Brunetti, 1990; Sato and Hatanaka, 1983). The fishery primarily targeted the feeding and spawning aggregations of this squid (Arkhipkin et al., 2015b). In 1986 the Falkland Islands Interim Conservation and Management Zone (FICZ) was established. This led to the operation of a regulated fishery in the area, where vessels were required to obtain licenses in order to participate. The current annual operation within the FICZ consists of ~105 Taiwanese and South Korean jigging vessels, and Spanish factory trawlers (Falkland Islands Government, 2021). In the Argentine EEZ, ~115 trawlers and jiggers operate under Argentine and Chinese flags, whereas up to 400 mainly Chinese-flagged fishing vessels operate in the international waters north of the FICZ and Falkland Islands Outer Conservation Zone (FOCZ), which are unregulated (Arkhipkin et al., 2022). Therefore, *I. argentinus* is considered a straddling stock, exploited by several nations (Arkhipkin et al., 2015b). Management measures are implemented independently by each country, wherein the Falkland Islands set an annual "precautionary minimum spawning biomass" of ~40, 000 tonnes, effort is limited by the number of vessels that are licensed (n=106), and a fishing season starting on the 1st of February until the 15th of June. Within the Argentine EEZ, the government has prohibited harvesting of *I. argentinus* north of 44˚S from beginning of February until the end of April and south of 44˚S from the beginning of July until the end of January (Arkhipkin et al., 2015b).

Species Background

Illex argentinus

The Argentine shortfin squid, *Illex argentinus* (Castellanos, 1960) (Fig. 1)*,* is a neritic-oceanic squid. Typical adult dorsal mantle length (DML) for this species ranges between 30 and 35 cm (maximum of 40 cm DML), with females attaining larger sizes than males (Haimovici et al., 1998). *Illex argentinus* is semelparous, with a year-long life cycle and individuals die after spawning (Rodhouse et al., 2013). The distribution range of this species is from 22˚S to 55˚S at depths between 80 and 400 m (Fig. 2). This squid occurs primarily along the Patagonian Shelf and slope, with highest concentrations south of 40˚S (Jereb and Roper, 2010). The oceanography of the primary habitat of *I. argentinus* is highly dynamic and varies seasonally (Goes et al., 2019).

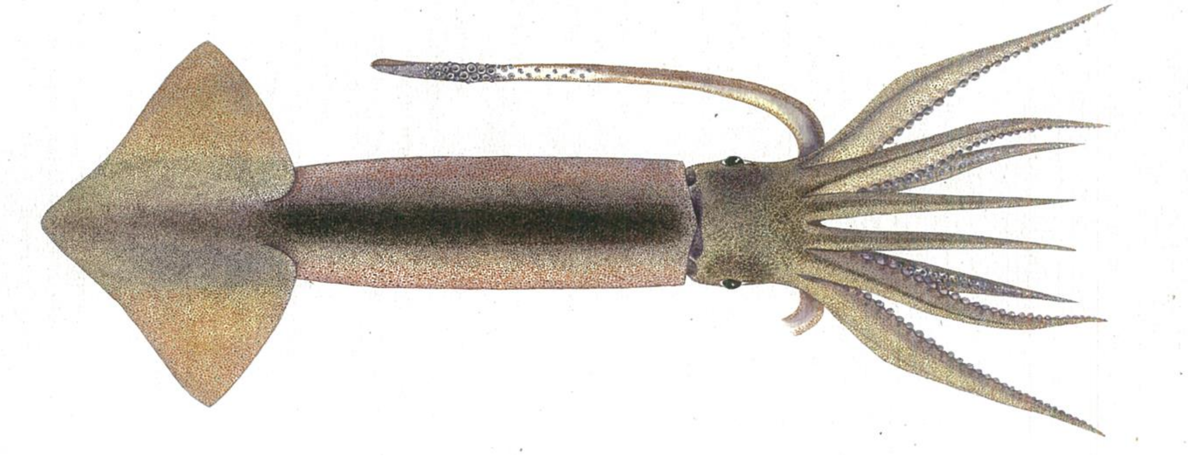

Figure 1. Argentine shortfin squid *Illex argentinus*, sourced from Alexeyev (2013).

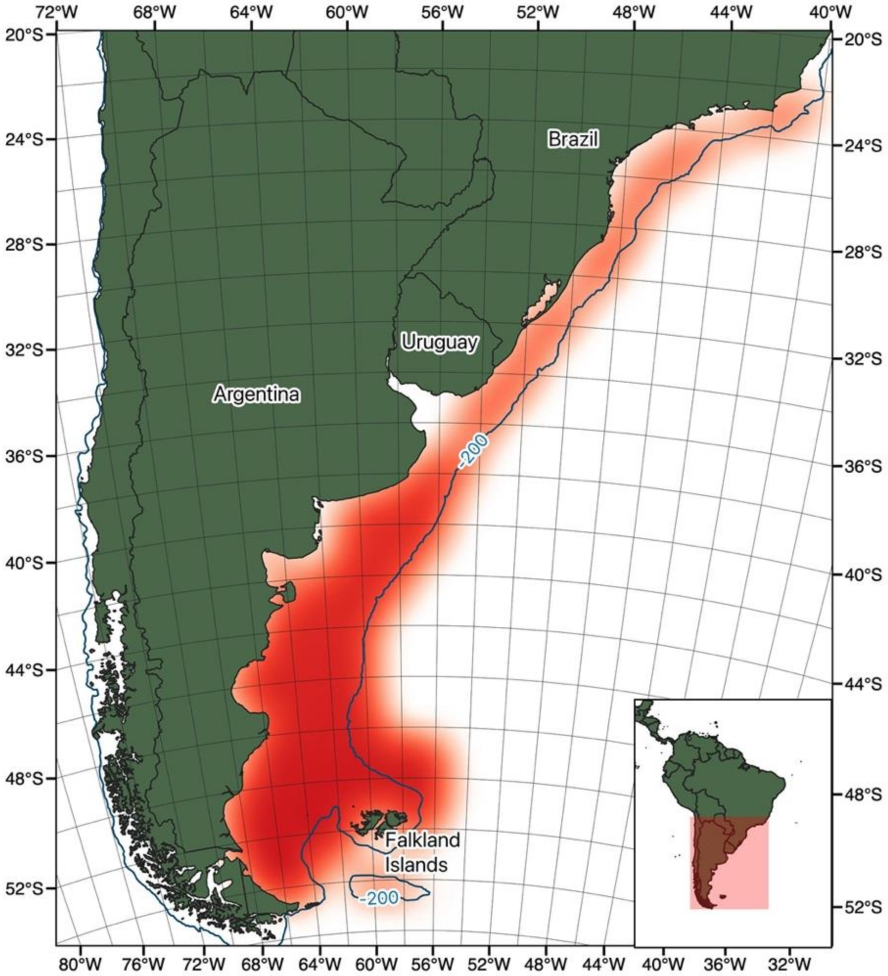

Figure 2. Map of *Illex argentinus* species range shaded in red, based on species range depicted in Jereb and Roper (2010).

Patagonian Shelf habitat and Illex argentinus

The oceanography of the Patagonian Shelf is characterised by two major western boundary currents. The Brazil Current (BC), flows southwards and carries warm subtropical waters (>20˚C) along the shelf. At approximately 38˚S the BC meets with the northward flowing, cold waters (<15˚C) of the Falkland Current (FC), a branch of the Antarctic Circumpolar Current (Fig. 3; Campos *et al.*, 1995; Piola and Matano, 2009). This convergence is termed the Brazil-Falklands Confluence (BFC), resulting in one of the most oceanographically dynamic regions of the world (Bouali et al., 2017). The confluence leads to the formation of numerous cold and warm-core eddies (Xu et al., 2011), some of which have been estimated to have a lifetime of over 2 months (de Souza et al., 2006). Furthermore, the position of the BFC varies seasonally, with the front moving north during the austral winter, and south during the austral summer (de Souza et al., 2019). The upwelling observed in the region is attributed to the interaction of the FC with the shelf break which results in numerous frontal zones (Acha et al., 2004; Belkin et al., 2009; Matano and Palma, 2008). Therefore, the Patagonian Shelf is considered one of the most productive marine ecosystems in the world (Sabatini et al., 2004). Chlorophyll-*a* blooms in the region reach their maximum at the shelf break, from 37˚S to 51˚S, during austral summer (Romero et al., 2006). These areas are widely utilised as feeding grounds by temperate fish (*e.g.,* hake, *Merluccius hubbsi*) and squid including *Doryteuthis gahi* and *I. argentinus* due to a peak in zooplankton abundance in the same period (Arkhipkin et al., 2013, 2012; Sabatini and Colombo, 2001). The environmental variability described above has the potential to affect various factors that may determine the annual abundance of *I. argentinus* available for harvesting. For example, variability in current speed or direction may affect dispersal patterns during early ontogeny (Cowen and Sponaugle, 2009), which may alter the overall proportions of the spawning groups that make up the complex population structure of this species. Furthermore, factors such as ambient temperature on the hatching grounds may alter population abundance or phenotypic characteristics in adults (Arkhipkin et al., 2015a; Waluda et al., 1999). However, it is unclear how inter-annual changes in environmental conditions on the Patagonian Shelf affect population-level characteristics (*e.g.,* abundance) or individual-level metrics (*e.g.,* DML and maturity) of *I. argentinus*.

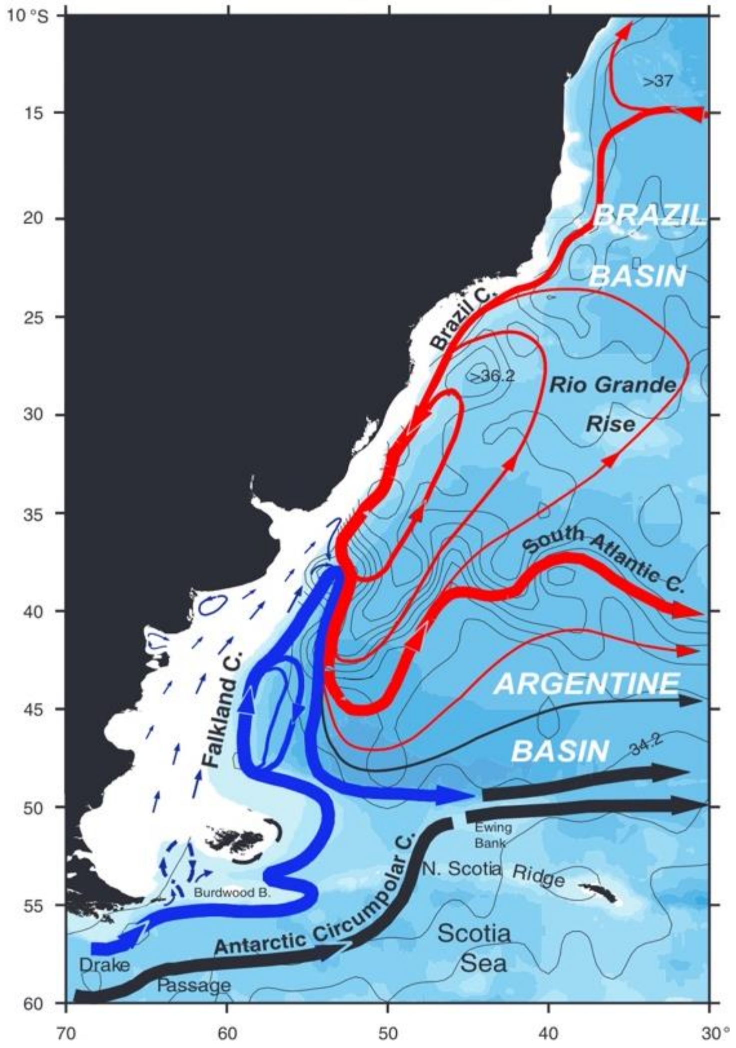

Figure 3. Oceanographic features of the Patagonian Shelf. The cold-water subantarctic Falkland Current is marked in blue, whereas the warm-water subtropical Brazil Current is depicted in red. Antarctic Circumpolar Current in black. Adapted from Piola and Matano (2009).

Population structure and life cycle

The population structure of *I. argentinus* may consist of up to four distinct seasonal spawning groups, which are frequently also referred to as stocks (*i.e.* fisheries management units) (Brunetti, 1988; Hatanaka, 1988). The largest of these groups is the winter-spawning one, as spawning occurs between June and August, during austral winter (Rodhouse et al., 2013). This group may be sub-divided into the Bonaerensis North Patagonian stock (BNPS) occurring north of 43°S and the South Patagonian stock (SPS), distributed south of 44°S (Brunetti, 1988). The SPS was considered to be composed of two subgroups: i) the shelf group, maturing at smaller sizes, with higher growth rates; and ii) the slope group, maturing at larger sizes, with slower growth rates, on the basis of statolith microstructure analysis (Arkhipkin, 1993). A similar sub-division of the slope SPS group has been previously hypothesised, with the two subgroups being called: i) early-maturing (ESPS) and ii) late-maturing (LSPS). The ESPS group is characterised by a north and north-eastern distribution in the Falkland Islands Conservation Zones (FICZ and FOCZ) during the month of March and attains small sizes at maturity. The LSPS group is primarily distributed in the western areas of the FICZ in the month of April and attains larger sizes at maturity (Falkland Islands Government, 2021). The SPS group as a whole is estimated to account for 95% of the total biomass of this species (Hatanaka, 1988), and has an approximately 1-year life cycle (Fig. 4; Arkhipkin, 1990; Rodhouse and Hatfield, 1990). Individuals of the SPS group complete the largest-scale migrations from 34°S – 38°S to 51°S (Arkhipkin, 1993). The post-larval period takes place near the Río de la Plata front from August until September (Leta, 1987; Santos and Haimovici, 1997). Followed by a juvenile period of feeding along the north part of the Patagonian Shelf from September until December (Brunetti, 1988; Parfeniuk et al., 1992). Subsequently, the feeding migration takes place southwards towards the Falkland and Patagonian shelves from January until April (Brunetti, 1988; Hatanaka, 1988; Sacau et al., 2005). Finally, the pre-spawning period is spent on the shelf edge and slope, from May until July off the coast of Argentina and Uruguay (Arkhipkin, 1993; Hatanaka, 1988, 1986). Fishing vessels generally follow the southward migration of the squid to the feeding grounds and then the northward migration towards the spawning grounds (Arkhipkin et al., 2015b; Basson et al., 1996; Nigmatullin, 1989). The location of their spawning grounds remains to be elucidated, as two areas have been proposed: i) South Brazil area, on the outer shelf and shelf-break north of 35˚S (Arkhipkin, 2013; Arkhipkin et al., 2015b; Haimovici and Pérez, 1990); and ii) Patagonian area, between 45˚S and 48˚S, on the outer shelf and shelf break (Haimovici et al., 1998; Torres Alberto et al., 2020).

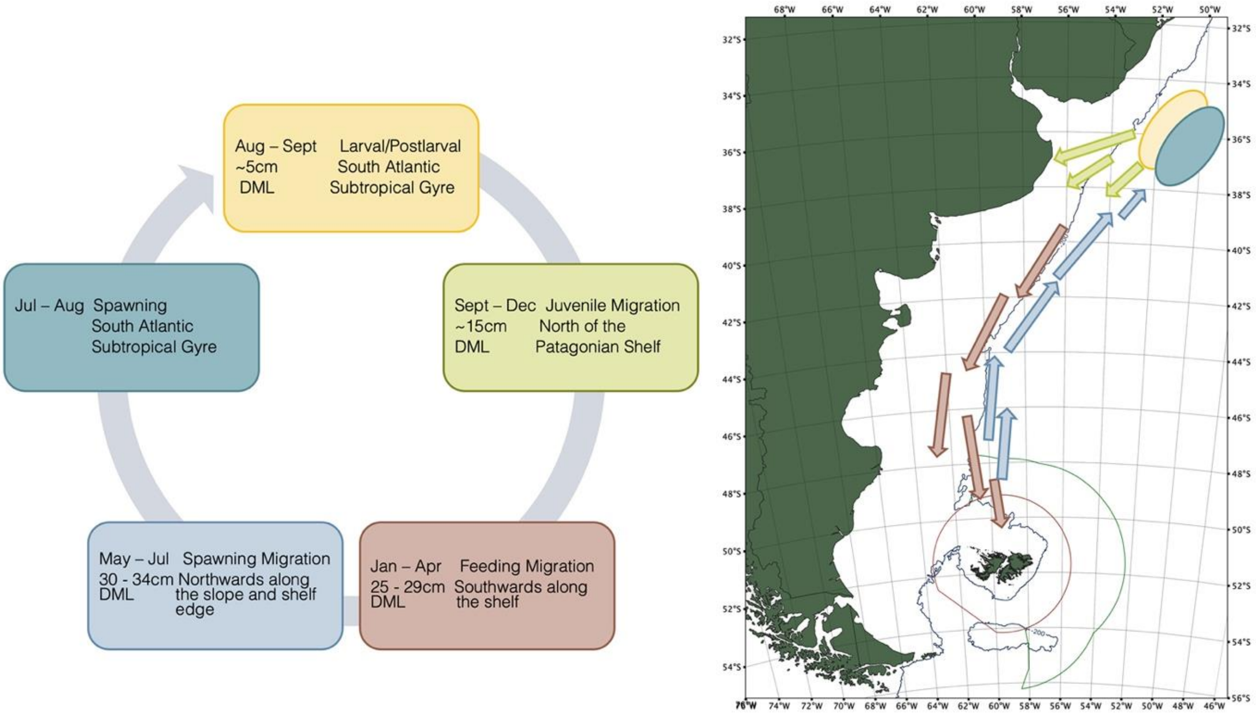

Figure 4. Life cycle of the winter-spawning South Patagonian Stock group across the Patagonian Shelf. DML, dorsal mantle length. FICZ, Falkland Islands Interim Conservation and Management Zone (red); FOCZ, Falkland Islands Outer Conservation Zone (green).

The remaining spring- and summer-spawning groups make up a smaller percentage of the overall population. Therefore, the information available regarding their spawning grounds, life cycle and ontogenetic migrations is limited. The San Matías Gulf (41°S to 42°S) is the coastal spawning ground hypothesised for the Spring Spawning Stock (SpSS) (Crespi-Abril et al., 2013, 2008; Crespi-Abril and Barón, 2012). During austral summer, the SpSS may also use the high seas areas between 45°S and 47°S (Arkhipkin et al., 2022). The Summer Spawning Stock (SSS) is believed to spawn on the shelf between 42°S and 46°S during the months of December to February. Occasionally, the SSS may migrate to the high seas (Crespi-Abril et al., 2010).

Finally, a local group north of 35˚S has been identified on the shelf and shelf break off the coast of Brazil (Alvarez Perez et al., 2009). This group is characterised by small DML sizes and a shorter life span of 6 months, with year-round spawning (Schwarz and Perez, 2013). Furthermore, a possible migratory group that grows to larger sizes has also been identified in the Brazilian slope area, individuals spawn primarily in the colder season, winter/spring, and have 10–11-month life spans (Haimovici et al., 2014; Schroeder et al., 2017). The origin of the migratory group is uncertain as it was previously hypothesised that it was an extension of the BNPS that migrated further north (Alvarez Perez et al., 2009; Haimovici et al., 1995). However, the possibility of large-sized migrant and non-migrant groups has been raised previously (Haimovici et al., 2014).

The approximate locations of the spawning and feeding grounds of the respective groups described here are represented in Figure 5, exemplifying the high level of spatial mixing between these groups. Due to the high migratory ability of this species, distinction between the seasonal spawning groups based on size, spatial or temporal distribution alone is challenging. Therefore, a combination of methods is required to provide the most accurate description of the population structure of *I. argentinus* (ICES, 2021).

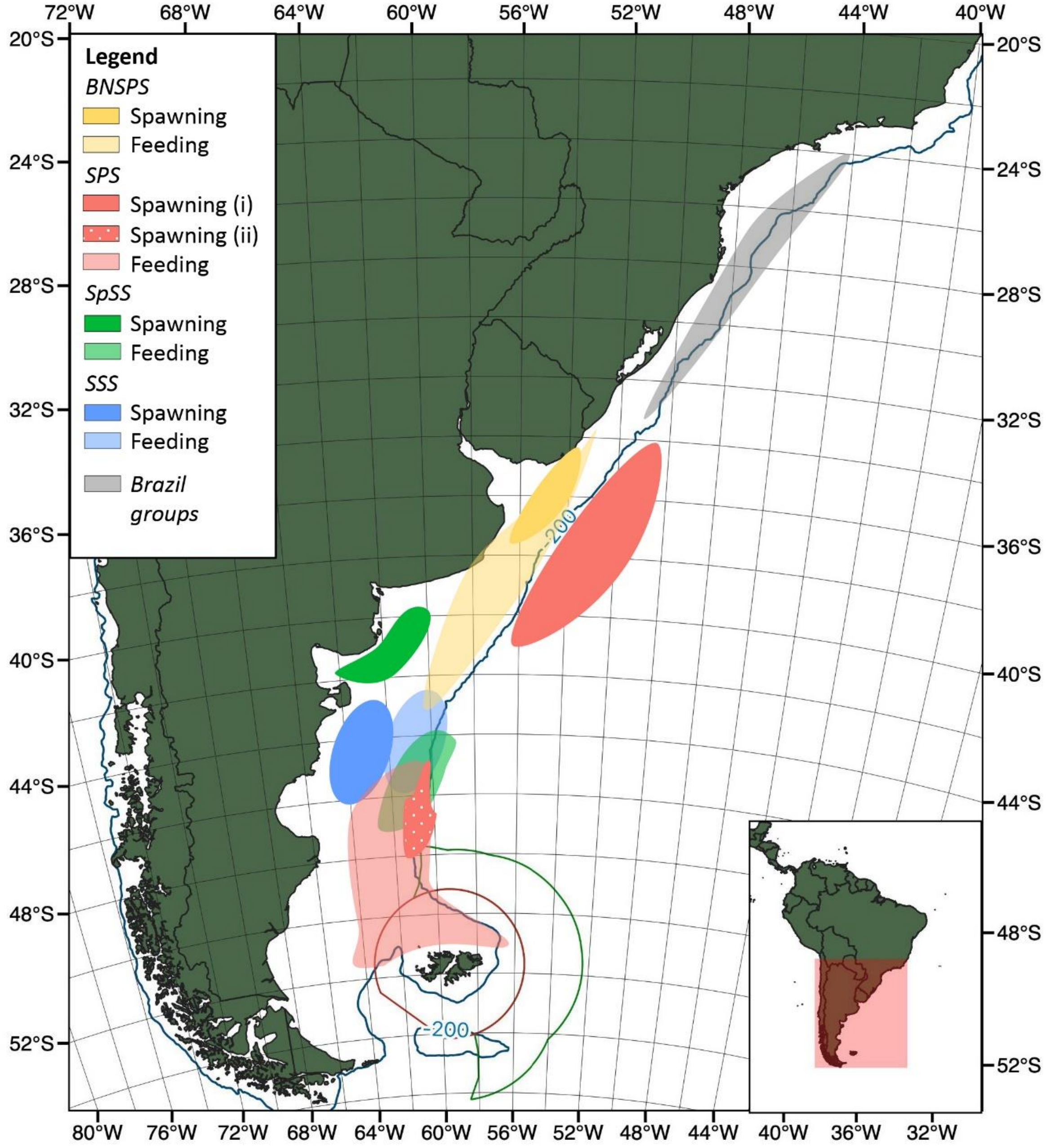

Figure 5. Map of the approximate spawning and feeding areas of the different *Illex argentinus* groups, based on information obtained from Haimovici *et al.* (2014) and Arkhipkin *et al.* (2022). The two hypothesised spawning grounds for the SPS stock are presented. Falkland Islands Interim Conservation and Management Zone in red; Falkland Islands Outer Conservation Zone in green. BNSPS, Bonaerensis North Patagonian stock; SPS, South Patagonian Stock; SpSS, Spring Spawning Stock; SSS, Summer Spawning Stock.

Discrimination of population structure

Accurate determination of the connectivity and structure of the aforementioned spawning groups is crucial for the successful management of the *I. argentinus* fishery. The response of these groups to changing environmental conditions during their life span may be similar across groups where connectivity is high. Conversely, if groups are distinct and the level of mixing is low they may respond differently, which in turn would different management strategies to focus on separate units instead.

The simplest method of discriminating between cephalopod stocks or groups within a population involves distinction on the basis of phenotypic traits (e.g., size, maturity, morphometric characteristics) and life history, such as spawning seasons and ontogenetic migrations (Boyle and Rodhouse, 2005). The spring and summer spawning-groups of *I. argentinus* were identified on the basis of size structure of mature individuals (Crespi-Abril et al., 2008; Nigmatullin, 1989). The difference between two groups of another valuable commercial species, *Uroteuthis edulis*, was initially described using morphological differences between the spring migrating group, deemed “kensaki-type” due to their slender bodies and the autumn migrating groups, termed “budo-type” due to their thick bodies (Yamaguchi et al., 2020). However, in *I. argentinus* recruitment occurs in successive waves over several months, and reliance on size structure alone may be misleading (Arkhipkin, 1993). The migratory ability of this squid (20 km/day) indicates the possibility that otherwise distinct seasonal spawning groups may mix in unknown proportions during feeding migrations (Arkhipkin et al., 2022), making geographic separation another unreliable metric. Squid size and spatial distribution are useful starting points for distinction between groups. Furthermore, they can be useful metrics for determining the response of groups to changes in environmental conditions (Chemshirova et al., 2021; Waluda et al., 1999). This information, coupled with obtaining detailed information on squid movements and connectivity would be beneficial for sustainable fisheries management. Nonetheless, obtaining detailed information on squid migrations over their short life span is challenging (Semmens et al., 2007).

Electronic tags have been previously used to track movements in cephalopods, with success in the large ommastrephid *Dosidicus gigas*, where an individual was tagged for 21 days (Gilly et al., 2006, 2012). The development of novel soft-bodied invertebrate tag (ITAG) and its use in smaller *Loligo forbesii* is promising (Flaspohler et al., 2019; Mooney et al., 2015). The smallest individuals tagged for both species were still larger than *I. argentinus*, *e.g.*, *D. gigas* 70 cm DML and *L. forbesii,* 52 cm DML. Therefore, the usage of electronic tags is currently not a viable option for investigating migration and connectivity of the seasonal spawning groups of *I. argentinus*.

Hard structures, such as statoliths, gladii, and beaks are archival structures in cephalopods that are present in hatchlings and grow during ontogeny (Roman Gonzalez, 2021). Therefore, they have the potential to provide life history information over the entire lifespan of an individual. Unfortunately, gladii and beaks are not entirely suitable for life history studies, as not all increments are visible in gladii (Arkhipkin et al., 2018). Beaks are used on a daily basis to feed and subject to surface erosion that may lead to underestimation of age (Rodríguez-Domínguez et al., 2013). Statoliths are suitable archival structures and may be used as “life recorders” in squid (Arkhipkin, 2005). Statoliths are paired calcareous structures located in the head, and make up a part of the equilibrium system required for balance maintenance and movement control in squid (Arkhipkin and Bizikov, 2000; Radtke, 1983). Statolith material is accreted throughout the squids’ life and the associated growth increments are deposited daily (Dawe et al., 1985; Hurley et al., 1985). Therefore, these archival structures may reveal a wealth of information about an individual’s life history (Arkhipkin, 2005). Age estimation with daily precision is particularly useful for species with such short life spans (>1 year) as it provides accurate hatching dates for individuals (Jackson, 1994). These dates can in turn be used to group individuals into seasonal groups, as groups growing in different seasons have previously been shown to have different growth rates and characteristics due to experiencing differing environmental conditions (Arkhipkin et al., 2015a, 2000).

Trace elements (*e.g.,* Strontium, Barium and Magnesium) are incorporated in the aragonite structure during statolith formation (Arkhipkin and Bizikov, 2000). The concentrations of these elements in the archival structures at the time of incorporation are considered to reflect the environmental conditions of the water body inhabited by the individual (Elsdon and Gillanders, 2003). Generally, marine taxa exhibit a negative correlation between temperature and Sr:Ca ratios (Beck et al., 1992; Campana, 1999). It is of note, that the relationship between temperature and Sr:Ca ratios has not been found to be consistent in cephalopods, as laboratory experiments on *Sepia officinalis* did not find an association (Zumholz et al., 2007). Further laboratory feeding experiments on *S. officinalis* found that food type may also contribute to changes in Sr:Ca ratio levels deposited in statoliths (Zumholz et al., 2006). Nonetheless, the combination of elemental signatures with ageing of archival structures, provides an opportunity to identify ontogenetic changes (Jones et al., 2018; Liu et al., 2015) and natal areas (Andrade et al., 2020; Macdonald et al., 2021) of species which are difficult to track in their natural habitat. Additionally, multiple elements can be used to determine the extent of spatial connectivity between seasonal groups throughout their ontogeny (Green et al., 2015). The hatching and spawning locations of the *I. argentinus* seasonal groups remain uncertain or are disputed. Therefore, investigating the trace elemental signatures of individuals during early ontogeny may improve our understanding on this issue. Finally, statolith shape, which may be the result of a combination between genetic and environmental influences can also reveal differences between seasonal groups (Fang et al., 2018).

Genomic methods have been successfully used to distinguish between different populations and allow the targeting of specific fishing stocks (Bekkevold et al., 2015). This is particularly useful in mixed-stock fisheries where individual stocks cannot be easily separated (Christensen et al., 2022). This can be achieved through targeting parts of the genome where a large proportion of species have shown variation (Allendorf et al., 2010). Previous population genomic studies of *I. argentinus* structure, using traditional markers, such as microsatellites and allozymes, did not identify significant differences (Adcock et al., 1999a, 1999b). However, sampling was primarily targeting the SSS and SPS groups, with a focus on latitudes south of 45°S, during the austral winter (Roldán et al., 2014). Overall, the markers used in these studies were few and provided low resolution in terms of the possible structure of the seasonal groups of *I. argentinus.* Two methods have been accepted to increase resolution in genomic studies: i) obtaining more samples; or ii) using more markers (Waples, 1998). High-throughput sequencing, particularly restriction site-associated DNA sequencing (RADseq), provides an efficient approach of achieving the latter, with the possibility of the discovery of thousands of markers, or single nucleotide polymorphisms (SNPs) (Luikart et al., 2003). Investigation of genome-wide SNPs is a method well-suited for population genomic studies of marine species as it does not require prior information on the genome of the species of interest (Andrews et al., 2016). This is particularly useful for cephalopods which are known for having large and complex genomes (Yoshida et al., 2011). Furthermore, SNPs have been successfully used to identify fine-scale genetic differences in another valuable commercial cephalopod species*, Doryteuthis opalescens* (Cheng et al., 2020). However, the combination of microsatellite markers and statolith shape in *L. forbesii* revealed a mismatch in the results of the two methods, the shape analysis identified fine-scale population structure whereas no genetic structure was found (Sheerin et al., 2022). Therefore, combining multiple methods for population (=stock) discrimination may reveal structuring on different scales.

The combination of the phenotypic and genotypic tools is powerful as it allows for investigations across different spatio-temporal scales. Phenotypic markers usually provide information on a short-term scale *i.e.*, the duration of an individual's life, whereas genotypic markers reveal information on a long-term scale *i.e.,* evolutionary (Conover et al., 2006). Therefore, a holistic approach, that incorporates multiple phenotypic and genotypic markers is required to understand the complicated population structure of *I. argentinus.*

Dissertation Outline

The complexity of the population structure described above and the extent of the fisheries interest in this species underpins the need for a more comprehensive evaluation of *I. argentinus*. This evaluation should assess the response of seasonal groups to environmental variation and the extent of connectivity between these groups on a spatio-temporal level.

Therefore, this dissertation addressed the following questions:

1) **Are key population- and individual-level characteristics of the most abundant seasonal group of *I. argentinus*, the SPS, affected by ambient temperature?**

The first chapter of the dissertation aimed to reveal the association of population-level and individual-level parameters and ambient temperature conditions. A 20-year period was investigated, with a focus on the areas of the Patagonian Shelf utilised by the SPS during their life-cycle. This chapter used data extracted from the Falkland Islands Fisheries Department database and related temperature data from the Patagonian Shelf extracted from the E.U. Copernicus Marine Service Information. The following hypotheses were tested:

- i) Population-level characteristics - abundance is positively correlated with temperatures in the hatching areas and the migration areas for the SPS group.
- ii) Individual-level characteristics - dorsal mantle length is negatively associated with increased temperatures on the hatching grounds, conversely and proportion of mature females is positively correlated with higher temperatures.

2) **Do the seasonal spawning groups belong to one genetically interconnected metapopulation, and how are they distributed in time and space?**

The second chapter of this dissertation aimed to identify the extent of the connectivity of the different seasonal spawning groups of *I. argentinus* using genomic and ageing methods. This chapter used extensive sampling of the study species, totalling 1,878 specimens. The sampling was conducted across the Patagonian Shelf with the aim to sample a broad range of possible spawning groups. All specimens were collected throughout 2020 to avoid interannual and long-term seasonal effects that may occur if sampling is performed over several years. Ageing and associated hatching dates were determined using quantification of the statolith growth increments of 191 individuals. Genomic analysis was performed using genome-wide scans for SNPs obtained from tissue samples of the same individuals following assignment to seasonal spawning groups based on the hatching dates and statolith microstructure obtained during ageing analysis. The following hypotheses were tested:

i) Individuals collected at different times and different locations belong to different seasonal spawning groups based on their hatching dates and statolith microstructure.

ii) All seasonal spawning groups identified belong to a single genetic metapopulation.

3) **Are individual statolith core elemental signatures correlated with *I. argentinus* seasonal spawning groups and how are these signatures associated with putative hatching grounds?**

The third and final chapter aimed to investigate the early ontogeny of the seasonal spawning groups of *I. argentinus* using statolith ageing and elemental signatures from the statolith core. This chapter used a subset (n=720) of the material collected for the entire dissertation. Additionally, the ages for 101 specimens which were aged for Chapter 2 were also used in Chapter 3. Ageing estimates were performed as described in Chapter 2. The same criteria were used to assign individuals to seasonal spawning groups. Statolith core elemental signatures were obtained using laser ablation-inductively coupled plasma mass spectrometry. The single and multi-elemental signatures of 625 statolith cores of specimens were analysed. The following hypotheses were addressed:

i) Seasonal groups have significantly different single elemental signatures that correspond to the environmental conditions of putative spawning grounds of each group.

ii) Multi-elemental signatures from statolith cores can be used to differentiate between seasonal spawning groups.

Data Collection and Contribution

This dissertation contains three chapters, based on three distinct first-author manuscripts. Chapter I has been published, Chapters II has been submitted and is under review, Chapter III is in preparation to be submitted.

Chapter I:

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.

This study was based on historical data extracted from the Falkland Islands Fisheries Department database and environmental data obtained from the E.U. Copernicus Marine Service Information.

	Authors
Conceptualisation	AA, HJH
Methodology	**IC**, AA
Data Collection and Curation	**IC**
Data Analysis	**IC**
Manuscript preparation – first draft	**IC**
Manuscript preparation – review and editing	**IC**, AA, HJH

Chapter II:

Chemshirova, I., Arkhipkin, A., Shaw, P.W., and McKeown, N.J. **Integrated statolith and genomic analysis reveals high connectivity in the nektonic squid *Illex argentinus*: Implications for management of an international cephalopod fishery**. ICES Journal of Marine Science (submitted and under review ICESJMS-2023-069).

A total of 1, 878 specimens of *I. argentinus* were collected for this dissertation. Tissue samples used for DNA extraction (n=191) and corresponding statoliths for age and microstructure analysis.

	Author
Conceptualisation	**IC**, AA
Methodology	**IC**, NJM, AA
Specimen collection	**IC**
Statolith processing and ageing	**IC;** AA^{+}; ZS*
Data processing	**IC**

Statolith and ageing data processing	**IC**
Sequencing and genotyping	NJM; PWS#
Data Analysis	**IC**, NJM
Manuscript preparation – first draft	**IC**, NJM
Manuscript preparation – review and editing	**IC**, NJM, HJH, AA

*ZS, Zhanna Shcherbich FIFD, provided training and support in statolith processing.

+AA, acted as second reader for statolith age validation and provided training in statolith reading.

#PWS, provided lab facilities required for genomic studies.

Chapter III:

Chemshirova, I., Brownscombe, W., Arkhipkin, A**. Identification of population connectivity of the Argentine shortfin squid *Illex argentinus* using trace elemental signatures of early ontogeny within statolith microstructure.** *Manuscript in preparation for submission*.

From the 1,878 specimens collected, 720 statoliths were used for this chapter. NB. There was an overlap of n=101 ages used for spawning group assignment of specimens in Chapter II and Chapter III.

	Author
Conceptualisation	**IC**, AA
Methodology	**IC**, AA
Specimen collection	**IC**
Statolith processing and ageing	**IC**; AA+; ZS*
Data processing	**IC**
Statolith chemistry with LA ICP-MS	**IC**; WB$
Data Analysis	**IC;** AZ#
Manuscript preparation – first draft	**IC**
Manuscript preparation – review and editing	**IC**, HJH, AA

*ZS, Zhanna Shcherbich FIFD, provided training and support in statolith processing.

+AA, acted as second reader for statolith age validation and provided training in statolith reading.

$^\$$WB, provided training in use of LA-ICPMS equipment and post-processing of raw chemistry data.

$^\#$AZ, Alain Zuur, Highland Statistics, provided guidance in data analysis.

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Chapter I

Temperature effects on size, maturity, and abundance of the squid *Illex argentinus* (Cephalopoda, Ommastrephidae) on the Patagonian Shelf

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1.1 Abstract

Cephalopod fisheries have expanded over the last decade. They are characterised by annual uncertainty in yield quantity and quality. This is attributed to changes in environmental conditions such as temperature combined with the semelparous life strategy (one reproductive cycle during life) of the majority of squid species. We determined the effect of environmental temperature on several individual- and population-level characteristics of *Illex argentinus*, a squid species in the South Atlantic that sustains one of the largest commercial squid resources in the world. The effect of temperature on dorsal mantle length, proportion of mature females, and abundance was determined in the context of the *I. argentinus* life cycle periods, and migration patterns. This was achieved by performing 520 linear regressions using a 20-year time series of fisheries data and monthly temperature data from the key regions associated with *I. argentinus* life cycle on the Patagonian Shelf in the Southwest Atlantic. Temperatures above 17.5°C in the hatching grounds lead to smaller and less mature females in the Falkland Islands waters, during the feeding period. Larger females, a higher proportion of mature and maturing females, and a lower abundance of *I. argentinus* were found in the Falkland Islands waters when temperatures in the migration areas were higher than 13.5°C. These findings will aid in the forecasting and assessment for future fishing seasons of this vital squid resource in the Southwest Atlantic.

1.2 Introduction

In the last fifty years, cephalopod landings have increased making up ~4% of global fisheries production (FAO, 2019). This may be due to a combination of increased effort and higher population abundance as a result of reduced competition from fish, as their stocks have been depleted globally (Arkhipkin et al., 2015b; Doubleday et al., 2016; Palomares et al., 2020). Cephalopod populations are characterized by short life cycles, rapid growth, high phenotypic plasticity, and population fluctuations reminiscent of "desert locusts" (Rodhouse, 2001). This makes them one of the most lucrative, yet challenging fisheries to manage (Arkhipkin et al., 2021). Moreover, cephalopod fisheries are not consistent. In the Falkland Islands fishery, the percentage of squid (*Illex argentinus* and *Doryteuthis gahi*) in the total catch varies annually from 20.5% in 2009 to as much as 83.9% in 2015 (Falkland Islands Government, 2021; Laptikhovsky et al., 2010). These fluctuations may be attributed to a combination of squid life history characteristics and changes in environmental conditions (Boyle and Rodhouse, 2005; Rodhouse et al., 2014).

Various abiotic factors may contribute to the fluctuations of squid populations worldwide. For example, increasing sea surface temperature during the hatching period was positively correlated with growth during the early life stages and accelerated maturation (Forsythe, 2004; Jackson et al., 1997). Recruitment abundance was positively correlated with temperature in *Illex illecebrosus* (Dawe, 2000), along with a shortened life cycle in *Dosidicus gigas* (Arkhipkin et al., 2015a; Hoving et al., 2013). *Illex argentinus* population abundance was primarily investigated in terms of temperatures on the hatching grounds in the year preceding the fishery, and a negative relationship between abundance and temperature was identified (Waluda et al., 1999). It should be noted that the opposite trend over a longer time period was also reported (Nigmatullin et al., 2004). Individuals that hatch during warmer seasons reach smaller terminal sizes as a trade-off for early onset of maturation, compared with their counterparts developing in colder seasons in both *Illex coindetii* and *D. gigas* (Arkhipkin et al., 2000; Keyl et al., 2011). Sex-specific responses to temperature were reported with males exhibiting plasticity in terms of size and growth and females in terms of somatic condition in *Sepioteuthis australis* (Pecl et al., 2004a).

The Argentine short-fin squid *(I. argentinus)* is a neritic-oceanic species, occurring on the shelf and slope of the Southwest Atlantic, from 22°S to 55°S, with maximum abundance recorded between 40°S and 52°S (Rodhouse et al., 2013). This abundant squid is the subject of one of the largest cephalopod fisheries in the world with total annual catch of > 1 million tonnes in productive years, such as 2015 (FAO, 2020). *Illex argentinus* is a medium-size squid with adults attaining 30-35 cm dorsal mantle length (DML) and exhibits sexual dimorphism with females growing larger than males (Arkhipkin, 1993; Haimovici et al., 1998). *Illex argentinus* has a complex population structure, including two major annual groups based on differences in their respective spawning seasons and locations: i) winter-spawning group and ii) summer-spawning group (Arkhipkin, 1990; Hatanaka, 1986; Rodhouse and Hatfield, 1990). The winter-spawning group was further divided into the Bonaerensis North Patagonian Stock (BNPS) and South Patagonian Stock (SPS), which have different feeding grounds and reach different adult sizes (Brunetti, 1988). SPS is the most abundant stock that is found south of 44°S, and the only one where individuals migrate to the southern part of the Patagonian Shelf to feed (Hatanaka, 1988). Following the hatching period (July until September) the juveniles migrate from their hatching grounds, on the outer Patagonian Shelf, towards the inner areas of the shelf from September until December. The feeding migration is performed by immature and maturing squid moving southwards along the Patagonian Shelf from January until April, to take advantage of the feeding opportunities available, reaching as far as 51°S (Haimovici et al., 1998; Hatanaka, 1988). The spawning migration north begins in late March/early April and follows the slope of the Patagonian Shelf (Arkhipkin, 1993). The exact location of the spawning grounds remains unclear, two possible areas have been proposed: i) South Brazil area, on the outer shelf and shelf break north of 35°S (Arkhipkin, 2013; Arkhipkin et al., 2015b; Haimovici and Pérez, 1990); and ii) Patagonian area, between 45°S and 48°S, on the outer shelf and shelf break (Haimovici et al., 1998; Torres Alberto et al., 2020).

The SPS spend their life cycle in one of the most oceanographically dynamic areas of the Southwest Atlantic. These dynamics are driven by the confluence between the warm, southward-flowing, Brazil Current; and the cold, nutrient-rich, Falkland Current, a branch of the Antarctic Circumpolar Current, flowing north (Fig. 1A) (Campos et al., 1995; Waluda et al., 2008). The Brazil-Falkland Confluence Zone varies seasonally, the mean position is estimated to be near 38°S and has been associated with seasonal variation in sea surface temperature gradients (Acha et al., 2004; de Souza et al., 2019). The gradients are particularly pronounced during the austral winter when the Falkland Current is the coldest during the spawning period of the SPS stock (Bouali et al., 2017). As the juveniles migrate south towards the feeding grounds during austral summer, they encounter lower temperatures compared with the hatching grounds. During the pre-spawning migration, the SPS stock moves from the cold and productive feeding grounds to the warm low-productivity spawning area north of 42°S (Arkhipkin, 2013). This means that every period of the SPS stock life history has the potential to be impacted by changes in sea surface temperature, which may, in turn, alter population abundance, maturity, size, and growth of individuals.

The present study aims to quantify the impact of temperature on key population parameters of the SPS stock of *I. argentinus* such as squid size and maturation. Additionally, we investigate how the abundance and population density depend on temperature conditions. The hypothesis that increased temperatures in the hatching grounds of *I. argentinus* will affect individual-level characteristics, and lead to smaller and more mature adults in Falkland Islands waters during the fishing season was tested. Additionally, we investigate whether increased temperatures may positively affect population-level characteristics, such as abundance. Each of these factors has the potential to influence the productivity of the fishery, as they determine the abundance and size of squid available to fishermen.

1.3 Material and methods

1.3.1 Data Collection

1.3.1.1 Biological Data

The biological data used for the present study was obtained from the Falkland Islands Fisheries Department Observer program, from the commercial monitoring of the jigging fleet. The dataset covered the years from 1999 until 2019, excluding the very poor 2009 season when only two vessels fished in the Falkland Islands Interim Conservation and Management Zone (FICZ) for one night and no observers went on board. Every night 200 squid were collected at random by the observer on board and sampled, measuring DML to the nearest 0.5 cm, and determining sex and maturity using the maturity scale by Lipinski (1979). Arkhipkin (2013) determined that the SPS stock arrives at their feeding grounds in the northern part of the FICZ in February and remains in the area until mid-April. As observer coverage for the duration of the season was not consistent across the years, the period of maximum coverage from 15 until 31 March was selected for every year, resulting in a dataset of 49, 022 specimens. Additionally, this time period was considered the optimal window for sampling only the SPS cohort, in order to avoid sampling multiple cohorts with different characteristics. In order to reduce noise we used the integer values for the DML. Using these data, the mean DML was calculated for every year, separately for males and females. The onset of maturation in females is defined as their transition from stage 2 to stage 3 (Lipinski, 1979). Therefore, we calculated the proportion of mature and maturing females (stages 3 to 5) from the total number of females sampled. Catch per unit effort (CPUE) was used as a proxy for abundance of *I. argentinus* (Waluda et al., 1999). CPUE (mt/h) was calculated using catch report data from the jigging vessels in the FICZ. Catch data for the period of 15-31 March was used for the calculation of CPUE (mt/h) and calculated for every year from 1999 until 2019.

1.3.1.2 Environmental Data

Sea water potential temperature data was obtained from the E.U. Copernicus Marine Service Information, specifically the Global Ocean Reanalysis and the Global Ocean Analysis and Forecast models, where sea surface temperature was an input for model predictions. The temperature dataset covered the years between 1998 and 2019. Temperature data layer from the surface depth level across the entire area studied was used. Predictions from the whole area of a box were used for boxes located entirely off the 200 m depth contour of the Patagonian Shelf (*e.g.,* H_33S_49W). In the case of boxes located partially on the 200 m depth contour (*e.g.,* MO_40S_57W), only temperature predictions for areas on the shelf were used *i.e.,* >200 m. A total of 26 boxes were selected, with spatial coordinates covering the region from 32°S to 51°S (Fig. 1B). The boxes between 32°S and 36°S were defined as the hatching grounds (H) of *I. argentinus* as identified by previous studies (Waluda et al., 2001, 1999). Juvenile period was considered to be from September until December. This was considered to entail the migration of the juveniles to the north of the Patagonian Shelf (*e.g.*, J_36S_55W) prior to the subsequent feeding migration south. Temperature data boxes south of 38˚S were divided into the possible feeding migration routes, which were selected as follows: Migration Offshore (MO) along the outer shelf and shelf edge, and Migration Inshore (MI) which covered the inner shelf and coastal areas (Fig. 1B). The mean monthly temperature was extracted at 1/12° (~8 km) grid resolution, per box, per year, with a total of 3, 253, 046 data points. Subsequently, the mean temperature for each box was calculated per month for every year to reduce the noise of the data. A final dataset of 6, 575 data points was compiled. The months were selected based on the aforementioned life cycle of the SPS stock (Arkhipkin, 2013; Hatanaka, 1986); therefore, May and June were not used for analysis. The months of July and August were considered to indicate the hatching period, September until December were deemed to be the juvenile period, and the feeding period was considered to last from January until April. The biological and temperature data were subsequently compiled whilst accounting for the life history of the SPS. Temperature data for the months of July until December of the previous year was used to account for the fact that individuals sampled in March would have encountered temperatures from July until December of the previous year during their juvenile period, as opposed to the year of sampling.

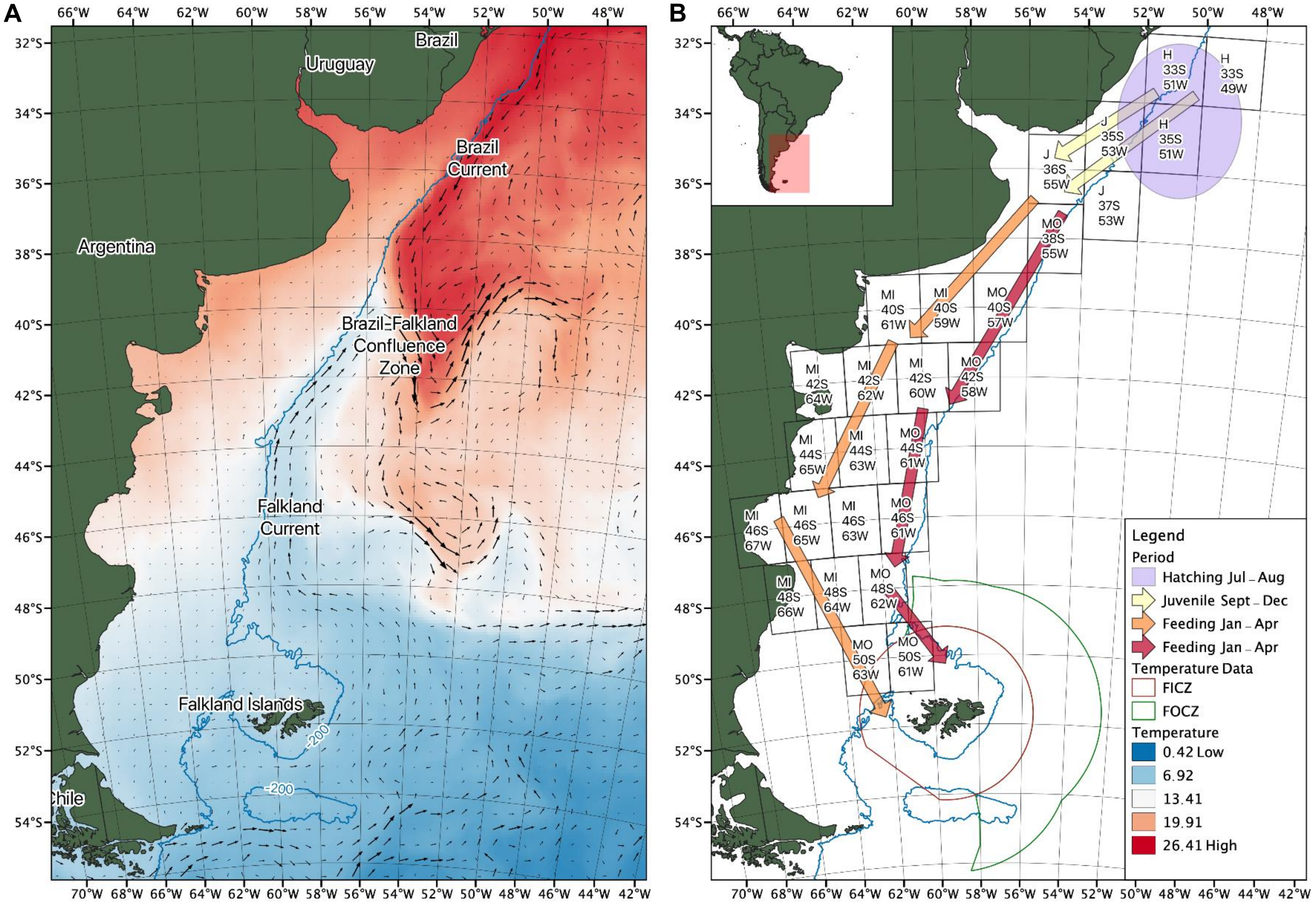

Figure 1. (A) Environmental conditions in the study area showing temperature and the western boundary current system in March 2019. (B) Temperature data boxes used for analysis and life cycle periods of *I. argentinus*. H, hatching grounds; J, juvenile grounds; MO, Migration Offshore; MI, Migration Inshore; FICZ, Falkland Islands Interim Conservation and Management Zone; FOCZ, Falkland Islands Outer Conservation Zone.

1.3.2 Statistical Analysis

Data exploration was performed following Zuur et al. (2010). As an additional step an ANOVA was performed on the raw data to determine if there were significant differences in the response variables between years, Year was used as the categorical variable. This tested for differences of DML between years and allowed for subsequent comparisons between the mean DML. Significant differences between mean DML in different years were identified. Subsequently, a Tukey's Honest Significant Difference test for the multiple comparisons was performed. More than 190 pairwise comparisons were carried out and no significant differences were identified for 31 of them. This was likely due to the way the p-values were calculated, as they were determined by the difference between groups and the sample size. Therefore, when years with a small sample size (*e.g.* 1999; n=647) were compared with years with a large sample size (*e.g.* 2006; n=2636), they were not found to be significantly different from one another. The same procedure was carried out to determine the differences in CPUE between years. From the 190 pairwise comparisons performed, 15 were not found to be significantly different. In order to show that there was a significant difference between the proportion of mature and maturing females present in different years a test of equal or given proportions was used. This showed that there were significant differences between the years of study.

Subsequently, the mean DML and abundance (CPUE) for the period of 15 - 31 March were calculated per year from 1999 until 2019 to use in further analysis. All statistical analysis was performed using R 3.6.1 (R Core Team, 2022). The number of mature and maturing females and the total number of females sampled was calculated for every year to use in subsequent analysis. The following packages were used: *tidyverse* (Wickham, 2019) for compilation of the dataset and visualisation of data; *broom* (Robinson and Hayes, 2020) for summarising linear model outputs; *measurements* (Birk, 2019) for converting spatial coordinates into decimal degrees; *fs* (Hester et al., 2021) for importing and cleaning of multiple temperature data files; *forecast* (Hyndman et al., 2019) for visualisation of diagnostic plots; *jtools* (Long, 2020) for visualisation of generalised linear models; and *patchwork* (Pedersen, 2020) for combining multiple graphs into figures. Spatial data was visualised using QGIS 3.14 (QGIS Development Team, 2022).

1.3.2.1 Linear Models

Linear models were used to determine whether mean temperature ($\bar{T}_i$; °C) in specific boxes affected the mean DML (Appendix A.1) and abundance (CPUE; App. A.2) of individuals sampled in the FICZ fishing grounds. The temperature data of neighbouring boxes were likely to be spatially and temporally auto-correlated. Therefore, the linear regressions were performed per box, per month, per sex. This resulted in a total of 520 models for each of the response variables. Model suitability was assessed using residual diagnostic plots as per Zuur and Ieno (2016). The residuals were assessed for temporal dependency using autocorrelation functions (ACF).

A linear model predicting mean DML as a function of abundance (CPUE) was used to determine if there was a density dependence effect on DML. Due to the sexual dimorphism of *I. argentinus* the linear model (App. A.3) was performed separately for each sex.

1.3.2.2 Generalised Linear Models (GLM)

To model the mature and maturing females as a function of $\bar{T_i}$, a quasibinomial GLM was applied using a logistic link function (Eq. 1). This allowed for the binomial distribution to be applied, as proportion data was used. This was expressed as a number of mature and maturing females ($matF_i$) out of the total number of females ($totF_i$) sampled. The variance in this model was overdispersed, thus a correction was applied using the quasi-likelihood method and an overdispersion parameter (ϕ) was introduced. Model suitability was assessed using diagnostic plots of Pearson residuals, temporal autocorrelation was examined using ACF.

$$matF_i \sim Binomial(\pi_i, totF_i)$$

$$E[matF_i] = totF_i \times \pi_i$$

$$var[matF_i] = \phi(totF_i \times \pi_i \times (1 - \pi_i))$$

$$logit(\pi_i) = \beta_1 + \beta_2 \times \bar{T}_i$$

(Eq. 1)

1.4 Results

1.4.1 Mean DML

Temperatures in the hatching grounds during the peak hatching period for *I. argentinus* (August until September), had no significant effect on the mean DML of males and females in the FICZ during the fishing season; however, a general negative trend was observed (*Fig. 2A, Box H_33S_51W, July,* $p = 0.157$, $F_{1,18} = 2.183$; *Fig. 2B, Box H_33S_51W, July,* $p = 0.192$, $F_{1,18}=1.839$; *Appendix B.1*). Throughout the juvenile period, temperatures above 17.5°C in the putative juvenile grounds were associated with reduced mean DML of males and females sampled in the FICZ, during the fishing season by ~2 cm (*Box J_35S_53W, November, Female,* $p < 0.05$, $F_{1,18} = 5.119$; *App. B.2B*). During the feeding migration period, a significant negative relationship between temperature in the hatching grounds and mean DML for females (*Box*

H_33S_49W, January, $p < 0.05$, $F_{1,18} = 6.802$; *App. B2.A; App. C1*) and males (*Box H_33S_51W, January,* $p < 0.05$, $F_{1,18} = 6.019$; *App. B.2A; App. C3*) was found. These trends were primarily driven by the mean DML in 2015, which was also the year with the highest abundance (4.11 mt/h).

No relationship was identified between temperatures in the offshore migration areas (MO) during the hatching period and the mean DML observed in the FICZ in March. During the juvenile period, a significant negative relationship between temperature in the northern part of the feeding migration areas and mean DML of males was recorded (*Box MO_38S_55W, October,* $p < 0.05$, $F_{1,18} = 5.355$; *App. B2.C; App. C.4*). A positive relationship was observed between temperature in the offshore migration areas during the feeding period and mean DML of females (*Fig. 2D; Box MO_48S_62W, February,* $p < 0.05$, $F_{1,18} = 5.275$; *App. B2.C*). This trend was primarily driven by the mean DML in 2004, which also had the lowest abundance (0.12 mt/h).

No significant relationship between temperature and mean DML for the inshore feeding migration areas (MI) was found during any of the life cycle periods investigated. However, a positive trend was identified for females during the feeding period (*Fig 2C; Box MI_44S_63W, January,* $p = 0.142$, $F_{1,18} = 2.294$; *App. B.3*).

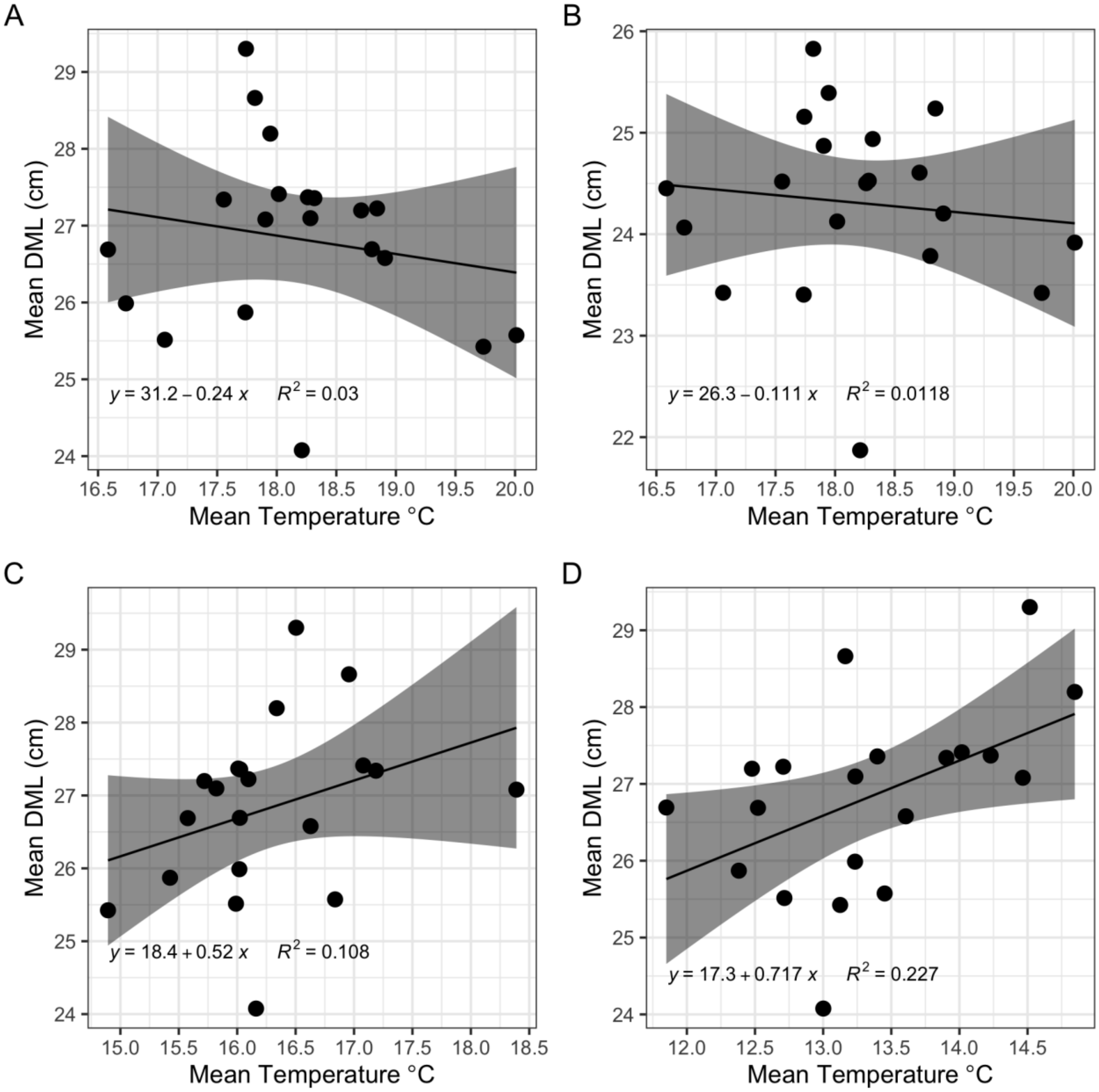

Figure 2. Mean DML as a function of mean temperature. Hatching grounds in July, during the hatching period, (Box H_33S_51W) for (A) females and (B) males. Feeding migration areas (C) inshore in January (Box MI_44S_63W) and (D) offshore (Box MO_48S_62W) in February, during the feeding period, for females. The prediction (solid line) and 95% confidence limits (shaded) from the linear model are shown. DML, dorsal mantle length.

1.4.2 Proportion of mature females

Temperatures above 19˚C in the hatching grounds during the hatching period lead to a significantly lower proportion of mature females being recorded during the fishing season in the Falkland zones (*Fig. 3A; Box H_33S_51W, July, $p < 0.05$; App. B.4A*). Increasing temperatures during the juvenile period in the same area also resulted in a significantly lower proportion of mature females observed during the fishing season (*Fig. 3B; Box H_35S_51W, October, $p < 0.05$;*

App. B.4A). Temperatures above 13˚C in the putative juvenile grounds, during the juvenile period a significant negative trend was identified (*Box J_37S_53W, October, p < 0.05; App. B.4B; App. C.5*).

During the feeding period, higher temperatures (15.5°C and above) in the inshore feeding migration (MI) areas resulted in a significantly higher proportion of mature females (>0.6) in the FICZ (*Fig. 3C; MI_46S_63W, February, p < 0.05; App. B.4C; App. C.6*). Similarly higher temperatures (> 14°C) in the offshore feeding migration areas (MO) were significantly associated with a higher proportion of mature females, >0.65 (*Fig 3D; MO_48S_62W, February, p < 0.05, App. B.4C*). During the feeding migration period, no significant relationships between mean temperature in the hatching grounds and proportion of mature females were found, although a general negative trend was identified (*H_33S_51W, February, p = 0.242; App. B.5*).

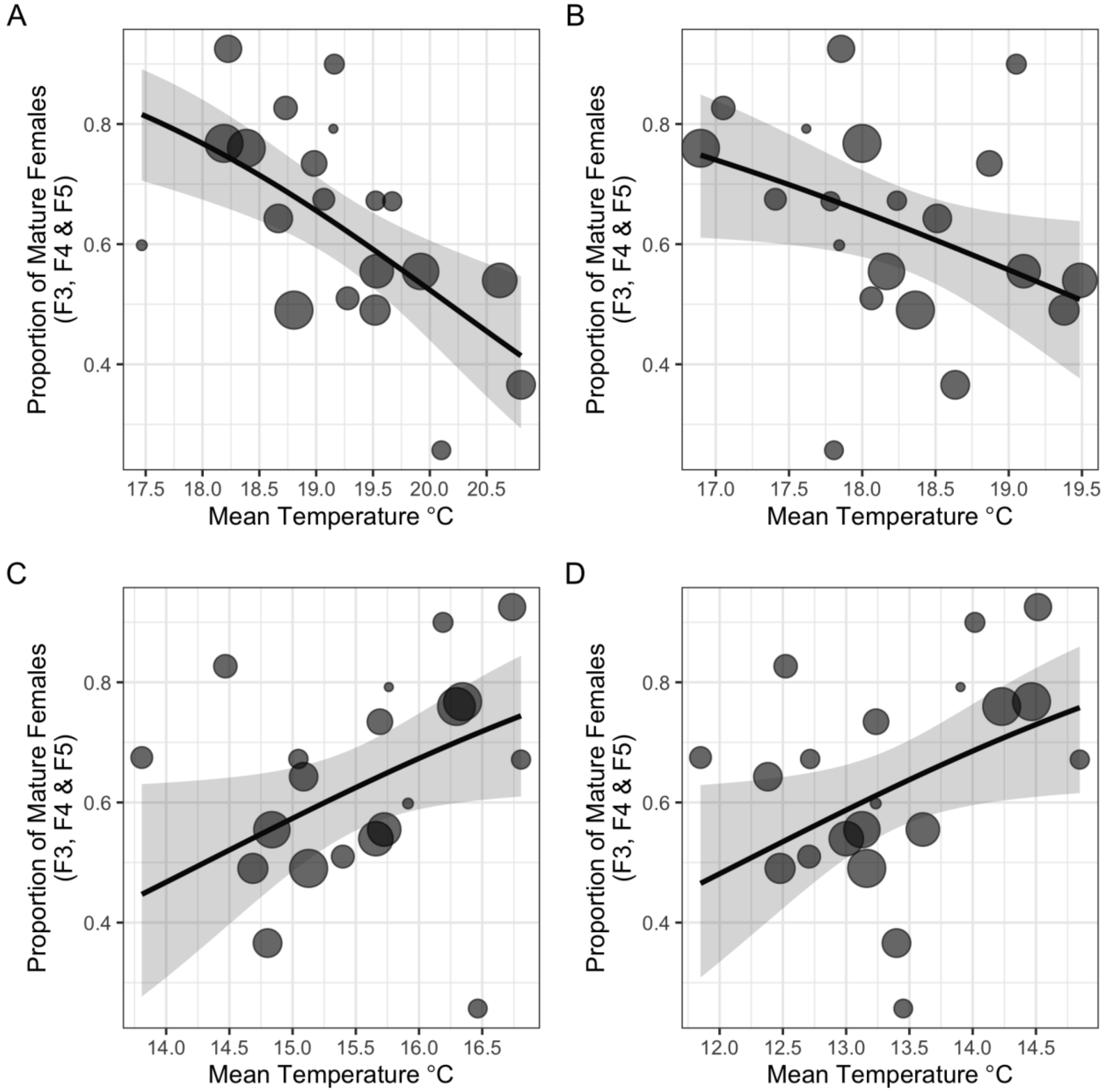

Figure 3. Proportion of mature females (F3, F4 & F5) as a function of mean temperature. Temperature in the hatching grounds in Boxes (A) H_33S_49W in July, during the hatching period, and (B) Box H_33S_51W during the juvenile period, in October. Temperatures and proportion of mature females during the feeding period (February) in the migration path areas for (C) Box MI_46S_63W and (D) Box MO_48S_62W. Predictions of quasibinomial generalised linear models (solid line) are shown with 95% confidence limits (shaded). Size of data points indicates the total number of females sampled, with larger data points representing a greater total number of females.

1.4.3 Abundance

Temperatures in the hatching grounds, during the hatching period, did not have a significant effect on *I. argentinus* abundance during the subsequent fishing season; however, a generally positive trend was identified (*Fig. 4A; H_33S_49W, July,* $p = 0.414$, $F_{1,18} = 0.699$, *App. B.6A*). It is of note that the box closest to the Brazil-Falkland Confluence Zone, shows the opposite pattern to the other hatching grounds boxes for the same time period (*Fig. 4B; July, H_35S_51W, $p = 0.553$, $F_{1,18} = 0.366$, App. B.6A*). Temperatures above 21.5°C in the juvenile grounds during the juvenile (*Fig. 4C; J_35S_53W, December, $p < 0.05$, $F_{1,18} = 6.415$; App. B.7B; App. C7*) and feeding migration period (*Fig. 4D; H_33S_49W, January, $p < 0.05$, $F_{1,18} = 9.220$; App. B.7A*) had a significantly positive effect on the *I. argentinus* abundance.

No significant trend was identified between temperatures in both migration areas, MO and MI, during the hatching period and *I. argentinus* abundance in the Falkland zones during the fishing season. No relationship was identified between temperature and abundance during the juvenile period for the same areas. Considering the migration areas during the feeding period, a significant negative relationship was observed between temperature and abundance for the MI (*Fig. 4E; MI_44S_65W, January, $p < 0.05$, $F_{1,18} = 4.661$; App. B.7C; App. C8*) and MO (*Fig. 4F; MO_46S_61W, February, $p < 0.05$, $F_{1,18} = 4.532$; App. B.7C; App. C8*) areas. This pattern appeared to be driven by data from 2004, which also had the largest mean DML values (Females, 28 cm; Males, 25 cm). Considering density dependence across the 20-year time period, females showed decreasing mean DML with increasing abundance (*Fig. 5A; $p < 0.05$, $F_{1,18} = 5.358$, App. B.8*). No pattern was identified for males. However, a general negative trend was present (*Fig. 5B; $p = 0.111$, $F_{1,18} = 2.815$; App. B.8*).

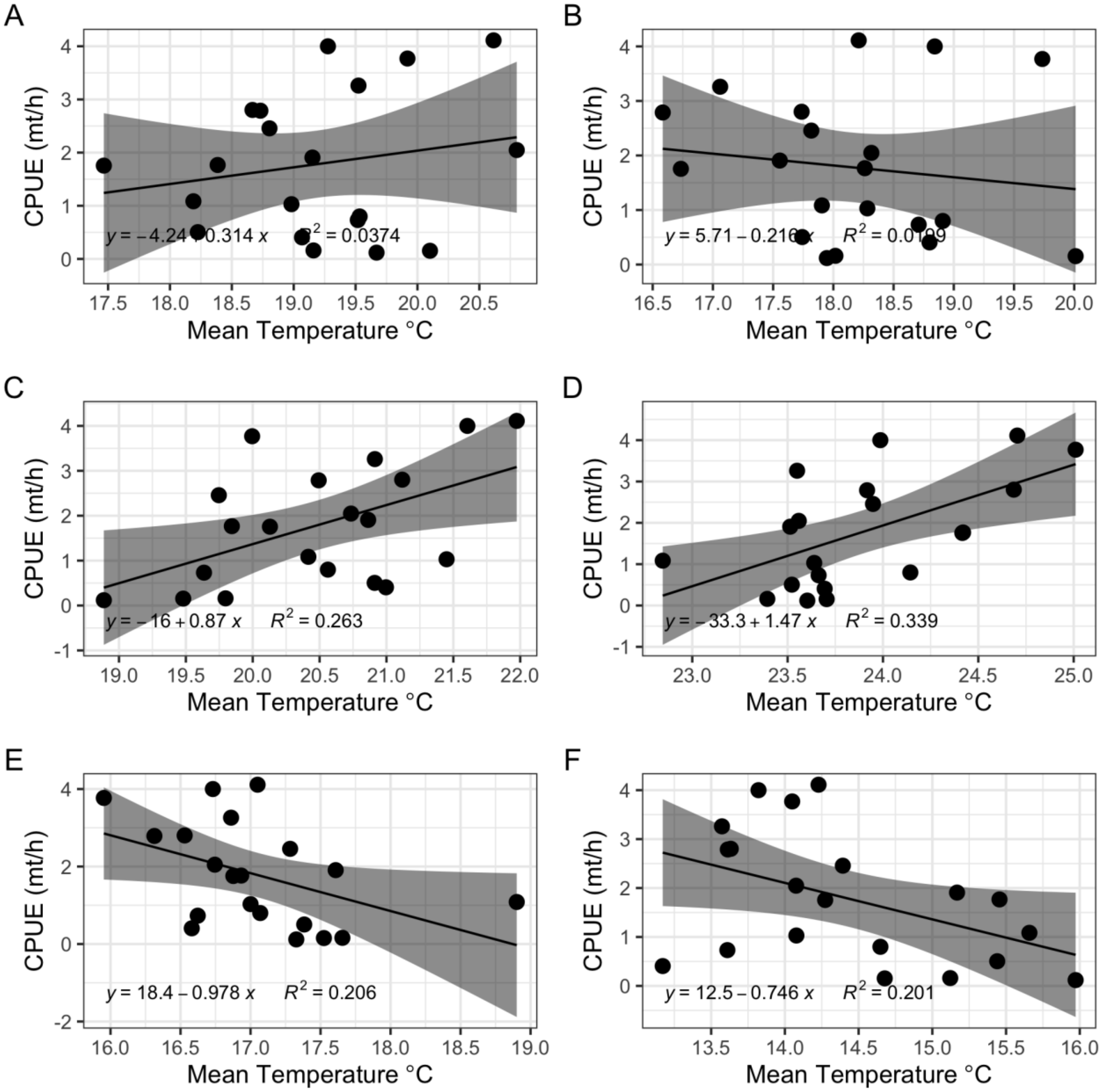

Figure 4. CPUE as a function of mean temperature. Hatching grounds temperatures in Boxes (A) H_33S_49W and (B) H_35S_51W in July, during the hatching period. Juvenile grounds temperatures in Box (C) J_35S_53W during the juvenile period, in December, and (D) H_33S_49W during the feeding period in January. Feeding migration temperatures in Boxes (E) MI_44S_65W (F) MO_46S_61W during the feeding period in January and February respectively. The predictions (solid line) and 95% confidence limits (shaded) from a linear model are shown.

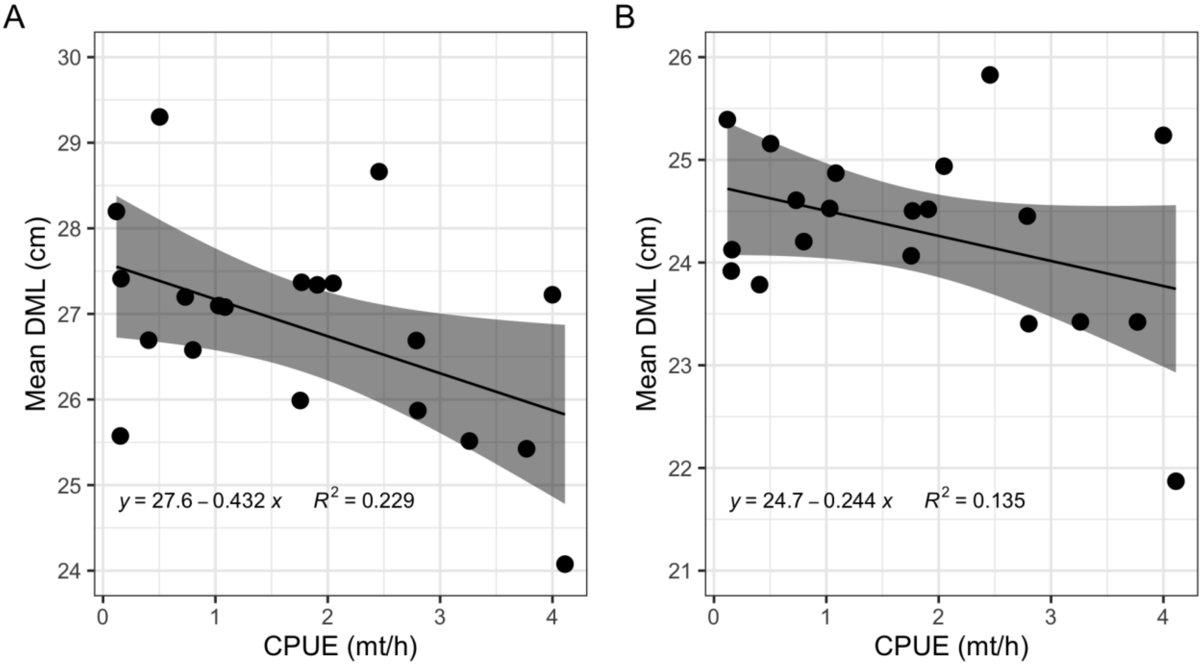

Figure 5. CPUE as a function of mean DML of (A) females and (B) males. The prediction (solid line) and 95% confidence limits (shaded) from the linear model are shown.

1.5 Discussion

The results of this study revealed that mean DML, the proportion of mature and maturing females, and abundance can be influenced by changing temperatures on the Patagonian Shelf. The characteristics recorded were the cumulative result of the temperature the SPS stock encountered during the different periods of its life cycle. No significant relationship between high water temperatures (> 17.5°C) in the hatching grounds and mean DML was identified. However, an overall negative effect on the mean DML and proportion of mature and maturing females throughout the *I. argentinus* life cycle was evident. Increasing temperatures on the hatching grounds during the juvenile and feeding periods also had a negative effect on abundance. Temperatures in the migration areas during the feeding period influenced both mean DML and proportion of mature and maturing females in a positive manner, whereas a negative effect was observed with respect to abundance.

1.5.1 Mean DML

High water temperatures (> 17.5°C) on the hatching grounds of *I. argentinus* during the hatching period lead to smaller mean DML for females during the subsequent fishing season. This is a well-established relationship in cephalopods, as higher temperatures have been shown to accelerate embryonic development and reduce hatchling size in various species, both in laboratory and field conditions (*I. coindetii*, Villanueva et al., 2011; *I. illecebrosus* O'Dor et al., 1982; *Loligo forbesii*, Gowland et al., 2002; *Uroteuthis noctiluca*, Ceriola and Jackson, 2010). Subsequently, small hatchlings could lead to small adults, which was predicted for *S. australis*, where small differences in growth during the early development of individuals were further increased over the course of the life cycle (Pecl et al., 2004b). It is of note, that large hatchlings have a generally higher chance of survival, as they are less vulnerable to predators and have better feeding opportunities (Boletzky, 1994; Vidal and PaulDiMarco, 2002). The present study shows that high temperatures in the hatching and putative juvenile grounds during the juvenile and feeding periods of *I. argentinus* lead to reduced mean DML in females. These findings indicate that temperatures in these areas are affecting subsequent life stages of *I. argentinus*. However, in order to determine the mechanisms behind this outcome, additional studies on how the oceanography in the hatching grounds is connected with the subsequent life cycle and migration patterns of *I. argentinus* are required. Further investigation on how the phenology of *I. argentinus* may be affected by temperature would also be valuable, as it has been shown that higher temperatures induced migration earlier than usual in *L. forbesii* (Sims et al. 2001).

When *I. argentinus* females encountered higher temperatures (> 15°C) in the offshore migration areas during the feeding period a positive relationship between temperature and mean DML was identified. This is primarily due to the fact that their feeding migration coincides with peak zooplankton abundance, and euphausiids are more dominant in the austral summer (Sabatini and Colombo, 2001). *Illex argentinus* are voracious predators, that feed primarily on crustaceans, including euphausiids and the amphipod *Themisto gaudichaudii* (Ivanovic, 2010; Mouat et al., 2001). Therefore, provided with sufficient resources, females were able to grow to larger sizes than males, regardless of temperature. These trends were not evident for males where the trends observed were primarily driven by outliers. This is likely due to the differing maturation and growth rates of males and females. Particularly as by March, when sampling took place, the males would have completed their maturation; therefore, it will be more challenging to disentangle the effects of temperature on their growth and maturation (Arkhipkin, 1990).

1.5.2 Proportion of mature females

The proportion of mature females observed during the fishing season was negatively correlated with the temperature in the hatching grounds during the *I. argentinus* hatching period. This is contrary to expectations, as high temperatures generally accelerate maturation in cephalopods; however, this generally means that the adult period of the life cycle is extended (Rodhouse et al., 2014). For example, *S. lessoniana* individuals from colder areas matured 17-20% slower than their warm area counterparts (Jackson and Moltschaniwskyj, 2002). Modelling the life history of *I. argentinus* showed that increased fishing pressure would also lead to earlier maturation (Murphy and Rodhouse, 1999). However, the model did not account for environmental variability or prey availability. Conversely, the present study may miss the effect that fishing intensity may have on the target species. Recent findings suggest that in addition to temperature, feeding may govern maturation in *I. argentinus*. Females primarily use direct feeding (and not energy storage) during the early stages of maturation (Lin et al., 2019). Therefore, it is possible that in years with insufficient resources the onset of maturation is delayed, resulting in lower proportions of mature females. Conversely, experimental data on *I. illecebrosus* have shown that starvation of females (20-25 cm DML) may induce maturation (Rowe and Mangold, 1975). This highlights the need to determine how changes in feeding opportunities throughout the *I. argentinus* life cycle periods affect maturation rates.

High temperatures (> 15.5°C) in the migration areas during the feeding period led to a higher proportion of mature females (>0.6) recorded during the fishing season. Similar patterns were previously observed for *Uroteuthis edulis* in the Tsushima Strait, where squid that migrated through the warmer waters exhibited accelerated maturation, and individuals that migrated via a different route with colder water temperatures remained immature (Yamaguchi et al., 2019). *Illex argentinus* proportion of mature females was previously shown to decrease at temperatures greater than 14°C, with the peak of mature females being recorded during the feeding period (February) (Sacau et al., 2005). Therefore, it is likely that the present study is looking at the snapshot of how population maturity develops over time. The Sacau et al., (2005) study investigated a different time period, ranging from 1988 to 2003, which is only a 4-year overlap with the present study. Additionally, they pooled data from the entire fishing season, which may also contribute to the differing results.

1.5.3 Abundance

High temperature (> 21.5°C) in all but one of the hatching ground boxes had a positive effect on *I. argentinus* abundance during the fishing season. The positive effect of temperature may be because squid that hatch in warmer areas tend to be smaller and can disperse further south during their juvenile period due to their smaller size allowing for more efficient transport by ocean currents (Villanueva et al., 2016). Therefore, the subsequent feeding migration may reach further south into the Falkland Islands zones, enabling the jigging fleet to catch a higher proportion of the SPS cohort. Positive sea surface temperature anomalies in the hatching grounds between August and October have been shown to successfully predict high abundance years for the SPS stock (Nigmatullin et al., 2004). In contrast, low abundance years were signified by the dominance of frontal waters (postulated to be Brazil-Falkland Current confluence) on the hatching grounds (Waluda et al., 2001). The negative trend observed for Box H_35S_51W in July is consistent with previous work in the area (Waluda et al., 1999). This may be due to that area of the hatching grounds having the greatest temperature and depth gradient and being the closest to the Brazil-Falkland Current confluence. Therefore, in years when the Falkland Current is stronger, it is more likely to have a negative effect on *I. argentinus* abundance as it may alter the usual feeding migration pattern leading to individuals migrating towards the Argentine Exclusive Economic Zone instead (Vanyushin and Barkanova, 2005).

Abundance of *I. argentinus* during the fishing season was negatively affected by high temperatures (> 13.5°C) in the migration areas during the feeding period. This is in contrast to previous findings which showed a positive trend for temperatures between 10 and 15°C (Chiu et al., 2017). This may be due to the fact that the data used were from the high seas and abundance data covered the whole fishing season as opposed to just March as in the present study, hence different seasonal spawning groups may have been pooled together. Previously, the highest abundance of *I. argentinus* has been recorded at temperatures between 7 and 15°C (Sacau et al., 2005). This relationship was non-linear, whereas the present study is focusing on areas where the temperature is greater than 15°C, this may be why a negative trend was observed. Another reason for this result may be due to the effect of density dependence. The negative trend found in the present study is primarily driven by the data from 2004, which has the lowest abundance (0.12 mt/h) and the largest mean DML. This could mean that unfavourable climatic conditions in that year led to a low abundance of individuals allowing them to grow to larger sizes. Unfavourable temperatures may have further exaggerated the density dependence effect. It may be that if resources are scarce, cannibalism may occur among cephalopods as a population regulation mechanism leading to population cycling (Claessen et al., 2004; Ibáñez and Keyl, 2010). Therefore, it is possible that cannibalism, combined with widely varying environmental conditions results in unpredictable population cycling, which is a prominent characteristic previously observed in *I. argentinus*.

The present study established the fundamental relationships between environmental temperature during the different periods of the life cycle of *I. argentinus* and the individual- and population-level characteristics of the SPS stock. This information can be used to make predictions for subsequent fishing seasons. Understanding the impact of abiotic factors such as temperature on squid growth and maturation is crucial for present-day cephalopod fisheries. This would inform predictions of the size and quality of squid available for human consumption (Mouritsen and Styrbæk, 2018). Investigating the biological characteristics of stocks that have encountered different environmental conditions may allow us to elucidate the mechanisms behind the observed temporal variation in fisheries yield of *I. argentinus*, a valuable squid species. Furthermore, the established relationships can be used to inform how the SPS stock may respond to future changes in climate. These findings lay the groundwork for more specific temperature life history investigations, that can be extracted using statolith ageing and trace element analysis. This may prove a valuable tool for assessment of the current stock composition of *I. argentinus*.

CRediT authorship contribution statement

Irina Chemshirova: Data Curation, Formal Analysis, Visualisation, Writing – original draft, Methodology. **Henk-Jan Hoving**: Supervision, Writing – review & editing, Resources. **Alexander Arkhipkin:** Conceptualisation, Supervision, Resources, Funding Acquisition, Methodology, Writing – review & editing.

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Chapter II

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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2.1 Abstract

The neritic-oceanic squid *Illex argentinus* supports one of the largest fisheries in the Southwest Atlantic. It is characterised 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 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.

2.2 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 fifty 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., 2017, 2015). 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 (EEZ) of multiple states and international waters (Arkhipkin et al., 2021, 2022), they may be exploited by multiple national fleets and at various life stages. The Argentine short-fin squid (*Illex argentinus*) is a nektonic cephalopod that supports one of the largest fisheries in the Southwest Atlantic, annual catches reached a peak over 1 million tonnes in 2015 (FAO, 2020) and up to 16.4% of global squid catch in 2020 (FAO, 2022). Furthermore, as this species is targeted by multiple international stakeholders, namely Taiwan; Republic of Korea; Spain; Argentina; People's Republic of China it is of a global importance (Arkhipkin et al., 2015). It is a neritic-oceanic species, distributed between 22°S and 55°S along the Patagonian Shelf and slope (Rodhouse et al., 2013). The population structure of *I. argentinus* appears to be complex. There may be up to four distinct seasonal spawning groups (Brunetti, 1988; Hatanaka, 1988) . The winter-spawning group has been identified as the largest and spawning occurs in austral winter (June-August) (Rodhouse et al., 2013). This group has been 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°S - 38°S to 51°S to feed during the austral summer (November - March) and subsequently return north to spawn in early April (Arkhipkin, 1993). 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 (ESPS) and late-maturing (LSPS) groups (Falkland Islands Government, 2021). The ESPS group has been primarily observed in the north and north-eastern areas of the Falkland Islands Conservation Zones (FICZ/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 Spring Spawning Stock (SpSS) are hypothesised to spawn in coastal waters, such as the San Matías Gulf (41°S to 42°S) (Crespi-Abril et al., 2013, 2008; Crespi-Abril and Barón, 2012), and may use the high seas areas between 45°S and 47°S during austral summer. The Summer Spawning Stock (SSS) is believed to spawn on the shelf between 42°S 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 strategies (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 number of loci (Adcock et al., 1999a, 1999b). Furthermore, these studies did not investigate individual membership to the different spawning groups 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). 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 (McKeown et al., 2020; Mullins et al., 2018).

Another tool for 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 allows 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 reveal fisheries management units (=stocks) within 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, allow 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*. Firstly, 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. Secondly, genome-wide SNP analysis of individuals assigned to these spawning groups was used to investigate neutral and non-neutral genetic structuring among these 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°S - 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, thus 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.

2.3 Materials and methods

2.3.1 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 program, 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 x 2 cm piece) was cut from the mantle, approximately 1/3 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 (Fig. 1; Table 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.

2.3.2 Statolith preparation and ageing

One statolith from each individual squid was mounted concave side up using thermoplastic resin, CrystalBond^{TM} 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^{TM} 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 Olympus BX51 compound microscope at x500 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 one 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 2nd degree polynomial locally weighted regression (loess) was performed with a span of 0.4 per spawning group in order to visualise the width of the growth increments.

2.3.2.1 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 visualisation of data; *measurements* (Birk, 2019) for converting spatial coordinates into decimal degrees; *lubridate* for calculation of hatching dates (Grolemund and Wickham, 2011). Spatial data was visualised 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; Eq 1) and average coefficient of variation (ACV; Eq. 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.

$$APE = 100 \times \frac{\sum_{j=1}^{n} \sum_{i=1}^{R} \frac{\left| x_{ij} - \overline{x}_j \right|}{\overline{x}_j}}{nR} \qquad \text{(Eq. 1)}$$

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

$$ACV = 100 \times \frac{\sum_{j=1}^{n} \frac{s_j}{\overline{x}_j}}{n} \qquad \text{(Eq. 2)}$$

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

2.3.3 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 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 quality 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. A 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 were reported at least 10 times, and each allele was represented in more than 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%.

2.3.4 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 10 000 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 hierarchical Bayesian approach implemented in BAYESCAN 2.1 (Foll and Gaggiotti, 2008). This method aims to separate locus-specific effects (*e.g.*, selection) from population effects (neutral gene flow and genetic drift) and has been shown to have a low Type I error rate (De Mita et al., 2013; Narum and Hess, 2011). 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 lower than 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).

2.4 Results

2.4.1 Statolith ageing & 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 (Fig. 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); iii) summer-hatched SSS (n=19) and Unassigned (UN; n=16).

2.4.1.1 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 (Fig. 1). Hatching peaked between late July and early September, overall individuals hatched continuously from end of June until the beginning of October (Fig. 2). Statolith microstructure presented narrow daily increments in the dark zone of the statolith, where more than 5 daily increments were observed per 20 µm (Fig. 3A; Fig. 4). Individuals with medium DML (15 ≤ 25 cm) were generally older when compared with other spawning groups (Fig. 5; Table 1)

2.4.1.2 Late-maturing South Patagonian Stock (LSPS)

For the LSPS group, individuals were found in the western part of the FICZ only (Fig. 1). Hatching occurred between late August and early September (Table 1; Fig. 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 6 increments per 20 µm (Fig. 3B; Fig. 4). Overall, individuals were older than the ESPS at similar DML (Table 1; Fig. 5).

2.4.1.3 High Seas Spring Spawning Stock (HS SpSS)

HS SpSS individuals were found to be characterised by distribution in the high seas, mainly north of 46˚S (Fig. 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; Fig. 2). The HS SpSS presented wide daily increments in the statolith dark zone with just over 5 daily increments per 20 µm (Fig. 3C, Fig. 4). This spawning group contained the oldest individual sampled at a maximum age of 230 days (Table 1; Fig. 5).

2.4.1.4 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 (Fig. 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 (Fig. 2). The statolith microstructure of this spawning group was distinct due to the narrow daily increments observed in the dark zone with more than 6 per 20 µm (Fig. 3D; Fig. 4). Individuals were older than their HS SpSS counterparts with small DML (≤ 15 cm) (Table 1; Fig. 5).

2.4.1.5 Summer Spawning Stock (SSS)

The SSS group was found in the high seas, primarily north of 46˚S (Fig. 1). Hatching dates ranged from the beginning of January until the end of March, with a peak in early January (Fig. 2). The statolith microstructure revealed the widest daily increments in the statolith dark zone when compared with other spawning groups, with less than 5 daily increments per 20 μm (Fig. 3E, Fig. 4).

2.4.1.6 Unassigned (UN)

The Unassigned spawning group was primarily sampled within the FICZ, from 48˚S to 53˚S with the largest number of individuals located in the "Loligo Box" (Fig. 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 (Fig. 2). Examination of the statolith microstructure revealed narrowing daily increments over the distance between 200 to 300 μm with more than 6 increments per 20 μm (Fig. 3F; Fig. 4).

Table 1. Summary of samples collected throughout 2020 and the spawning groups identified. 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; HS, High Seas.

Sample Location	Spawning Group	Hatching Period	Collection Period	N	DML Range (cm)	Age Range (days)
FICZ/FOCZ	ESPS	15 Jul - 14 Oct	1 - 14 Feb	4	19 - 25	161 - 187
			15 - 29 Feb	9	22 - 28	161 - 215
			1 - 14 Mar	9	24 - 31	164 - 211
			15 - 31 Mar	5	25 - 31	176 - 215
			1 - 14 Apr	5	27 - 33	192 - 211
	LSPS	15 Aug - 30 Sept	15 - 31 Mar	10	28 - 32	190 - 222
	Unassigned	15 Jan - 29 Feb	15 - 31 Jul	3	12 - 20	146 - 184

LOLBOX	FI SpSS	1 Sept - 31 Oct	1 - 14 Feb	3	12 - 14	132 - 153
			15 - 29 Feb	7	12 - 17	140 - 160
			1 - 14 Mar	7	12 - 20	134 - 181
			1 - 14 Apr	5	26 - 29	170 - 202
	Unassigned	15 Jan - 14 Apr	1 - 14 Jul	2	10 - 16	122 - 161
			15 - 31 Jul	10	11 - 26	129 - 186
			1-14 Sept	1	15 - 15	149 - 149
HS	ESPS	15 Jun - 14 Sept	15 - 31 Jan	9	16 - 27	147 - 210
			1 - 14 Feb	5	20 - 28	177 - 203
			15 - 29 Feb	5	21 - 26	175 - 205
			1 - 14 Mar	4	26 - 28	180 - 207
			15 - 31 Mar	4	26 - 29	185 - 212
	HS SpSS	1 Sept - 31 Dec	15 - 31 Jan	2	15 - 16	138 - 144
			1 - 14 Feb	6	11 - 23	118 - 166
			1 - 14 Mar	3	20 - 23	150 - 167
			15 - 31 Mar	3	22 - 26	147 - 172
			1 - 14 May	10	20 - 32	161 - 215
			15 - 31 May	29	20 - 32	141 - 209
			1 - 14 Jun	10	26 - 32	179 - 211
			15 - 31 Jul	2	30 - 33	212 - 230

SSS	1 Jan - 31 Mar	1 - 14 Jun	1	24 - 24	148 - 148
		15 - 31 Jul	18	10 - 31	122 - 198

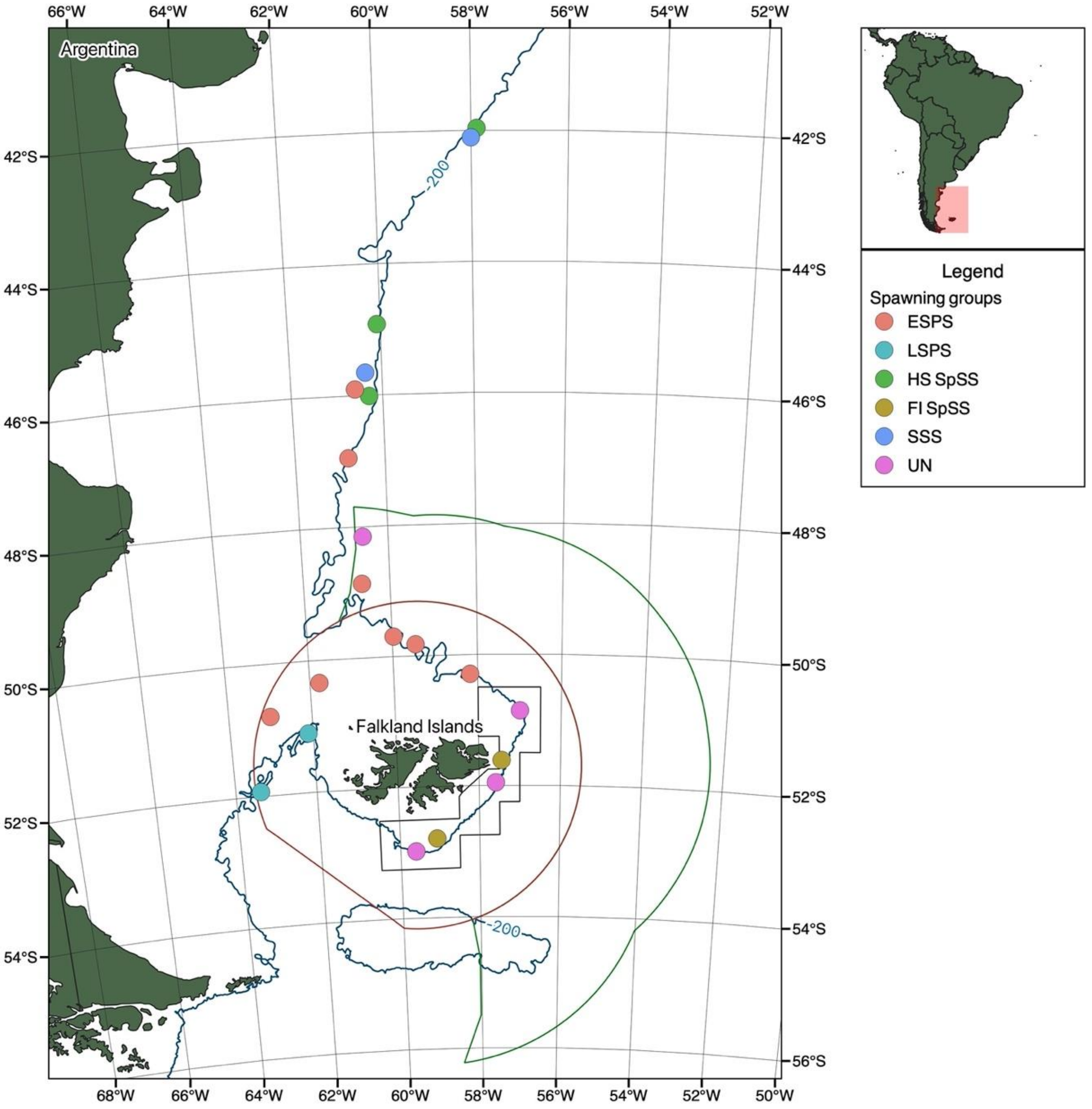

Figure 1. Map showing the sampling locations of *Illex argentinus* and the occurrence of the spawning groups identified. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; HS SpSS, High Seas Spring Spawning Stock; FI SpSS, Falkland Islands Spring Spawning Stock; SSS, Summer Spawning Stock; FICZ, Falkland Islands Interim Conservation and Management Zone (red); FOCZ, Falkland Islands Outer Conservation Zone (green); LOLBOX, Loligo Box (black).

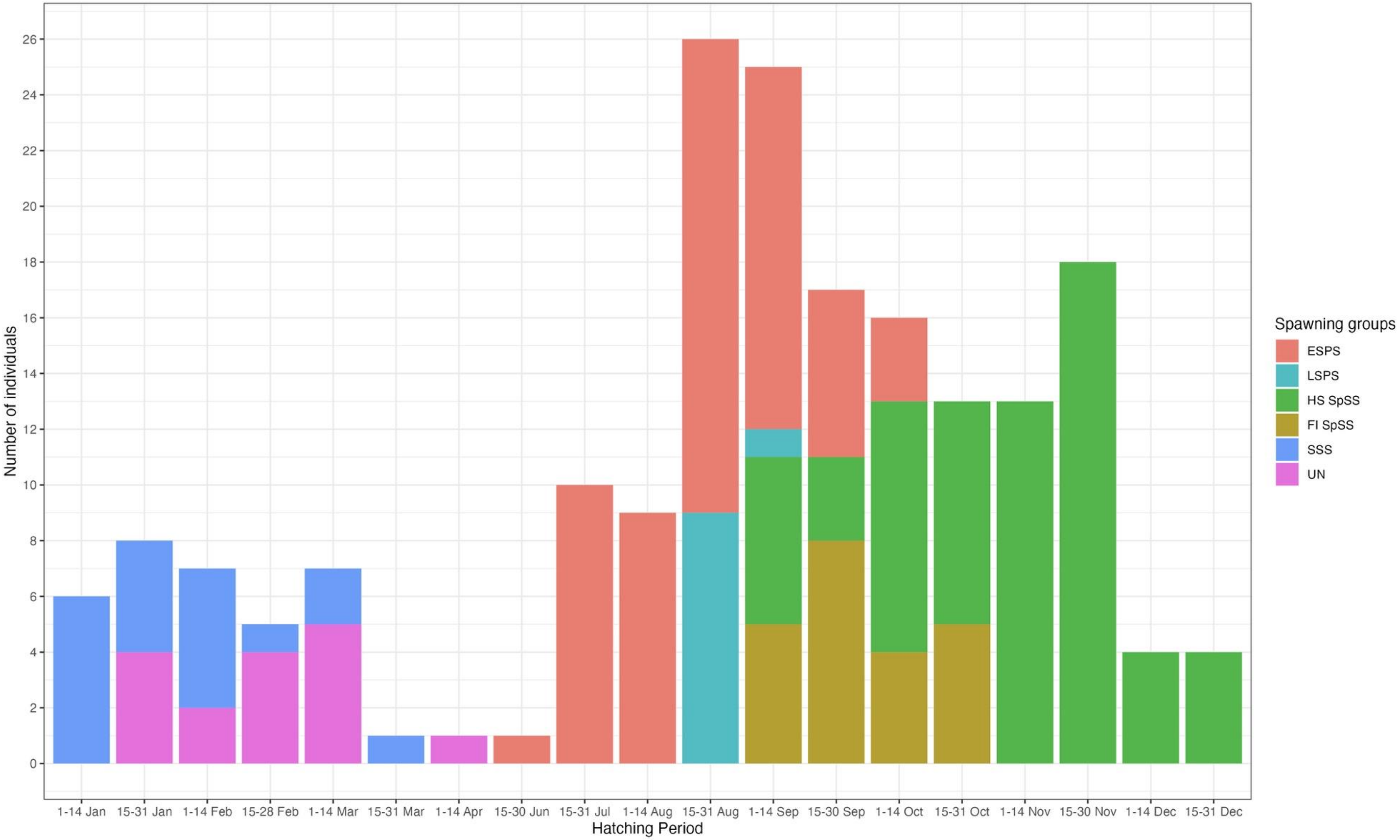

Figure 2. Hatching date distribution of spawning groups identified. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; HS SpSS, High Seas Spring Spawning Stock; FI SpSS, Falkland Islands Spring Spawning Stock; SSS, Summer Spawning Stock; UN, Unassigned.

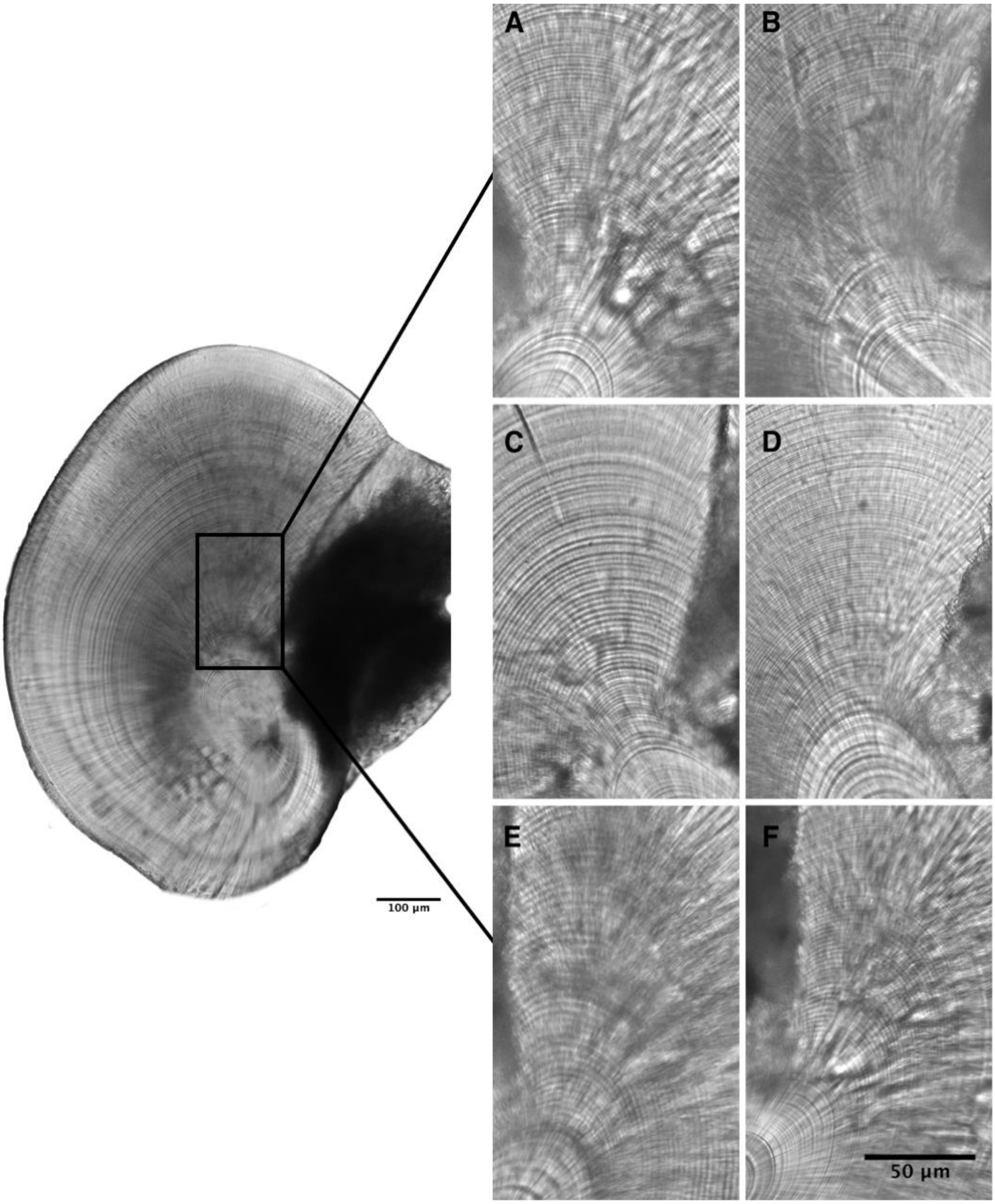

Figure 3. Statolith microstructure of the identified spawning groups: (A) ESPS; (B) LSPS; (C) HS SpSS; (D) FI SpSS; (E) SSS; (F) UN. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; UN, Unassigned.

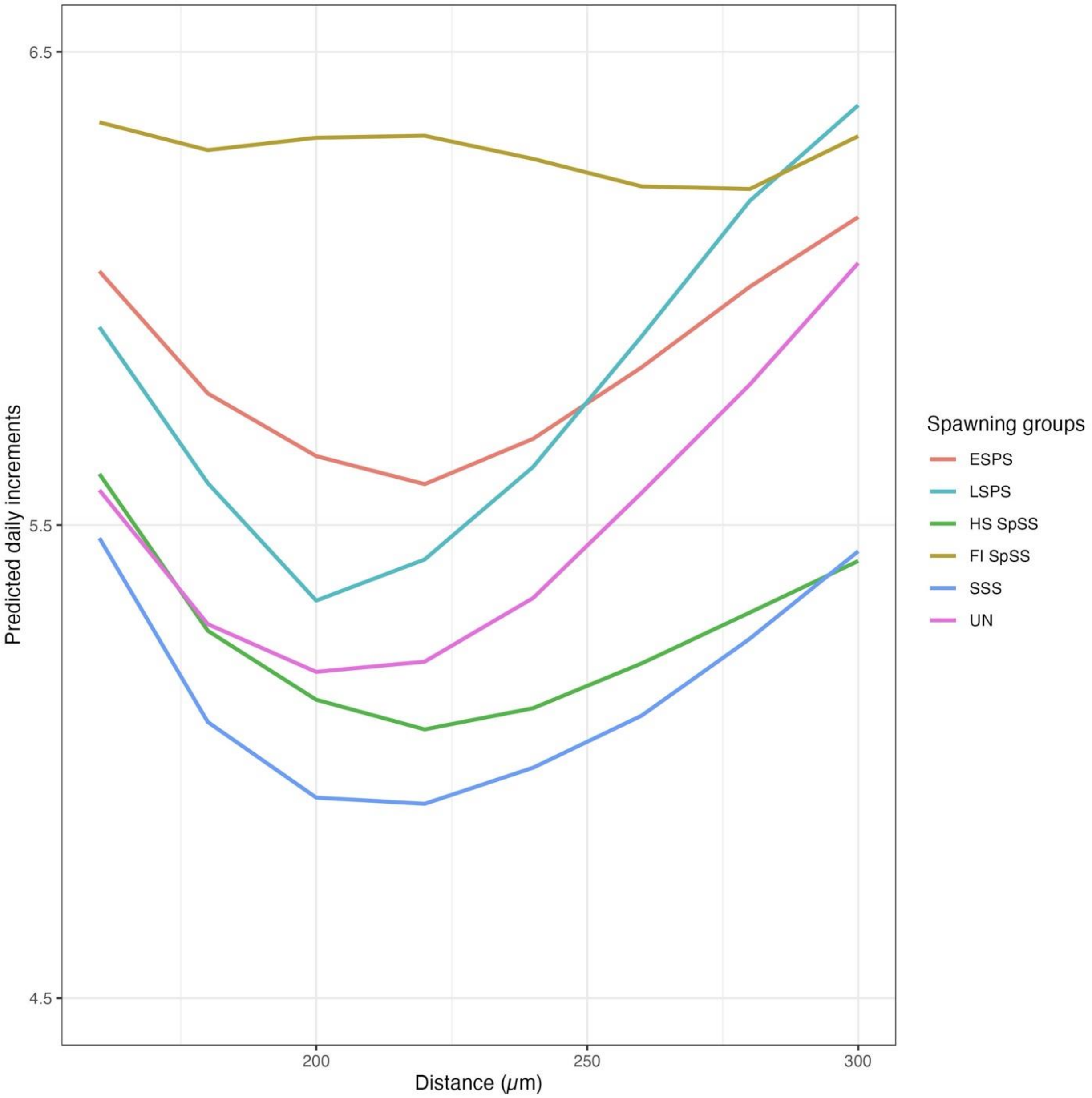

Figure 4. Predicted number of daily growth increments using loess smoothing (span=0.4) in a subset of the dark zone of the statolith as a function of distance for the spawning groups identified. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland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

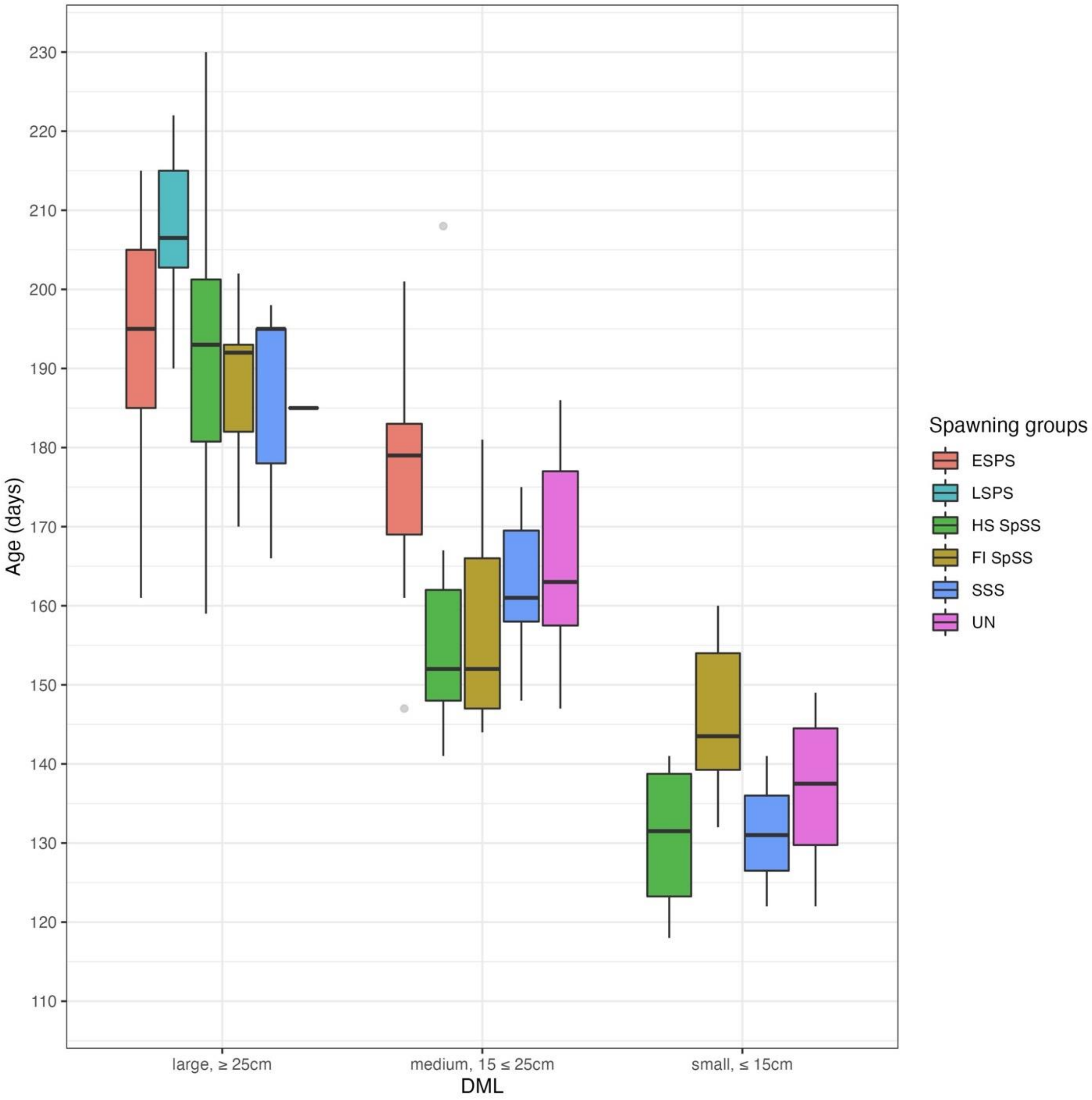

Figure 5. Age range per spawning group as a function of DML groups. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; UN, Unassigned; DML, dorsal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

2.4.2 Genomic diversity

A total of 2x 528,267,065 sequence reads were obtained with an average of 2x 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 MAF of 5% resulted in 10, 353 biallelic SNPs which were used for downstream analysis.

As each genotyped individual had been included in the statolith ageing we initially grouped samples according to their membership to the 6 groups revealed in that analysis. These groups exhibited similar levels of multi-locus variability (Table 2). Each group exhibited a significant deviation from HWE 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 (Fig. 6). BAYESCAN analysis did not identify any loci that deviated from neutral expectations.

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

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

Table 3. Pairwise F_{ST} based on all loci. Significance was assessed after 10 000 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; UN, Unassigned.

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	-

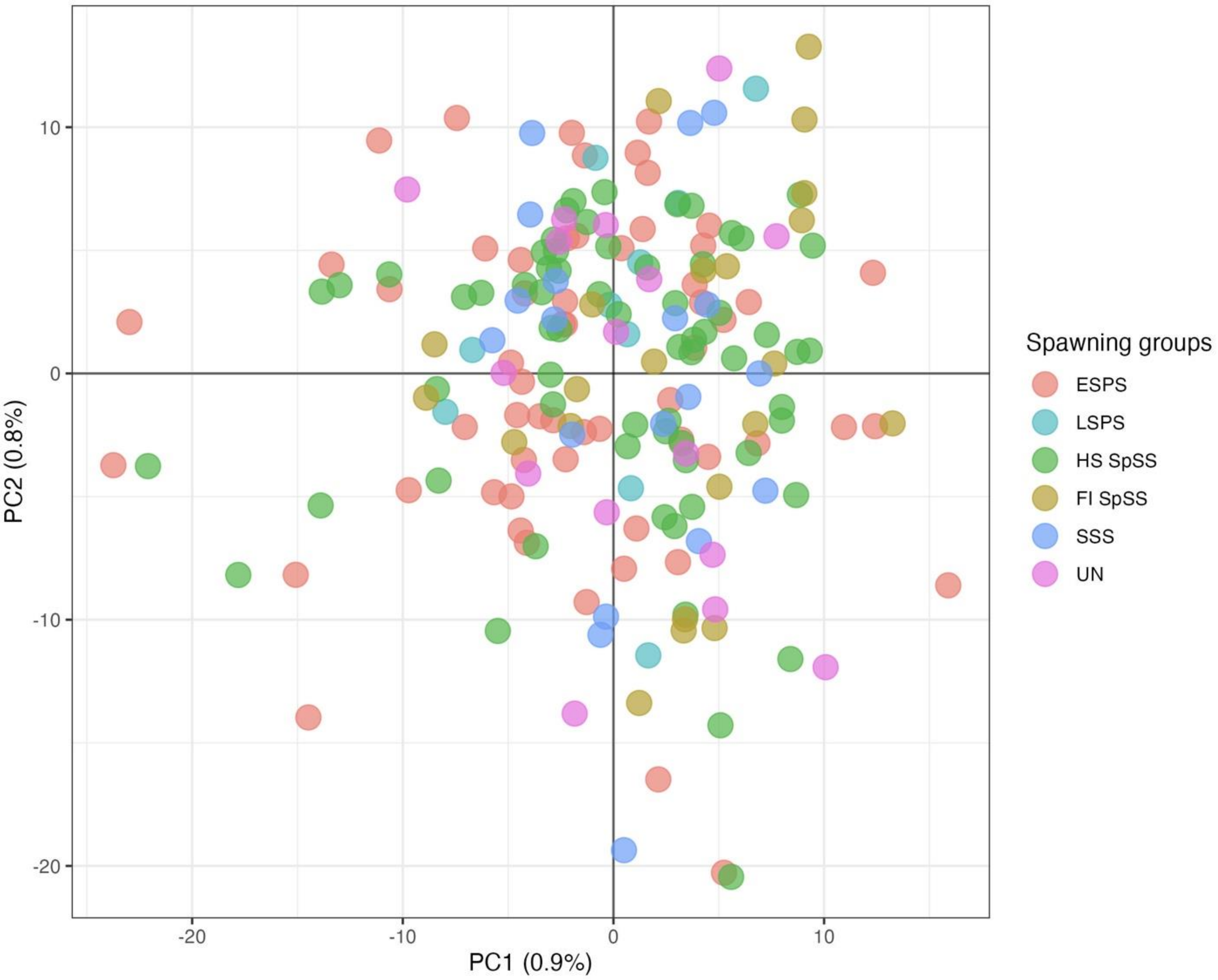

Figure 6. Principal component scatter plot with individuals denoted by spawning group assignment. ESPS, early-maturing South Patagonian Stock; LSPS, late-maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; UN, Unassigned.

2.5 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 (Kerr et al., 2017; Reiss et al., 2009). Given the complexity of the processes that shape stock structure, fisheries managers are increasingly combining information obtained from different stock identification methods. This 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); iii) spring-hatched (HS SpSS and FI SpSS); and iii) summer-hatched (SSS and UN). While these groups have been previously described in other studies (Arkhipkin et al., 2022; Brunetti, 1988; Crespi-Abril and Barón, 2012), 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. F_{ST}, individual clustering and outlier analyses reported a lack of genetic structure and no outlier loci among spawning groups supporting the aforementioned hypothesis. 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 characterised using phenotypic and genotypic markers to reveal a high level of population connectivity.

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. Therefore, 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, that showed that there were several “waves of abundance” of squid passing through the fishing grounds from 52°S 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. coindetii* (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 temperature 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 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 SSS and UN groups, in which 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 difference 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, 1999b). However, such studies may have been limited in terms of resolution owing to the small number of loci analysed (<9 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˚S 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. An allozyme study by Carvalho *et al.* (1992) reported significant differences among samples which contradicts the overall pattern of genetic homogeneity revealed here and by 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 (Bekkevold et al., 2015; Bradbury et al., 2013; 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). These 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.

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 forbesii* (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 (McKeown et al., 2019; Sandoval-Castellanos et al., 2007; Staaf et al., 2010). 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. *Illex argentinus* individuals undertake a feeding and spawning migration across several large marine ecosystems in the Southwest Atlantic (Arkhipkin, 2013). Furthermore, the most numerous spawning group (SPS) completes a 2,000 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, this 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.

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 stocks sizes are maintained the genetic drift is not sufficient to reduce genetic variation.

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 population as causes. Interestingly, both Carvalho *et al.* (1992) and Adcock *et al.* (1999b) reported significant genetic differentiation between temporal samples despite 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 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 subsequent fishing season (Chemshirova et al., 2021; Waluda et al., 1999). 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.

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. Firstly, 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. Secondly, the lack of genetic structuring in *I. argentinus* confirms that the fishery should be considered a straddling stock, as individuals migrate through several EEZ and international waters. Nonetheless, no regional organisation 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.

CRediT authorship contribution statement

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; Writing – review & editing. **Niall J McKeown**: Investigation, Data Curation, Formal Analysis, Supervision, Writing – original draft; Writing – review & editing.

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Chapter III

Identification of population connectivity of the Argentine shortfin squid *Illex argentinus* using trace elemental signatures of early ontogeny within statolith microstructure

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3.1 Abstract

Ommastrephid squid are highly migratory species which are a pivotal part of marine food webs, that act as links between ecosystems, and sustain the world's largest invertebrate fisheries. *Illex argentinus* has a complex population structure of up to four seasonal spawning groups and its ontogenetic migrations span multiple EEZs in the Southwest Atlantic. To improve understanding of the potential hatching grounds of these groups of, we investigated the seasonal variation of early ontogeny and connectivity of *I. argentinus*. To do so we require data throughout the lifespan of individuals. Analysis of archival structures, *i.e.*, statoliths provides comprehensive life history information, such as hatching dates. This, combined with microstructure analysis was used to identify the seasonal spawning groups in the present study. We investigated if the elemental signatures within the statolith core of the groups vary, and if these differences are congruent with the ambient temperature on the putative hatching grounds. We also aimed to identify distinct multi-elemental signatures for spawning groups and establish whether these signatures correlate with the temporal and/or spatial patterns of the groups under investigation. Statolith core elemental signatures (^{88}Sr, ^{24}Mg, ^{137}Ba, ^{55}Mn, ^{7}Li ^{57}Fe) from 625 specimens were analysed using LA-ICP-MS. We found seasonal differences between spawning groups that were underscored by significant differences of single-elemental signatures. Furthermore, the summer-hatching group had higher Sr:Ca ratios, which suggested they encountered lower ambient temperatures than expected. The environmental heterogeneity due to seasonal hatching times and subsequent ontogenetic migrations may explain the seasonal groups identified. Multi-elemental signatures suggested a high level of connectivity in *I. argentinus* during early ontogeny.

Mixing of spawning groups during early life stages identified the need for cohesive management of the *I. argentinus* fishery across the Southwest Atlantic.

3.2 Introduction

Nektonic squid have a vital role in marine ecosystems and their fisheries have become more valuable over time (Arkhipkin et al., 2015; Xavier et al., 2014). These cephalopods are characterised by the long-range ontogenetic horizontal migrations that have the important role of linking otherwise disparate ecosystems and hence act as “biological pumps” (Arkhipkin, 2013). Additionally, the extent of these migrations makes them more vulnerable to overexploitation as they pass through the jurisdictions of several coastal countries and international waters, leading to the exploitation of multiple cohorts by various nations during a single life cycle (Arkhipkin et al., 2022).

The Argentine shortfin squid (*Illex argentinus*) is a nerito-oceanic species that has a range from 22˚S to 55˚S in the Southwest Atlantic, primarily along the Patagonian Shelf and slope (Rodhouse et al., 2013). *Illex argentinus* is the subject of one of the largest fisheries in the Southwest Atlantic, with landings accounting for 16.4% of worldwide squid catch in 2020 (FAO, 2022). This squid species is semelparous and attains adult sizes of 30 – 35 cm in dorsal mantle length (DML), with females being larger than males (Arkhipkin and Laptikhovsky, 1994).

The complexity of *I. argentinus* population structure is exemplified by the fact that the existence of up to four spawning groups is currently hypothesised: i) Bonaerensis North Patagonian Stock (BNPS); ii) South Patagonian Stock (SPS); iii) Spring Spawning Stock (SpSS) and iv) Summer Spawning Stock (SSS) (see Brunetti, 1988; Arkhipkin *et al.*, 2020, 2022 for detailed descriptions). BNPS and SPS are winter-spawning, with SPS being the most numerous stock, contributing up to 95% of the total biomass, mainly distributed south of 44˚S (Brunetti, 1988; Hatanaka, 1988). The SpSS is believed to be largely distributed between 45˚S and 47˚S, occurring primarily during the austral spring, with spawning areas hypothesised to be in coastal waters (Crespi-Abril et al., 2013, 2008; Crespi-Abril and Barón, 2012). The SSS occasionally migrates to the high seas from the Argentine EEZ and is assumed to spawn on the shelf between 42˚S and 46˚S in austral summer from December until February (Crespi-Abril et al., 2010). The description of four seasonal groups, each with subgroups in *I. argentinus* underscores the need for improved understanding of the extent of the connectivity between these spawning groups. Basic information such as location of spawning grounds is still disputed, the SPS is hypothesised to spawn north of 35˚S in the south Brazil area of the shelf and shelf-break (Arkhipkin, 2013; Haimovici and Pérez, 1990) or between 45˚S and 48˚S, on the Patagonian outer shelf (Haimovici et al., 1998; Torres Alberto et al., 2020). These conflicting hypotheses may be resolved by the use of artificial tags, as previously demonstrated in *Loligo forbesii*, where individuals were successfully tagged using a novel soft-bodied invertebrate tag (ITAG) (Mooney et al., 2015). However, the smallest individual tagged had a DML of 52 cm. This is almost twice the maximum DML of *I. argentinus* and indicates that further development is required prior to wider use of ITAGs. Therefore, natural tags (or archival structures) in the form of squid statoliths are the only viable alternative for clarifying the potential spawning or hatching grounds of the seasonal groups of *I. argentinus* (Arkhipkin, 2005).

Squid statoliths are calcareous deposits, analogous to fish otoliths (Radtke, 1983; Arkhipkin and Bizikov, 2000). As statoliths grow continuously throughout the squid's life the daily deposition of increments enables accurate ageing, which when combined with microstructure analysis may reveal seasonal differences between individuals (Arkhipkin, 2005). Furthermore, statoliths may be used as a record of life history of individuals due to chronological incorporation of trace elements in the aragonite matrix (Arkhipkin and Bizikov, 2000; Radtke, 1983; Reis-Santos et al., 2022). The uptake of trace elements in these hard structures is believed to be a reflection on the bio-availability of those elements in seawater and environmental conditions encountered by the individuals at the time of incorporation (Bettencourt and Guerra, 2000; Elsdon and Gillanders, 2003; Yamaguchi et al., 2018). For example, Sr:Ca ratios have been found to be negatively associated with temperature in various marine taxa (Beck et al., 1992; Campana, 1999; Ikeda et al., 1998). Elemental signatures have previously been combined with ageing techniques to distinguish between different stocks (Arkhipkin et al., 2004; Avigliano et al., 2020; Ching et al., 2017; Nazir and Khan, 2021), disentangle ontogenetic migrations (Arbuckle and Wormuth, 2014; Jones et al., 2018; Liu et al., 2016) and identify natal areas (Burns et al., 2020; Green et al., 2015; Pecl et al., 2011). Therefore, the elemental signatures of statolith cores from spawning groups identified through statolith ageing and microstructure analysis may reveal differences in the hatching areas of *I. argentinus*.

We hypothesize that: i) there are differences between the single elemental signatures from the statolith core of different seasonal spawning groups; and ii) these differences are consistent with the environmental conditions in the putative hatching grounds for the relevant groups. To test these hypotheses, the present study used statolith ageing techniques to identify seasonal spawning groups of *I. argentinus* and trace elemental signatures from the statolith core to reveal possible environmental conditions experienced by individuals in early ontogeny. Additionally, we aimed to identify a unique multi-elemental signature that characterises each spawning group and determine if the signatures correspond to temporal and/or spatial patterns of the seasonal spawning groups of interest.

3.3 Materials and methods

3.3.1 Sample collection and preparation

The majority of squid samples were collected fortnightly from the 15th January until the 14th of October 2020, by scientific observers deployed on commercial vessels. Samples were also collected during routine Falkland Islands Fisheries Department research cruises aboard F/V Castelo and F/V Argos Cies both in February and July 2020. Finally, in the event that no members of the scientific team were present onboard a vessel, a subsample of *I. argentinus* catch was randomly collected by crew when fishing vessels were operating in international waters. Spatially, the area studied ranged from 42˚S to 53˚S (Fig. 1; Table 1). All samples were immediately frozen to be processed on land, in the departmental laboratory. Subsequently, a total of 1,878 individuals were processed, 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). Statoliths were extracted and stored in 95% ethanol. A sub-sample of 720 individuals was selected for trace elemental and age analyses. Individuals were selected based on DML, maturity, sample location and date in order to include a wide range of seasonal spawning groups, with an equal number of males and females where possible (Table 1). One statolith per individual was mounted concave side up using thermoplastic resin, CrystalBond™ 509 (AREMCO Products, Inc., USA), and ground and polished on one side using P1200 and P2400 wet paper. Individual statoliths were placed on glass slides in groups of 30. Statoliths were placed in randomised order on the glass slides to avoid elemental concentration measurements being biased by instrument drift or failure (Kerr and Campana, 2014). Subsequently the statoliths were orientated with the dorsal dome towards the short side of the glass slide, fixed with thermoplastic resin, and mounted as flat as possible for laser ablation processing. Following placement, statoliths were cleaned from any residue using pliable mini card (House of Marbles, Devon, UK).

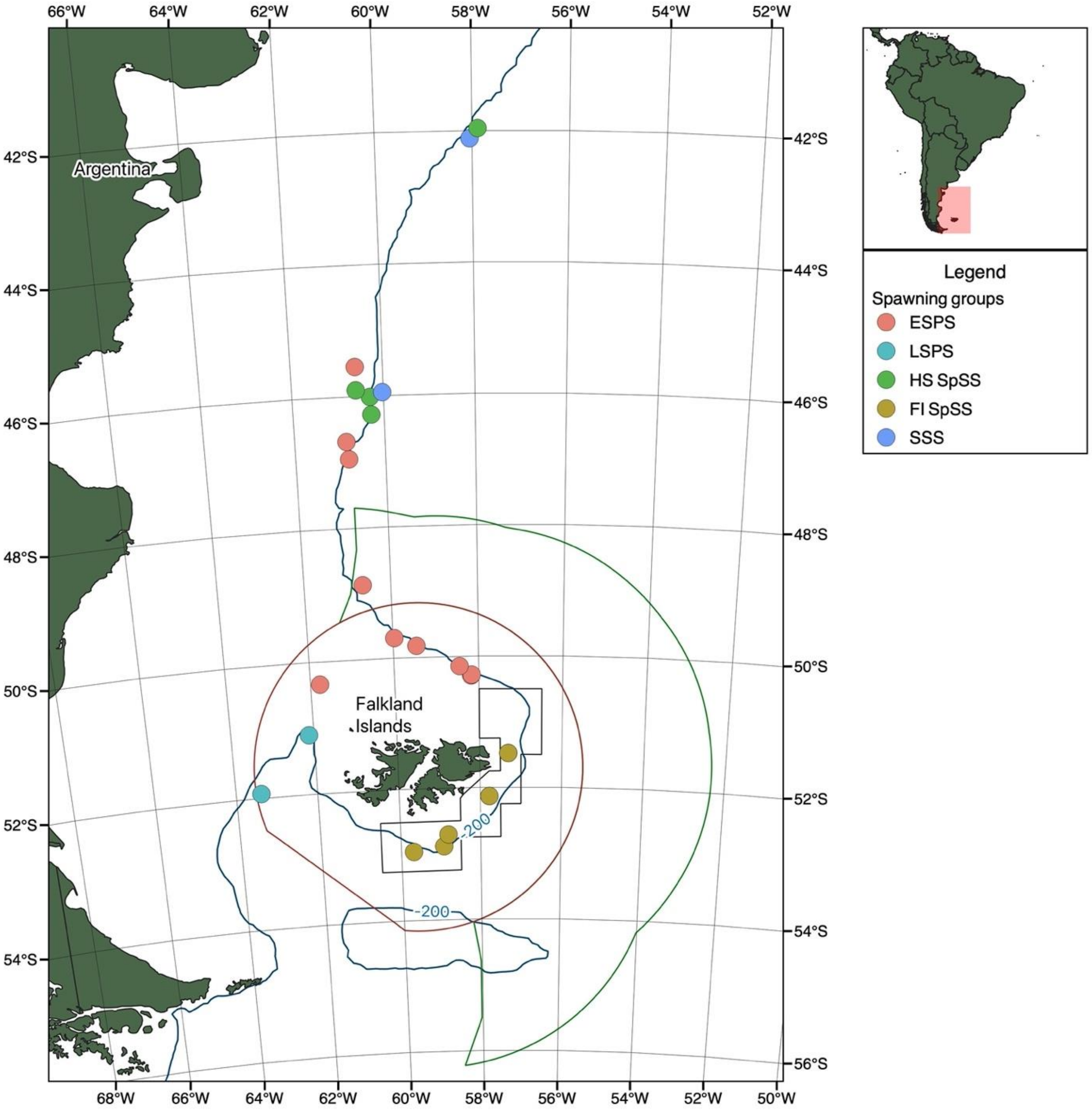

Figure 1. Map of sampling locations for *I. argentinus* on the Patagonian Shelf, locations of seasonal spawning groups are presented. FICZ, red outline; FOCZ, green outline; Loligo Box, black outline. ESPS, early-maturing South Patagonian Stock; LSPS, late maturing South Patagonian Stock; FI SpSS, Falkland Islands Spring Spawning Stock; HS SpSS, High Seas Spring Spawning Stock; SSS, Summer Spawning Stock; FICZ, Falkland Islands Interim Conservation and Management Zone; FOCZ, Falkland Islands Outer Conservation Zone. Adapted from Chapter 2 to include all sampling locations used in for microchemistry analysis.

Table 1. Sample information. 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; FOCZ, Falkland Islands Outer Conservation Zone; LOLBOX, Loligo Box; HS, High Seas; F, Female; M, Male; N, number of individuals collected

Sample Location	Spawning Group	Sex	N	DML Range (cm)	Age Range (days)
FICZ/FOCZ	ESPS	F	133	22 - 32	160 - 226
		M	73	21 - 28	167 - 230
	LSPS	F	27	28 - 32	187 - 222
LOLBOX	FI SpSS	F	27	12 - 17	132 - 181
		M	16	12 - 16	137 - 177
HS	ESPS	F	54	14 - 28	152 - 212
		M	47	16 - 27	148 - 212
	HS SpSS	F	121	12 - 33	114 - 230
		M	75	12 - 28	110 - 211
	SSS	F	21	18 - 31	148 - 203
		M	31	18 - 26	133 - 193

3.3.2 Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)

All 720 statoliths were analysed for trace elements using LA-ICP-MS at the Natural History Museum, London, United Kingdom. The excimer-based laser ablation system comprised an ESI New Wave Research 193 laser (Elemental Scientific Lasers, LLC, Bozeman, MT, USA) combined with an Agilent 7700x ICP-MS (Agilent Technologies, Inc., Santa Clara, CA, USA). Each statolith was sampled using a line transect from the statolith core (hatching) to the dorsal dome edge (time of collection) of the statolith (Fig. 2). Statoliths were ablated in the direction of maximum growth to obtain temporally resolved life history chronology. Each statolith was ablated using a continuous 25 μm diameter laser beam, with a 10 Hz repetition rate, 4.5 Jcm^{-2} laser flux, at a scan speed of 3 μm/s. Three glass slides were placed in the laser ablation cell, which was then purged with Helium.

Prior to the ablation of each statolith transect, a blank sample of gas was quantified for elemental isotope counts per second (CPS) for 30 s to provide average background counts of the relevant elemental isotopes, that were subtracted from the sample counts for each transect ablation. The elemental intensities in CPS from the ICP-MS were converted to elemental concentrations in parts per million (ppm) using Igor version 7.0.8.1 (WaveMetrics, Inc., CA, USA) with Iolite version 3 (Elemental Scientific Lasers, LLC). The following elemental concentrations were measured for each statolith transect: ^{7}Lithium (^{7}Li); ^{11}Boron (^{11}B); ^{23}Sodium (^{23}Na); ^{24}Magnesium (^{24}Mg); ^{27}Aluminium (^{27}Al); ^{55}Manganese (^{55}Mn); ^{57}Iron (^{57}Fe); ^{63}Copper (^{63}Cu); ^{66}Zinc (^{66}Zn); ^{87}Strontium (^{87}Sr); ^{88}Strontium (^{88}Sr); ^{111}Cadmium (^{111}Cd); ^{137}Barium (^{137}Ba); ^{208}Lead (^{208}Pb); and ^{43}Calcium (^{43}Ca) was used as an internal standard, which accounted for variation in ablation yield. National Institute of Standards and Technologies 612 and 610 standard reference material (NIST–SRM 612 and 610) were used as calibration standards at the beginning of each laser ablation session and after every 5 statolith transects, to account for instrumental drift. NIST-SRM 612 used to assess the accuracy of measurements, as it was used as an unknown sample, whereas NIST-SRM 610 was used to calibrate instrument sensitivity (Limbeck et al., 2015). Limits of detection (LOD) were calculated as three standard deviations (SD) of the background signal measured for each elemental concentration for every statolith sampled. The following elements were consistently below the calculated LOD and were subsequently dropped from further investigation: ^{27}Al, ^{63}Cu, ^{66}Zn, ^{111}Cd, and ^{208}Pb. The elemental concentrations in ppm were subsequently converted to Element:Ca (μmol mol^{-1}) using the formula as presented in Eq. 1.

$$X{:}\,Ca = \frac{\left(\frac{ppm_x}{Z_x}\right)}{\left(\frac{ppm_{Ca}}{Z_{Ca}}\right)} \qquad \text{(Eq. 1)}$$

Where X is the analysed element; $\mathrm{ppm_x}$ the measured concentration of the analysed element; $\mathrm{Z_x}$, atomic mass of analysed element; $\mathrm{ppm_{Ca}}$, fixed number of ppm of Ca set at 40,000; $\mathrm{Z_{Ca}}$, atomic mass of ^{43}Ca.

Once the laser ablation processing was completed, all statoliths were transferred to individual slides, flipped over, and ground on the other side. To achieve maximum visibility of growth rings and laser ablation transect, statoliths were embedded in Canada Balsam™ mounting medium and covered with a glass cover slip. Ready preparations were then dried in a temperature-controlled cabinet at 30°C for at least 1 week. Subsequently, the statoliths were examined under an Olympus BX-51 compound microscope (Olympus Corporation, Tokyo, Japan) at x100 magnification combined with an Olympus DP70 camera and Olympus CellSens 3.1 to measure distance from the core to the statolith edge along the laser ablation transects.

This measurement was used in data processing and cleaning, to remove elemental concentration readings that were before the statolith core, or after the statolith edge. The location of the statolith core was cross-referenced with plots of Mg:Ca concentration against laser ablation transect distance for every statolith analysed, as the core area has previously been identified to be Mg rich (Ruttenberg et al., 2005).

As the present study aimed to investigate the early ontogeny of individuals, only the first 20 μm of the laser ablation transect (equivalent to 3 to 5 days post-hatch) were used in the subsequent analysis (Fig. 2). The laser ablation transect was found to be off-centre from the statolith core for some individuals. To quantify the extent of the deviation, the distance from the centre of the transect to the statolith core was measured. Subsequently, Mg:Ca elemental profiles of each individual where a deviation was observed were plotted in order to determine a cut-off distance beyond which the laser ablation line was deemed to have missed the statolith core as a Mg:Ca peak was absent. A distance greater than 7.5 μm was determined to be the cut-off, and individuals were dropped from further investigation.

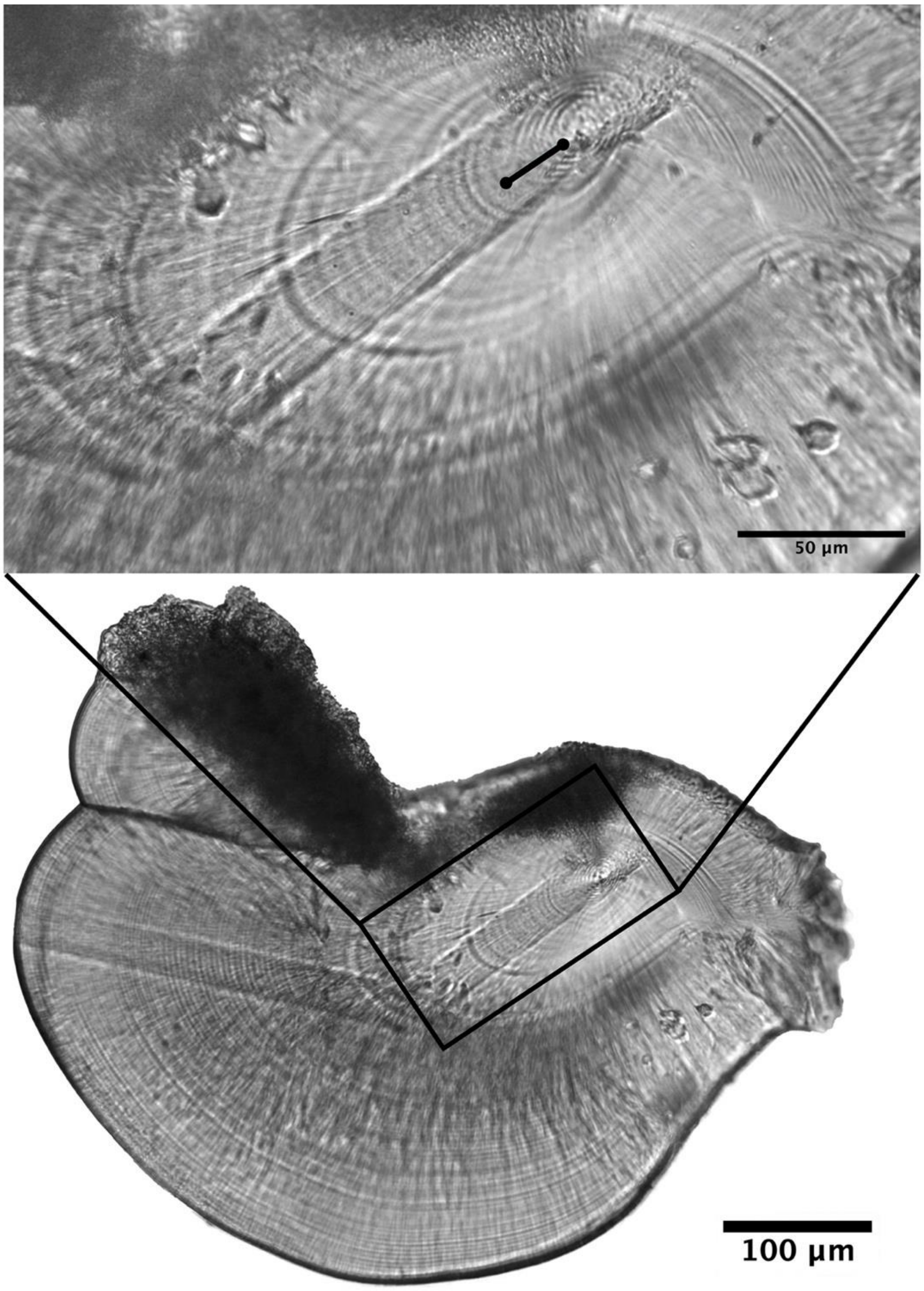

Figure 2. Example of an ablated *Illex argentinus* statolith, the trace element ablation transect was positioned from the statolith core to the edge of the dorsal dome. Solid line in the inset depicting the 20 µm distance from the core region used in the present study.

3.3.3 Statolith Ageing

Following preparation described above, statoliths were read using an Olympus BX51 compound microscope at x500 magnification, with a phase-contrast Nomarski effect to improve readability as previously described in Arkhipkin and Shcherbich (2012). The number of growth increments were counted at 20 µm intervals from the first natal ring to the statolith dorsal dome edge, following the laser ablation transect as closely as possible. One growth increment was assumed to represent one day, following the "one ring = one day" hypothesis previously validated for *Illex illecebrosus* (Dawe et al., 1985; Hurley et al., 1985). The total number of increments recorded was considered to be the age of an individual in days post-hatch. The precision of the ageing estimates was evaluated by a randomly selected subsample of 50 statoliths which were aged a second time by the primary reader (IC), and a third time by the second reader (AA), without any prior knowledge of the specimens to verify the age estimates. Average percent error (APE; Eq. 2) and average coefficient of variation (ACV; Eq. 3) were calculated for all three readings using the methods described by Beamish and Fournier (1981), and Chang (1982), respectively.

$$APE = 100 \times \frac{\sum_{j=1}^{n} \sum_{i=1}^{R} \frac{|x_{ij} - \overline{x}_j|}{\overline{x}_j}}{nR} \qquad (\mathrm{Eq.\,2})$$

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

$$ACV = 100 \times \frac{\sum_{j=1}^{n} \frac{s_j}{\overline{x}_j}}{n} \qquad (\mathrm{Eq.\,3})$$

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

The hatching date of individuals was back-calculated from the date of collection. Assignment of individuals to relevant seasonal spawning groups was carried out based on DML, hatch date, sampling location, and statolith microstructure (Arkhipkin, 1993). Statolith microstructure was inspected visually while ageing. Ground-truthing was performed by plotting the number of growth increments counted per 20 μm distance intervals along the ageing direction focusing on the dark zone area of the statolith. A 2^{nd} degree polynomial locally-weighted regression (loess) was performed (span = 0.4) for each seasonal spawning group in order to determine the overall width of the growth increments.

3.3.4 Statistical Analysis

The present study focused on Sr:Ca, Mg:Ca, Ba:Ca, and Mn:Ca ratios for univariate analysis as these elemental ratios have been previously used to identify the natal environment and population structuring in cephalopods (Green et al., 2015). Li:Ca and Fe:Ca were added for the purpose of the multivariate analysis.

During data exploration performed as per Zuur *et al.* (2010), a total of 16 individuals were recorded to have levels of ^{55}Mn (n=16) and ^{7}Li (n=1) below the calculated LOD. Therefore, they were excluded from subsequent analysis. Elemental ratios ±3 SD from the mean per statolith, per element, were removed as they were considered to be outliers (Lee, 2022). Negative values for elements of interest (e.g., ^{55}Mn) were also identified. Therefore, elemental measurements (~35 data points per statolith core) were also compared with the LODs calculated, values below the overall statolith LOD were excluded. Finally, if a statolith remained with fewer than 10 elemental measurements, it was dropped from analysis.

A total of 720 statoliths were successfully prepared, ablated and read, of which 625 (86.8%) were used in the univariate analysis (except ^{55}Mn, n=620), and 514 (71.4%) were used in the multivariate analysis. Statoliths were removed for the following reasons: i) statolith was lost during transfer from LA-ICP-MS slides to individual slides or overground during ageing preparation (n=6); ii) ablation transect was found to miss the statolith core as aforementioned (n=73); iii) overall levels of ^{55}Mn and ^{7}Li in statolith were below LOD (n=16); iv) fewer than 10 elemental measurements remained following filtering of data below LOD (only in ^{55}Mn for univariate data, n=5; multivariate data n=111).

3.3.4.1 Single-elemental signatures - generalised additive mixed effects models (GAMM)

The datasets used for the univariate analysis contained multiple observations per statolith core. Therefore, a mixed modelling approach was used, and *Statolith ID* was treated as a random variable, with a normal distribution (mean of 0, and variance of σ^2), to model the dependency between elemental ratio observations from the same statolith. Elemental ratios for ^{88}Sr (Eq. 4), ^{24}Mg (Eq. 5), ^{137}Ba (Eq. 6), and ^{55}Mn (Eq. 7) were modelled as the response variables. Data exploration revealed that a Gaussian distribution with an identity link function may be applied to the response variables. The possibility of collinearity between response variables was investigated by calculation of Pearson correlation coefficients, and pair plots. Seasonal spawning group and sex were included as fixed categorical covariates. Distance was included as a covariate to account for the temporal dependency of the elemental ratio readings over the 20 µm statolith core section of the laser ablation transect. Data exploration indicated non-linear patterns for distance; therefore, a smoother with cubic regression spline was fitted. Model validation was performed by plotting normalised residuals against the fitted values, each covariate included the model, and each covariate not in the model (Zuur and Ieno, 2016). The optimal GAMM models were selected using a backwards selection procedure and Akaike Information Criterion (AIC), the necessity for a smoother for distance was investigated for each elemental ratio, and restricted maximum likelihood estimation was used for the optimal models. The optimal models for the elemental ratios investigated are shown below.

$$({}^{88}Sr\colon Ca_{ij}) \sim \alpha\ + Statolith\ ID_i + f(Distance_{ij}) + Spawn\ group_{ij} + \varepsilon_i \qquad \text{(Eq. 4)}$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

$$({}^{24}Mg\colon Ca_{ij}) \sim \alpha\ + Statolith\ ID_i + f(Distance_{ij})\colon Spawn\ group + Spawn\ group_{ij} + Sex_{ij} + \varepsilon_i \text{(Eq. 5)}$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

$$({}^{137}Ba\colon Ca_{ij}) \sim \alpha\ + Statolith\ ID_i + f(Distance_{ij})\colon Spawn\ group + Spawn\ group_{ij} + \varepsilon_i \text{ (Eq. 6)}$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

$$({}^{55}Mn\colon Ca_{ij}) \sim \alpha\ + Statolith\ ID_i + f(Distance_{ij}) + Spawn\ group_{ij} + \varepsilon_i \qquad \text{(Eq. 7)}$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

where $X\colon Ca_{ij}$ is the elemental ratio, which is the j^{th} observation for *Statolith ID* i; α is the intercept, $f()$ denotes smooth term applied with cubic regression spline, which for ^{137}Ba and ^{24}Mg was varied by *Spawning group*.

3.3.4.2 Multi-elemental signatures - multivariate generalised linear latent variable model (MGLLVM)

A MGLLVM was applied to identify an overall elemental fingerprint that may be used to distinguish between the seasonal spawning groups (Niku et al., 2019). The complete dataset (n=514) was defined as a matrix of trace elemental concentrations with n rows (statoliths with multiple measurements) and m columns of trace elements (^{88}Sr, ^{137}Ba, ^{24}Mg, ^{55}Mn, ^{7}Li, and ^{57}Fe). The elemental ratio of the j^{th} element of the i^{th} statolith was denoted as y_{ij}. Distance in the form of the 20 µm statolith core region was modelled as a set of distance measurements recorded for each statolith as the vector $x_i = (x_{i1}, \ldots, x_{ik})^{\top}$. The MGLLVM was used to regress the mean trace elemental ratio μ_{ij} against Distance $(x_i)^{\top}$ and a vector of 2 latent variables (LVs) $u_i = (u_{i1}, \ldots, u_{id})^{\top}$

$$g(\mu_{ij}) = \eta_{ij} = \alpha_i + \beta_{0j} + \boldsymbol{x}_i^{\top}\boldsymbol{\beta}_j + \boldsymbol{u}_i^{\top}\boldsymbol{\gamma}_j \qquad \text{(Eq. 8)}$$

where β_j and γ_j are vectors of element-specific coefficients associated with covariates (β_j) and LVs (γ_j). The LVs were considered to be unmeasured variables which represent the main axes of covariation observed in the elemental ratios after the effect of the observed predictor (*Distance*) has been accounted for. The LVs were assumed to be independent across the statoliths sampled. β_{0j} denotes the element-specific intercepts and α_i was modelled as random effect representing *statolith ID* with a normal distribution $N(0, \sigma^2)$. A Gaussian distribution with an identity link was deemed suitable for the data based on prior data exploration. Model estimations were performed using Laplace approximations. Several models of increasing complexity were fitted, using forward selection, and the final model was selected based on the lowest AIC. Model validation was performed using Dunn-Smyth residual plots (Niku et al., 2019). However, due to the size of the complete dataset (n=514) and the computational power required it was not possible calculate the standard errors for the optimal model. Therefore, a second MGLLVM was carried out, using a subset of the data (n=115), where 23 individuals were randomly selected from each seasonal spawning group.

3.3.4.3 Hierarchical cluster analysis

Hierarchical cluster analysis was performed on the latent variables (LVs) extracted from the MGLLVM performed on a subset of the data with 23 individuals randomly selected from each seasonal spawning group. This was used to determine if variables not included in the multivariate model (e.g., Spawning group; Hatching Period) may account for the unexplained variation. The clustering tendency of the resulting LVs was investigated using the Hopkins statistic (Lawson and Jurs, 1990), which measures the probability that a given dataset is generated by a uniform data distribution and tests the spatial randomness of the data. Hierarchical clustering was performed using a Euclidean distance matrix and the complete method. Subsequently, the optimal number of clusters was determined by calculating 30 validity indices that predicted the number of clusters based on the data, the rule of majority was used to select the optimal number of clusters (Charrad et al., 2014). The final hierarchical clustering was visualised using a dendrogram. The percentage contribution of the seasonal spawning groups to each cluster was calculated. Subsequently, the original elemental ratios were plotted against the corresponding hatching periods and individuals were highlighted by their cluster assignment, in order to investigate the possibility of a temporal effect underlying the LVs extracted from the MGLLVM.

All statistical analysis was performed in R 4.2.0 (R Core Team, 2022). The following packages were used: *tidyverse* (Wickham et al., 2019) for compiling the dataset and visualisation of data; *measurements* (Birk, 2019) for converting spatial coordinates into decimal degrees; *lubridate* for calculation of hatching dates (Grolemund and Wickham, 2011); *FSA* for calculation of APE and APC (Ogle et al., 2022); *fs* for compilation of LA-ICP-MS output files (Hester et al., 2021); *ggforce* for visualisation during data exploration and cleaning of trace elemental data (Pedersen, 2022); *mgcv* for GAMM implementation (Wood, 2017, 2011); *gratia* for GAMM visualisation and diagnostics (Simpson, 2022); *gllvm*, for MGLLVM implementation (Niku et al., 2021, 2019); *factoextra* for cluster analysis and visualisation (Kassambara and Mundt, 2020); and *NbClust* for determination of the number of clusters most representative of the final dataset (Charrad et al., 2014). Spatial data was visualised using *QGIS 3.4.12 Madeira* (QGIS Development Team, 2022).

3.4 Results

3.4.1 Statolith ageing & spawning group assignment

Three independent statolith age estimates were successfully performed on a subsample of 50 individuals, an APE of 2.54% and an ACV of 3.45% were calculated, which is consistent with previous ageing estimates for *I. argentinus* (Bainy and Haimovici, 2012). The methods used for statolith microstructure analysis were confirmed as these values indicated a high level of reproducibility.

A total of 720 individuals were successfully aged, of which 625 were used for subsequent analysis, with a mean age of 181 days and a range of 110– 230 days. The back-calculated hatching dates revealed that continuous hatching occurred throughout the year, with a distinct peak from early August until late October. The following seasonal spawning groups, and associated subgroups were identified: i) winter-spawning ESPS (n=307) and LSPS (n=27); ii) spring-spawning High Seas SpSS (HS SpSS; n=196) and Falkland Islands SpSS (FI SpSS; n=43); and iii) summer-spawning SSS (n=52).

The winter-spawning groups (ESPS and LSPS) are characterised by peak hatching in austral winter (July – September), with individuals predominantly distributed south of 46˚S. Statolith microstructure analysis revealed a notable difference between the two subgroups, where the LSPS group presented narrow daily increments in the latter part of the dark zone of the statolith (250 µm onwards) up to 6 increments per 20 µm, and for ESPS >5 daily increments were observed per 20 µm.

The spring-spawning groups (HS SpSS and FI SpSS) exhibited the longest hatching period over the austral spring starting from early September until late December. The two subgroups were identified on the basis of the distinct statolith microstructure observed in the statolith dark zone, HS SpSS presented wide daily increments (5 per 20 µm), whereas FI SpSS had >6 daily increments per 20 µm. Furthermore, the two groups were found to occur at two distinct locations, HS SpSS was distributed north of 46˚S, and FI SpSS was distributed south of 50˚S.

There was only one summer spawning group (SSS) identified in the current study, occurring primarily north of 46˚S, with a distinct hatching peak identified in early January. Statolith microstructure analysis of this group revealed the widest daily increments compared with other groups, at <5 per 20 µm. The relevant information used to assign individuals to spawning groups described was presented in *Appendix A.* More detailed description of the spawning groups can be found in **Chapter II** of this dissertation.

3.4.2 Trace elemental signatures

The element with the highest concentration in the 20 µm statolith core region investigated was ^{23}Na, followed by ^{88}Sr (Table 2). ^{55}Mn was found to have the lowest concentration from all the elements investigated. No correlation coefficient above 0.35 was identified between the elemental ratios investigated. All data exploration, model selection, and model validation procedures are presented in *Appendix B*.

Table 2. *Illex argentinus* statolith elemental concentrations of the 20 µm statolith core region standardised to Ca and expressed as µmol:mol^{-1}. SD, standard deviation

Elemental ratio	Minimum	Maximum	Mean ± SD
Na:Ca	15,070.87	34,223.37	23179.97 ± 1862.92
Sr:Ca	5,860.31	15,787.19	9621.57 ± 1057.44
Mg:Ca	73.35	1,392.02	442.25 ± 180
Fe:Ca	33.39	1,656.57	315.99 ± 156.18
B:Ca	9.79	819.40	129.62 ± 83.53
Li:Ca	1.22	155.12	20.22 ± 13.05
Ba:Ca	0.43	27.32	8.54 ± 3.32
Mn:Ca	0.28	18.23	3.78 ± 2.06

3.4.2.1 Single-elemental signatures

The optimal GAMMs of elemental ratios confirmed the importance of distance from the statolith core, even over the relatively short 20 µm distance (Table 3). Additionally, the spawning groups were significantly associated with Element:Ca ratios for all univariate models presented.

For Sr:Ca ratios, the optimal model contained spawning group. The effect of distance was small, where an initial decline was observed until 10 µm region of the statolith core, followed by a slight increase. It is of note, that during model selection the option of removing the smoother was investigated, nonetheless the smoother was shown to be important and was retained in the model (edf=1.46; $p<0.01$). Sr:Ca levels were significantly different in each spawning group (F=4.73; $p<0.05$; Table 3), the SSS group had the highest Sr:Ca levels (>10, 000 µmol:mol^{-1}), whereas the HS SpSS group had the lowest levels at 9, 500 µmol:mol-1 (Fig. 3A).

The optimal model for Mg:Ca ratios included spawning group (F=6.75; $p<0.0001$; Table 3; Fig. 3B) and sex (F=4.16; $p<0.05$) as significant main terms. A smoother for the 20 µm distance over the statolith core region that was varied by spawning group was included. Minor heterogeneity was observed during model validation (see App. B3). The autocorrelation plot of normalized residuals suggested autocorrelation may be an issue. Autoregressive functions were considered but deemed beyond the scope for the present study. Therefore, the findings regarding Mg:Ca ratios presented here should be treated with caution. Overall, Mg:Ca ratios showed a decline in all spawning groups over the 20 µm distance, with ESPS, FI SpSS and SSS presenting almost linear trends. For LSPS and HS SpSS the decrease in Mg:Ca was more gradual at the beginning of the core region, up to 10 µm and sharply decreased thereafter (Fig. 3B). Mg:Ca ratios differed significantly between spawning groups, with FI SpSS exhibiting the highest concentration starting at ~550 µmol:mol^{-1}, and SSS had the lowest concentration ~400 µmol:mol^{-1}. It is of note that Mg:Ca ratios were higher in all spawning groups for males (Table 3).

For Ba:Ca ratios, separate distance smoothers were included for each spawning group in the final model along with spawning group as a fixed effect (*Table 3; Fig. 3C*). ESPS, LSPS and FI SpSS exhibited a steady increase of Ba:Ca ratios from the statolith core at ~ 7 µmol:mol^{-1} to under 9 µmol:mol-1 over the 20 µm distance. HS SpSS and SSS showed more interesting patterns over distance, as for HS SpSS ratios started low (>8.5 µmol:mol^{-1}) increased until 5 µm only to decline again at 10 µm, and finally increase to 9.5 µmol:mol^{-1} at 20 µm. Conversely, SSS exhibited the opposite pattern, starting at higher Ba:Ca ratios in the immediate core (9.5 µmol:mol^{-1}), followed by a decrease below 9 µmol:mol^{-1} until 10 µm, and subsequent levelling off by 20 µm. Overall Ba:Ca ratios significantly differed between spawning groups (F=4.08; p<0.01; Fig. 3C), where HS SpSS had the highest levels on average (>9 µmol:mol^{-1}) and FI SpSS ratios were low at >8 mol:mol^{-1}.

The optimal model identified for Mn:Ca ratios included spawning groups as a significant covariate (F=5.40; p<0.001; Table 3; Fig. 3D) and a smoother for the 20 µm distance from the statolith core (edf=3.14; p<0.0001). The distance smoother presented a slightly U-shaped pattern with the lowest Mn:Ca levels recorded at 10 µm. The LSPS group had the highest overall levels with >4 µmol:mol^{-1}, and the lowest levels were recorded in the SSS group at 3 µmol:mol^{-1}.

Table 3. Estimated parameters for generalised additive mixed models for the response variables as presented in Eq 4 to Eq 7. edf, expected degrees of freedom; DE, deviance explained; 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.

Response variable	Explanatory variable	edf	F-value	p-value	DE (%)
Sr : Ca	Spawning group	-	4.73	<0.001***	53.3
	Distance	2.45	0.93	<0.05*	
Mg : Ca	Spawning group	-	6.75	<0.0001***	90.2
	Sex	-	4.16	<0.05*	
	Distance:ESPS	2.18	322.14	<0.0001***	
	Distance:LSPS	2.8	25.43	<0.0001***	
	Distance:HS SpSS	3.7	387.21	<0.0001***	
	Distance:FI SpSS	1.05	87.50	<0.0001***	
	Distance:SSS	1.63	20.29	<0.0001***	
Ba : Ca	Spawning group	-	4.08	<0.01**	36.0
	Distance:ESPS	1.01	8.55	<0.0001***	
	Distance:LSPS	0.66	0.20	0.09	
	Distance:HS SpSS	3.24	8.56	<0.0001***	
	Distance:FI SpSS	0.96	2.34	<0.0001***	
	Distance:SSS	1.91	0.69	<0.05*	
Mn : Ca	Spawning group	-	5.40	<0.001***	33.3
	Distance	3.14	3.27	<0.0001***	

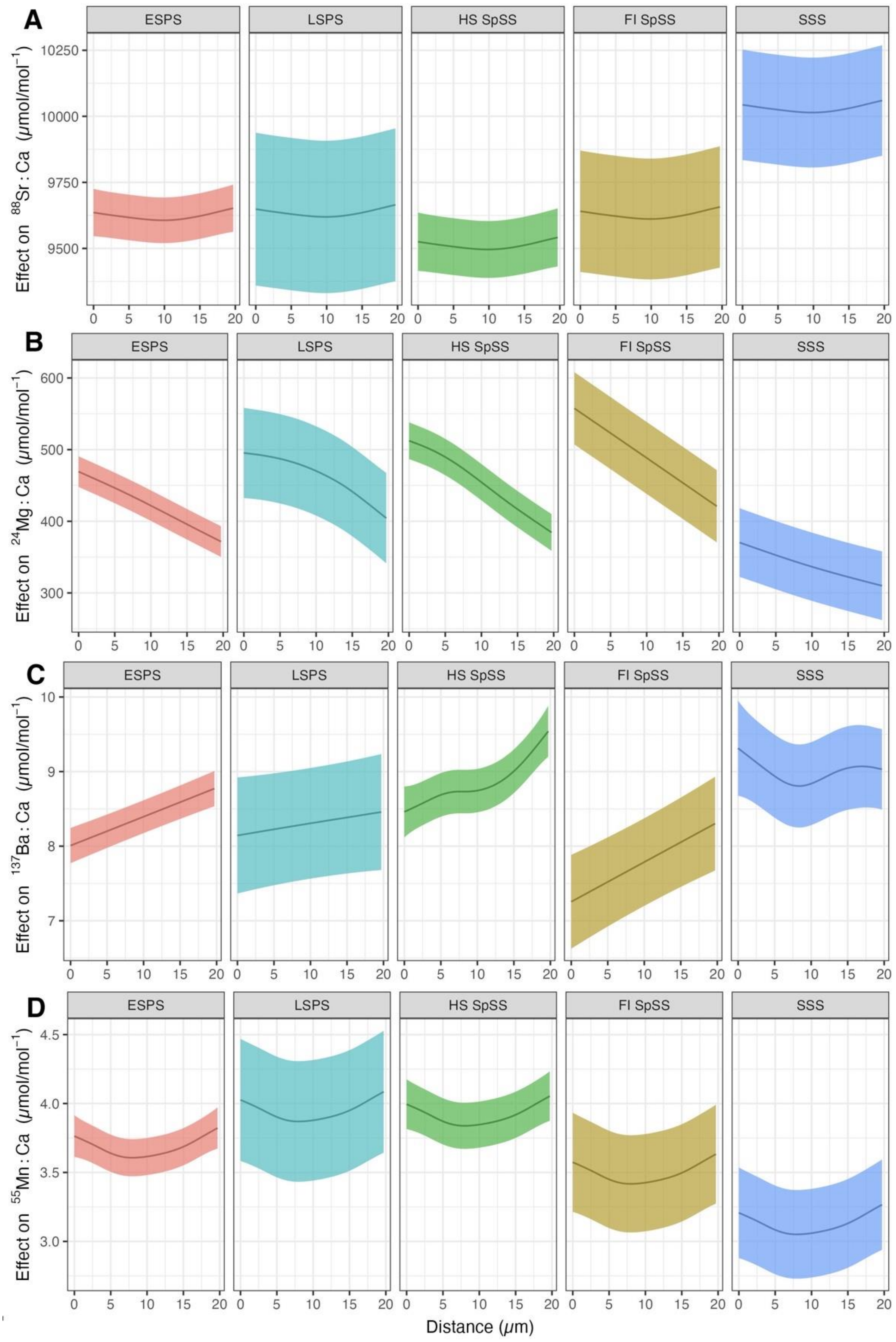

Figure 3. Generalized additive mixed model results of (A) Sr:Ca, (B) Mg:Ca ratios (smoothing curves fitted per spawning group) of females, (C) Ba:Ca (smoothing curves fitted per spawning group), (D) Mn:Ca, as a function of the 20 µm statolith core region for the spawning groups, with 95% confidence intervals shaded. 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.

3.4.2.2 Multi-elemental signatures

The optimal MGLLVM model for both the complete (n=514) and subsampled (n=115) dataset included 20 µm distance and two latent variables. As the standard errors could not be calculated for the optimal model using the complete dataset due to excessive computing requirements, the findings from subsampled dataset are presented. The subsequent ordination of the latent variables from the optimal model was consistent across the complete dataset and the subsampled one (*Fig. 4; App. B*).

Model validation was carried out by examination of Dunn-Smyth residuals. The majority of the confidence intervals included zero; however, a strong association between distance and Ba:Ca ($p<0.05$) and Mg:Ca ratios ($p<0.0001$) was identified. Two latent variables were predicted, suggesting that there is a common pattern of statolith core elemental ratios, after accounting for the distance effect and the random effect of multiple measurements per statolith. The ordination plot of the latent variables revealed that spawning group did not account for the unexplained variation represented by the latent variables (*Fig. 4*).

The clustering tendency of the LVs extracted from the MGLLVM model of the subsampled dataset was calculated as a Hopkins statistic of 0.94, a value higher than 0.75 indicated a clustering tendency at the 90% confidence level. Therefore, the clusters identified subsequently, were considered to be meaningful. Three clusters were identified to optimally represent the data using the majority rule following the calculation of 30 indices using hierarchical clustering of Euclidean distance matrix and the complete method (*Fig. 5)*. Clusters 1 and 2 contained a balanced quantity of each of the spawning groups, whereas cluster 3 (the smallest cluster) was primarily comprised of the SSS group (*see App. C*). This confirmed that the LVs extracted from the MGLLVM are not representative of spawning groups. No fine-scale temporal patterns in cluster assignment were evident upon investigation of Sr:Ca ratios plotted against hatching dates as all three clusters were present throughout the different hatching periods (*Fig. 6*). This lack of temporal pattern was also observed for the remaining elemental ratios included in the MGLLVM.

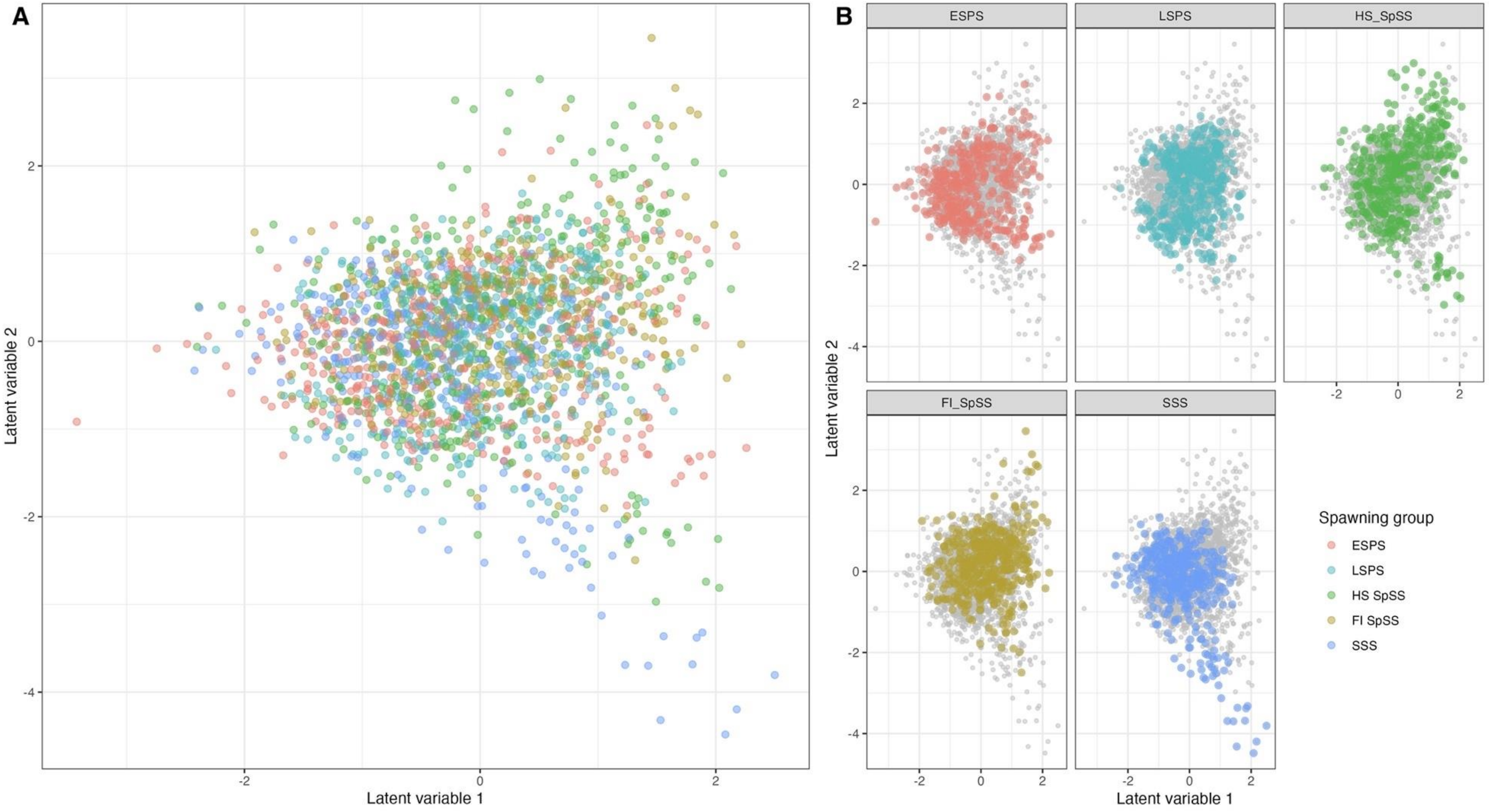

Figure 4. (A) Ordination plot based on the Gaussian multivariate generalised linear latent variable model fitted to the subsampled dataset. The spawning groups were highlighted in different colours, (B) Individual ordination plots with spawning group highlighted to avoid over plotting. ESPS, early-maturing 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.

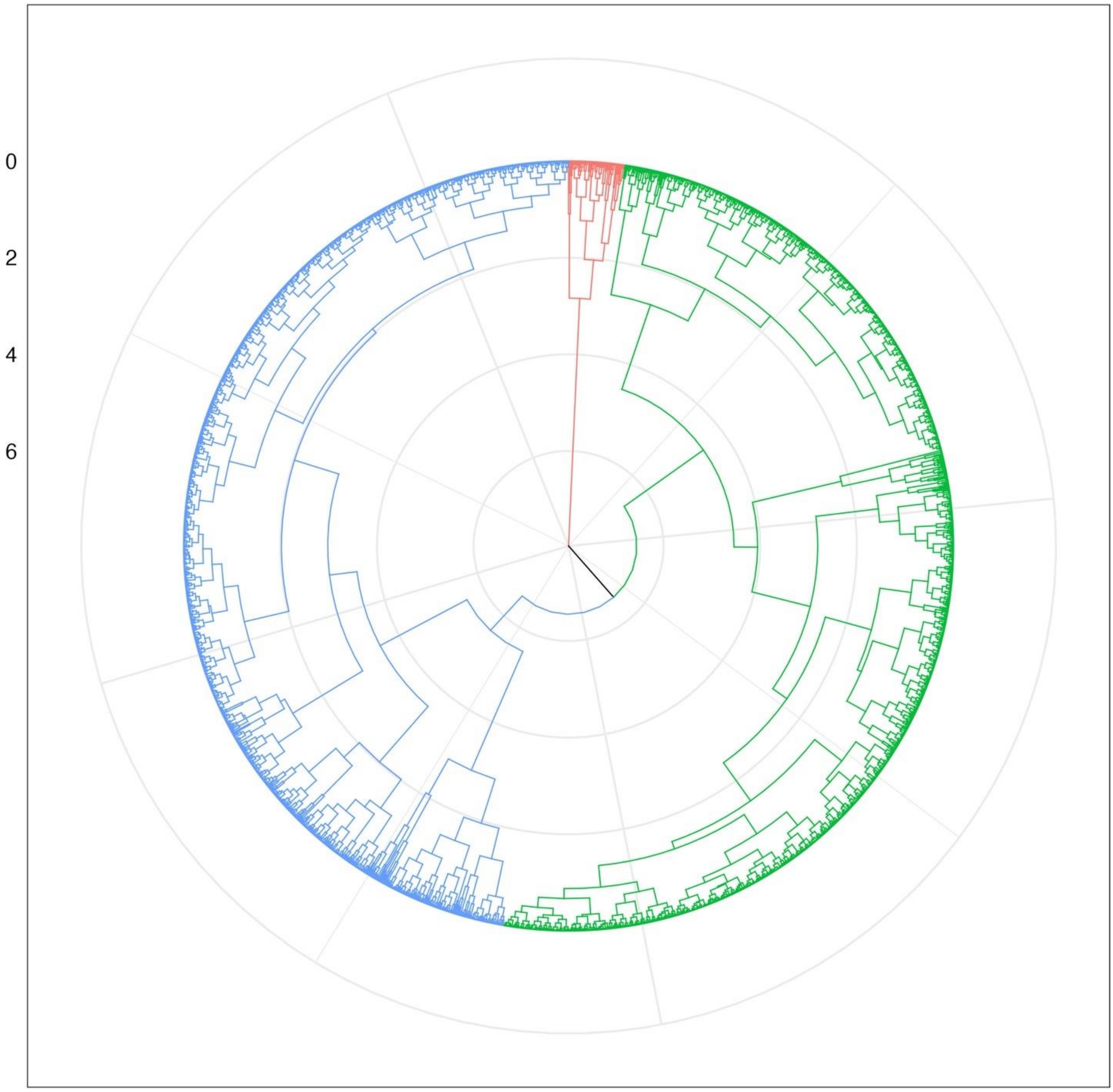

Figure 5. Dendrogram of hierarchical cluster analysis of latent variables extracted from the Gaussian multivariate generalised linear latent variable model fitted to the subsampled dataset. Cluster 1 in green; Cluster 2 in blue; Cluster 3 in red.

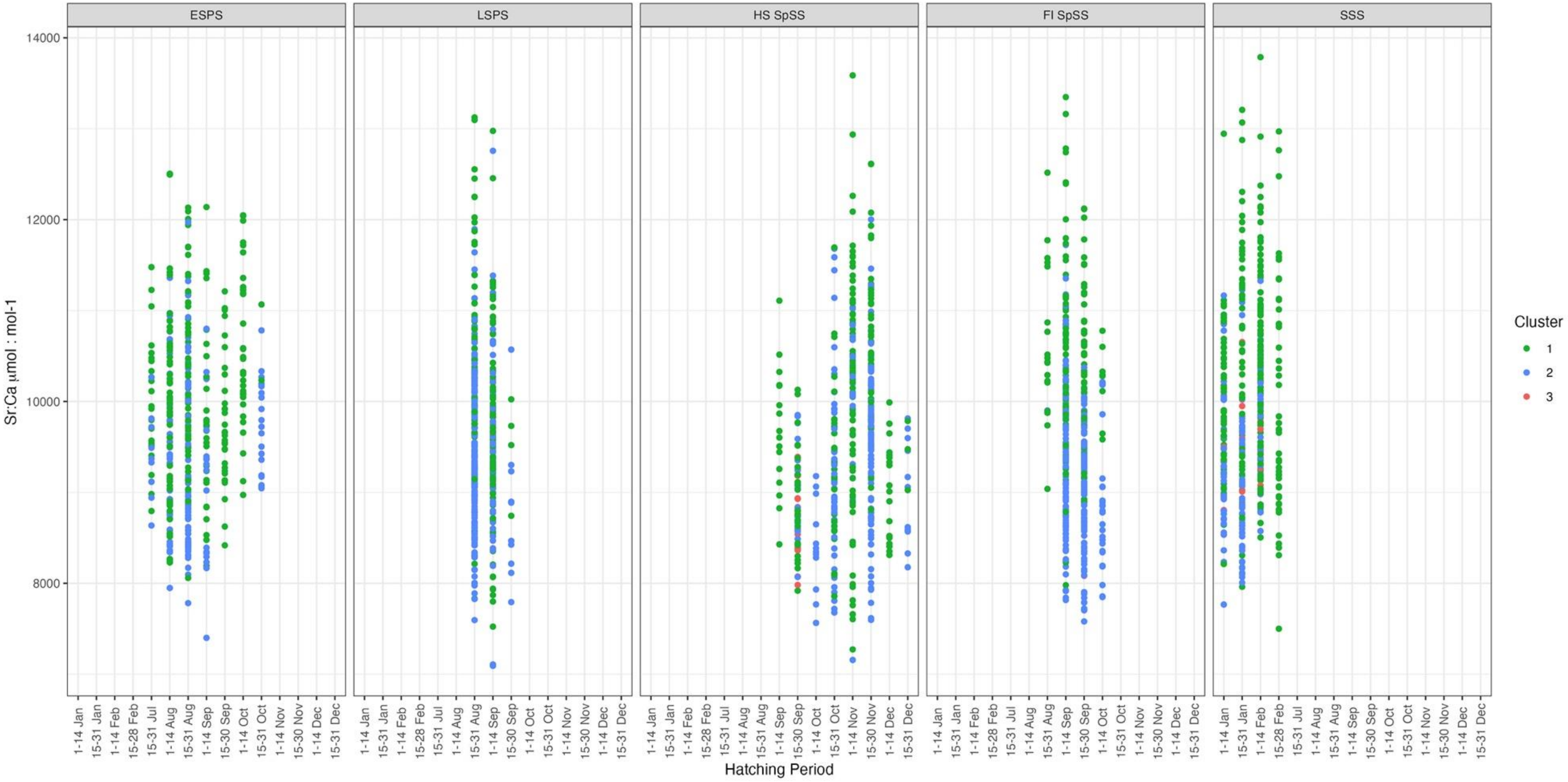

Figure 6. Scatter plots of Sr:Ca ratio levels of *Illex argentinus* 20 µm core region as function of hatching period of the spawning groups. Individual readings were coloured by the clusters identified by hierarchical cluster analysis of the latent variables extracted from the Gaussian multivariate generalised linear latent variable model fitted to the subsampled dataset. 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.

3.5 Discussion

The present study used a novel approach of combining statolith elemental signatures with ageing and microstructure analysis to identify variation between the early ontogeny of *I. argentinus* seasonal spawning groups. These differences were associated with the environmental conditions individuals may experience in the putative hatching grounds. This was achieved through the identification of three seasonal groups: winter, spring; and summer-spawning. Additionally, two subgroups were identified, LSPS from the winter-spawning and FI SpSS from the spring-spawning group. Significant differences in single elemental signatures from the statolith core confirmed the hypothesis that seasonal spawning groups have distinct single elemental signatures. These differences were found to be consistent with environmental conditions (*e.g.*, ambient temperature, local productivity) on the putative hatching grounds of the respective groups. Sr:Ca ratios highlighted possible environmental heterogeneity in hatching conditions, confirming warmer waters as the likely hatching areas of the winter-spawned groups (ESPS and LSPS), compared with the summer-spawned group (SSS). The multi-elemental signatures suggested a high level of connectivity between seasonal spawning groups during early life stages. However, the ordination of multiple elemental signatures revealed two axes of covariation that may be representative of fine-scale structuring within spawning groups. This structuring may be attributed to minor heterogeneity in hatching location within groups as opposed to variation in hatching seasons between groups. Therefore, our results suggest that *I. argentinus* exhibits a high level of connectivity throughout its range and the extent of this spatial connectivity is greater than the temporal disparity introduced through individuals hatching in different seasons.

3.5.1.1 Single elemental signatures

Sr:Ca ratios have frequently been shown to have a negative association with temperature in the hard structures of various marine taxa - scleractinian corals (Beck et al., 1992; Smith et al., 1979); fish *Gadus morhua* (Elsdon and Gillanders, 2002; Stanley et al., 2015); and loliginids *Doryteuthis gahi* (Arkhipkin et al., 2004; Jones et al., 2018) and *Uroteuthis edulis* (Yamaguchi et al., 2015); and ommastrephids *Dosidicus gigas* (Liu et al., 2013, 2016) and *Todarodes pacificus* (Ikeda et al., 1998). In our study, we have observed a seemingly controversial result. The SSS group had the highest Sr:Ca ratios, which would imply that individuals in this group have experienced the lower ambient temperatures despite their summer hatching dates compared with the winter-hatched groups *i.e.*, LSPS and ESPS. This is contrary to expectations, as individuals hatching in the austral summer would be more likely to experience higher ambient temperatures in the first few days of life than those hatched in winter if all have hatched in the same location. However, the SSS group is assumed to spawn and hatch on the shelf south of the Río de la Plata area (Crespi-Abril et al., 2008), where the summer water temperatures do not exceed 12°C (Barré et al., 2006). The main spawning and hatching area for winter spawning ESPS and LSPS groups is assumed to be southern part of the Brazil current (Arkhipkin et al., 2015; Haimovici and Pérez, 1990), where the winter water temperatures reach 26-27°C (Goes et al., 2019). Therefore, the findings of this study indirectly confirm these areas as the hatching locations of the summer- and winter-spawning groups of squid. A similar pattern was observed for the winter and summer spawning stocks of *I. illecebrosus*, where the Sr:Ca ratios during the first few days of life of the summer-spawning group were higher compared with the winter spawning group (Jones and Hendrickson, 2022).

The plausibility of the winter-spawning groups hatching in warmer waters (i.e., southern part of the Brazil Current) is further supported by the Mg:Ca ratios of the ESPS and LSPS being higher than in the SSS group. These differences are unlikely to be due to spatial variation in the occurrence of paralarvae, as Mg has the third longest residence time in the world's oceans after B and Na (Lécuyer, 2016). Mg:Ca ratios have previously been positively associated with deposition of organic matter in the statolith (Bettencourt and Guerra, 2000); which in turn has been hypothesised to be associated with growth rates (Arkhipkin et al., 2004). Mg:Ca ratios have also been associated with high metabolic activity and somatic growth in *G. morhua* otoliths (Heimbrand et al., 2020).Therefore, if the SSS group is occupying colder waters during early ontogenesis (*i.e.*, the Río de la Plata region) they are also more likely to exhibit slower growth rates (Forsythe, 2004; Jackson et al., 1997), which would explain the low Mg:Ca ratios observed for this group in the present study. Additionally, the current study found a significant difference in Mg:Ca ratios between males and females; however, as we are considering only the initial period of a squid's life (3-5 days post hatch) this is difficult to explain in the present study.

The present study also found similarities between the single elemental signatures (Sr:Ca and Mg:Ca) of the spring hatched groups that were sampled in Falkland Islands waters (FI SpSS) and on the high seas (HS SpSS). This is interesting as it would suggest that both groups hatched in similar environmental conditions. It is likely that the similarities observed in the single elemental signatures of these two groups support the idea of a transport of juveniles from the Brazil-Falkland Current Confluence by warm water eddies through the Argentine Basin towards the Falkland Islands. Followed by subsequent migration across the eastern branch of the Falkland Current onto the southern part of the Patagonian Shelf (Parfeniuk et al., 1992).

The patterns observed for Ba:Ca and Mn:Ca ratios were opposite to those of Mg:Ca and Sr:Ca ratios, respectively. The HS SpSS and SSS had the highest Ba:Ca ratios. This is likely to be due to the putative hatching areas of these groups, presumably located in productive shelf waters (Crespi-Abril et al., 2010). Conversely, the putative hatching area of winter-spawning groups is located in oligotrophic waters of the Brazil Current, (Arkhipkin, 2013; Leta, 1992). With regards to Mn, the LSPS group exhibited the highest overall Mn:Ca levels, and SSS the lowest. These patterns are the opposite to those observed for Sr:Ca ratios. This may be explained by the positive association between Mn:Ca ratios and sea surface temperatures previously identified for *D. gigas* (Liu et al., 2013). Manganese is believed to have a “scavenger-type” distribution in the worlds’ oceans, meaning it is characterised by short residence time and distribution that decreases with depth, and distance from sources such as rivers and hydrothermal vents. (Bruland and Lohan, 2006). It has also been suggested that uptake of Mn in statoliths is taxon-specific (Arkhipkin et al., 2004). Therefore, it is possible that is it not taken up in high quantities in *I. argentinus* statoliths in general.

3.5.1.2 Multi-elemental signatures

The present study was unable to identify unique elemental signatures for the seasonal spawning groups. This is contrary to expectations, as multi-elemental signature analyses have previously revealed population structuring based on seasonal and geographic differences in loliginids, including *D. gahi* (Arkhipkin et al., 2004) and *D. opalescens* (Warner et al., 2009). It is also possible that this is due to the low correlation coefficients observed between the elemental ratios, as ordination analyses rely on high correlation between variables (Abdi and Williams, 2010). However, the lack of grouping was consistent with the ommastrephid *D. gigas* as the multi-elemental signature of the paralarval region of the statolith did not reveal differences between the geographic regions sampled (Liu et al., 2015). It is of note, that the paralarval region in the previous study was covering a longer time period and later in an individual’s life (18 to 30 days) compared with the present study (3 to 5 days post-hatch).

Overall, two latent variables were identified using the multi-elemental ratios. This indicates that there was a common characteristic descriptive of the multi-elemental signatures of the individuals presented in the current study. It is possible that the unexplained variation and subsequent clustering are indicative of spatial differences in hatching location within each seasonal group. Individuals may spawn at a common location and common time; however, egg mass dispersal may be influenced by various oceanographic processes, such as currents and eddies (Staaf et al., 2011). This is particularly important as the putative spawning grounds of

I. argentinus are believed to be located in one of the more dynamic areas of the Southwest Atlantic (Acha et al., 2004; Bouali et al., 2017). Therefore, it is plausible that fine-scale spatial heterogeneity of hatching locations exists.

It is of note, that the present study is not directly comparable with a previous study on *I. argentinus* statolith trace elemental ratios by Avigliano *et al.* (2020) as that study did not perform age analysis, thus the assignment to spawning groups was less robust. Furthermore, the laser ablation transect of the previous study did not include the statolith core region, which is the focus of the present study.

3.5.2 Conclusion

To the best of our knowledge, the present study is the first to investigate the early ontogeny of *I. argentinus* spawning groups using statolith core elemental ratios. Single-elemental ratios highlighted potential differences in the environmental conditions that the spawning groups are exposed to post-hatching. However, these seasonal differences were outweighed by the potential spatial effects of hatching locations within spawning groups as the multi-elemental signatures suggested high levels of mixing. This has wider implications for fisheries management, as individual spawning groups may respond to seasonal heterogeneity during early ontogeny and this in turn may result in variable fishery yield (Chemshirova et al., 2021). Furthermore, the high degree of mixing between these seasonal groups indicates that the entire population should be monitored closely in order to ensure the sustainable harvesting of this species (Arkhipkin et al., 2022).

Future studies should consider investigation of the trace elemental signatures of *I. argentinus* paralarvae sampled across the Patagonian Shelf, and comparison with adults of the same year to confirm the hatching grounds of the spawning groups. Further investigation of temporally-resolved elemental profiles from the entire statolith may reveal ontogenetic migrations, which would be invaluable for the confirmation of these ecological groups. Finally, the extent of genetic connectivity between the seasonal spawning groups should also be explored in order to determine the level of interbreeding of these groups and how that is associated with the phenotypic plasticity of *I. argentinus*.

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CRediT authorship contribution statement

Irina Chemshirova: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Writing – original draft; Writing – review & editing. **Will Brownscbombe:** Methodology, Data Curation; **Alexander Arkhipkin**: Conceptualization, Supervision, Resources, Funding Acquisition, Methodology, Writing – review & editing.

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General Discussion

The *Illex argentinus* fishery in the Southwest Atlantic is considered a “straddling stock” (Arkhipkin et al., 2022). This is largely due to the long-range ontogenetic migrations of this species, which leads to individuals passing through the Exclusive Economic Zones of four states (namely Brazil, Uruguay, Argentina, and Falkland Islands) as well as international waters (Arkhipkin et al., 2021, 2015). This fishery is vulnerable to overexploitation, partly due to the cephalopod life history characteristics such as short life, spans, rapid growth rates, and phenotypic plasticity in response to environmental change (Rodhouse, 2001). To safeguard against overexploitation, it is crucial to improve our understanding of population structure, connectivity, and responses to abiotic factors (*e.g.,* temperature) (Doubleday et al., 2016), which can be used to make well-informed management decisions. Therefore, the overarching aim of this dissertation was to investigate the phenotypic plasticity response to changing environmental conditions and to determine the population structure and connectivity of *I. argentinus* on the Patagonian Shelf.

Chapter I investigated the effect of ambient temperature on individual- and population-level characteristics, *i.e.*, dorsal mantle length and proportion of mature females, and abundance, respectively. This chapter focused on the most abundant group of *I. argentinus,* the winter-spawning SPS. The hypothesis that higher temperatures in the hatching grounds would lead to smaller and more mature adults, and increased abundance of *I. argentinus* in Falkland waters was tested. A 20-year dataset obtained from the Falkland Islands Fisheries Department (FIFD) monitoring of the *I. argentinus* jigging fishery was used in conjunction with monthly temperature data from areas along the Patagonian Shelf that correspond to key life stages of the SPS group. The results revealed the importance of the sea surface temperature on the hatching grounds. Temperatures above 17.5˚C were associated with higher abundance and the harvesting of smaller and immature females during the fishing season in the Falkland Islands Interim Conservation and Management Zone (FICZ). The finding of more immature females is of a particular interest. High temperatures are generally expected to lead to smaller adults that mature at a faster rate compared with their cold-water counterparts resulting in an extended adult phase of the life cycle (Jackson and Moltschaniwskyj, 2002; Rodhouse et al., 2014). It is possible that additional factors govern maturation of individuals, such as resource availability. Starvation of *Illex illecebrosus* females in an experimental setting led to acceleration of maturation (Rowe and Mangold, 1975). However, feeding, as opposed to energy storage, in *I. argentinus* significantly contributes to the onset of maturation (Lin et al., 2019). Therefore, in years where food availability is low, maturation in *I. argentinus* may be delayed, leading to lower proportions of mature females.

Following from this, it is possible that for *I. argentinus,* differences in feeding regimes at different life stages lead to different maturation rates later in life. This possibility is underscored by the finding that temperatures above 15.5˚C in the migration areas led to larger individuals and higher proportion of mature females being harvested in the FICZ. The prospect of the existence of two migration routes with different ambient temperatures resulting in two groups: i) large and mature; and ii) small and immature individuals as previously observed in *Uroteuthis edulis* should also be considered (Yamaguchi et al., 2019). Temperatures above 21.5˚C in the hatching grounds also had a positive effect on the abundance of *I. argentinus* in the fishing area. This is consistent with previous findings that positive sea surface anomalies on the hatching grounds, during peak hatching times (August until October) resulted in high abundance of the SPS group during the fishing season (Nigmatullin et al., 2004). This chapter identified a baseline response to variability in ambient temperature of population and individual-level characteristics of *I. argentinus*. This may allow for improved forecasting of fishery yield that may in turn inform harvesting thresholds set by fishery managers.

Chapter II aimed to distinguish between seasonal spawning groups using statolith ageing and microstructure analysis and apply this information to subsequent population genomic investigation in order to determine the degree of connectivity between groups. Two hypotheses were tested: i) individuals collected at different times and locations have different hatching dates and different statolith microstructures, thus belong to different seasonal groups; and ii) *Illex argentinus* consists of one single genetically connected population. Statolith ageing and microstructure analyses revealed three seasonal groups, each with respective subgroups: i) winter-hatching (ESPS and LSPS); ii) spring-hatching (HS SpSS and FI SpSS); and iii) summer-hatching (SSS and UN). These seasonal groups are consistent with findings of previous studies on *I. argentinus* population structure (Brunetti, 1988; Crespi-Abril and Barón, 2012; Nigmatullin, 1989). However, this chapter provided the first description of the aforementioned subgroups. The individuals assigned to the FI SpSS and UN groups are particularly interesting as this is the first record of spring and summer-hatched individuals occurring as far as 52˚S. This finding supports the hypothesis put forward by Parfeniuk, Froerman and Golub (1992) of a southward transport of spring-hatched juveniles from the Brazil-Falkland Confluence, located at ~38˚S, to the southern part of the Argentine Basin. Followed by an active migration through the eastern branch of the Falkland Current to the southern part of the Patagonian Shelf.

This level of dispersal also contributes to the explanation of the lack of neutral and non-neutral genetic structure that was found following the analysis of over 10 000 genome-wide SNPs. This finding is consistent with previous genomic studies in commercial loliginid species including *Doryteuthis opalescens* (Reichow and Smith, 2001) and *D. gahi* (McKeown et al., 2019). Investigation of the genetic structure in the ommastrephid *Dosidicus gigas* using traditional markers (Sanchez et al., 2016) and genome-wide SNPs (Ibáñez et al., 2022; National Institute of Fisheries Science, 2022) also found high gene flow and connectivity between groups. Another notable finding of this chapter was the lack of heterozygosity evident across all samples. This may indicate variability in recruitment success across the seasonal groups identified. Fine-scale temporal structuring has been recently reported in *D. opalescens* (Cheng et al., 2020) which was attributed to recruitment pulses. Therefore, it is plausible that the heterozygote deficits observed presently in *I. argentinus* are due to a similar mechanism, where transient genetic differentiation is triggered due to the failure or success of a subgroup to survive to adulthood. Subsequently the differentiation is erased due to mixing of adult individuals in spawning grounds.

The high level of population connectivity described in **Chapter II** was confirmed by findings in **Chapter III**. The aim of this chapter was to investigate the differences between the seasonal groups in relation to the putative hatching grounds, and the population connectivity of *I. argentinus* individuals during this life stage. This was achieved by pairing statolith ageing and microstructure analyses with elemental signatures from the statolith core (representing the first 3-5 days of life). The hypothesis that different seasonal groups would present different single elemental signatures was tested, and significant differences were found between groups. Furthermore, this chapter evaluated if these differences were consistent with the environmental conditions on the putative hatching grounds. The final aim of this chapter was to identify multi-elemental signatures, which are unique to the identified seasonal groups. Statolith ageing and microstructure analysis identified the same seasonal groups as previously described in **Chapter II** using the same criteria for assignment to seasonal spawning groups. The UN subgroup was not included in **Chapter III** as there was only a small number of specimens collected that may belong to this subgroup (n=16) and including them may have reduced the statistical power of the subsequent analysis.

All single-elemental ratios investigated revealed significant differences between the seasonal spawning groups. The negative association of Sr:Ca ratios with temperature has been previously established in the ommastrephids *D. gigas* (Liu et al., 2013, 2016) and *Todarodes pacificus* (Ikeda et al., 1998). Upon initial consideration, the findings of **Chapter III** would appear to contradict this relationship, as summer-hatched individuals (SSS group) had the highest Sr:Ca ratio. This may suggest that they encountered lower temperatures in their early ontogeny during the austral summer, compared with their winter-hatching counterparts. However, it is possible that this finding is an indirect confirmation of the respective hatching areas of these seasonal groups. The putative hatching area of the summer-hatching group has been proposed to be the shelf south of the Río de la Plata area (Crespi-Abril et al., 2008) and here summer water temperatures are rarely above 12˚C (Barré et al., 2006). Conversely, the winter-hatching groups are purported to hatch in the southern part of the Brazil Current (Arkhipkin et al., 2022; Haimovici and Pérez, 1990), where winter water temperatures are ~27˚C (Goes et al., 2019). In addition, the higher Sr:Ca ratio found for the SSS group contradicts the proposed hatching area for the winter-hatching groups (*e.g.,* ESPS and LSPS) as suggested by Haimovici et al. (1998) and Torres Alberto et al. (2020). According to their hypothesis the winter groups should hatch between 45˚S and 48˚S on the outer shelf and shelf-break. However, if this was the case the LSPS and ESPS groups in the present study should have higher Sr:Ca ratios than the SSS group, whereas our findings showed the opposite.

Multi-elemental signatures could not be identified for the seasonal spawning groups. However, two latent variables of unexplained variation were derived from the ordination of the multi-elemental ratios. This suggests that there was a commonality in these chemical variables that was not explained by any of the variables measured in **Chapter III**. The clustering of these latent variables may reveal fine-scale heterogeneity in hatching location within seasonal spawning groups. Differences in dispersal of egg masses may lead to this result, as the egg masses of individuals that spawn at a shared time and location may be affected by numerous oceanographic features, such as eddies and currents (Staaf et al., 2010). This is consistent with the findings of **Chapter II**, where the lack of heterozygosity may be indicative of variable breeding success within groups; further confirming the importance of environmental conditions on the hatching grounds described by **Chapter I**. The confirmation of the high level of population connectivity in **Chapters II and III** may allow for the extrapolation of the fundamental relationships established in **Chapter I** to the remaining spawning groups, provided information on the location of relevant hatching grounds and subsequent ontogenetic migrations becomes available.

Direction of future work

There are numerous possible directions future research of *I. argentinus* may take due to the complexity and difficulty of studying migratory marine species. Therefore, the following suggestions could build upon the findings described in this dissertation.

Firstly, there is a need for examination of the entire trace elemental chronologies of statoliths, in order to elucidate the migration patterns of seasonal groups described in this dissertation. Based on the microstructure analysis presented in **Chapters II and III**, individuals from different seasonal spawning groups may experience different environmental conditions throughout ontogeny, possibly due to ontogenetic migrations. Understanding the migration patterns of seasonal spawning groups and how environmental factors impact individuals is crucial in determining the spatial and temporal connectivity between groups. Furthermore, the use of the entire elemental profiles may provide insight into important life history change points that would otherwise be difficult to detect (Rooker et al., 2021).

The application of statolith elemental signatures can be extended if paralarvae are sampled throughout the Patagonian Shelf and compared with adults in the same year. This may confirm the existence of fine-scale spatial structuring of the seasonal groups during early ontogeny hypothesised in **Chapter III**. Furthermore, the identification of the elemental composition of paralarval statoliths may allow for the application of statolith "chemoscapes" (Burns et al., 2020). This method produces a continuous-surface profile of the microchemistry of marine regions, which has the potential highlight important areas for the early life stages of *I. argentinus*. Furthermore, these chemoscapes may determine the extent of connectivity between hatching regions and areas of fishery activity, which may inform fishery management strategies *i.e.,* closures of certain areas. These paralarvae studies may also allow us to pinpoint the hatching grounds of the different seasonal spawning groups with more certainty. Subsequently, the relationships between fishing yield (abundance) and quality (size and maturity) of squid and temperatures in areas important for the life cycle of this species established in **Chapter I** could be verified for the remaining seasonal spawning groups.

Statolith shape should also be considered in comparisons between the seasonal spawning groups described. It may reveal fine-scale structuring that the methods used presently were unable to detect, as previously found in *L. forbesii* (Sheerin et al., 2022), as statolith shape is the result of both genetic and environmental influences combined (Lishchenko and Jones, 2021).

The sampling for this dissertation was extensive; however, it was not possible to obtain samples from the southern Brazil range of *I. argentinus* during 2020. A comparison of specimens between this region and those occurring on the southern part of the Patagonian Shelf would be of interest, as a bottom trawl fishery has been operating off the coast of Brazil since the early 2000s (Alvarez Perez et al., 2009). Another investigation combining genetic approaches with statolith life history data as presented in **Chapter II**, may reveal whether the individuals found in this part of the species' range are a peripheral population that is reproductively isolated or if they should also be considered part of the metapopulation (Fisher et al., 2022). The extent of the connectivity between these groups would allow for discussions regarding a possible regional management organisation in the Southwest Atlantic. If there is evidence of potential adaptive divergence in this population, these findings may in turn be useful for untangling the response of this species to future climate change (Spies et al., 2021).

Conclusion

This dissertation revealed the extent of the connectivity and interbreeding of the seasonal spawning groups of *I. argentinus* through a combination of life history information, statolith ageing, microchemistry, and genomics. The significant effects of temperature on individual and population characteristics determined in **Chapter I** and the high level of the connectivity between seasonal groups in this species confirmed by **Chapters II and III** show the imperative need for data sharing across the Southwest Atlantic. Only with the participation of all coastal states and the international distant water fleets involved in harvesting of *I. argentinus* would it be possible to ensure the successful and sustainable management of the fishery. In conclusion, the findings presented in this dissertation, provide fisheries managers and government officials with the scientific support required to improve communication and data sharing between stakeholders worldwide.

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Chapter I: Appendices

Appendix A

$$DML_i \sim Normal(\mu_i, \sigma^2)$$

$$E[DML_i] = \mu_i$$

$$\mu_i = \beta_1 + \beta_2 \times \overline{T}_i + \varepsilon_i$$

$$\varepsilon_i \sim Normal(0, \sigma^2)$$

(Eq. A.1)

$$CPUE_i \sim Normal(\mu_i, \sigma^2)$$

$$E[CPUE_i] = \mu_i$$

$$\mu_i = \beta_1 + \beta_2 \times \overline{T}_i + \varepsilon_i$$

$$\varepsilon_i \sim Normal(0, \sigma^2)$$

(Eq. A.2)

$$DML_i \sim Normal(\mu_i, \sigma^2)$$

$$E[DML_i] = \mu_i$$

$$\mu_i = \beta_1 + \beta_2 \times CPUE_i + \varepsilon_i$$

$$\varepsilon_i \sim Normal(0, \sigma^2)$$

(Eq. A.3)

Appendix B

Appendix B.1. Model coefficients, standard errors and significance tests for linear models of mean DML as a function of mean temperature in (A) Hatching grounds and (B) Juvenile grounds, during the hatching period. DML, dorsal mantle length; SE, standard error; RSE, residual standard error; H, hatching grounds; J, juvenile grounds.

Box	Month	Period	Sex		Slope	SE	t value	p-value	$F_{1,18}$	RSE	R^2
(A)											
H_33S_51W	Jul	Hatching	Female	Intercept	34.446	5.160	6.676	<0.001	2.183	1.168	0.108
				meanT	-0.464	0.314	-1.477	0.157			
H_33S_51W	Jul	Hatching	Male	Intercept	29.495	3.826	7.708	<0.001	1.839	0.866	0.093
				meanT	-0.316	0.233	-1.356	0.192			
H_33S_51W	Aug	Hatching	Female	Intercept	28.980	3.205	9.043	<0.001	0.452	1.221	0.025
				meanT	-0.137	0.204	-0.673	0.51			
H_33S_51W	Aug	Hatching	Male	Intercept	26.142	2.346	11.143	<0.001	0.613	0.894	0.033
				meanT	-0.117	0.149	-0.783	0.444			
H_33S_49W	Jul	Hatching	Female	Intercept	37.001	6.186	5.982	<0.001	2.707	1.153	0.131
				meanT	-0.530	0.322	-1.645	0.117			
H_33S_49W	Jul	Hatching	Male	Intercept	30.867	4.626	6.672	<0.001	2.011	0.862	0.100
				meanT	-0.342	0.241	-1.418	0.173			
H_33S_49W	Aug	Hatching	Female	Intercept	37.712	6.846	5.509	<0.001	2.529	1.158	0.123
				meanT	-0.576	0.362	-1.590	0.129			
H_33S_49W	Aug	Hatching	Male	Intercept	32.060	5.055	6.342	<0.001	2.352	0.855	0.116
				meanT	-0.410	0.268	-1.534	0.142			
H_35S_51W	Jul	Hatching	Female	Intercept	31.184	5.835	5.344	<0.001	0.557	1.218	0.030

				meanT	-0.240	0.321	-0.747	0.465			
H_35S_51W	Jul	Hatching	Male	Intercept	26.319	4.331	6.078	<0.001	0.215	0.904	0.012
				meanT	-0.111	0.238	-0.464	0.648			
H_35S_51W	Aug	Hatching	Female	Intercept	28.673	4.216	6.801	<0.001	0.192	1.230	0.011
				meanT	-0.105	0.241	-0.438	0.667			
H_35S_51W	Aug	Hatching	Male	Intercept	25.826	3.096	8.342	<0.001	0.240	0.903	0.013
				meanT	-0.087	0.177	-0.490	0.63			
(B)											
J_35S_53W	Jul	Hatching	Female	Intercept	32.722	4.555	7.184	<0.001	1.678	1.183	0.085
				meanT	-0.425	0.328	-1.295	0.212			
J_35S_53W	Jul	Hatching	Male	Intercept	28.748	3.341	8.604	<0.001	1.768	0.868	0.089
				meanT	-0.320	0.241	-1.330	0.2			
J_35S_53W	Aug	Hatching	Female	Intercept	29.526	2.678	11.024	<0.001	1.022	1.203	0.054
				meanT	-0.204	0.201	-1.011	0.325			
J_35S_53W	Aug	Hatching	Male	Intercept	26.327	1.967	13.383	<0.001	1.060	0.884	0.056
				meanT	-0.152	0.148	-1.029	0.317			

Appendix B.2. Model coefficients, standard errors and significance tests for linear models of mean DML as a function of mean temperature in the (A) Hatching grounds, (B) Juvenile grounds and the (C) Migration paths. DML, dorsal mantle length; SE, standard error; RSE, residual standard error; H, hatching grounds; J, juvenile grounds; MI, migration inshore; MO, migration offshore.

Box	**Month**	**Period**	**Sex**		**Slope**	**SE**	**t value**	**p-value**	**$F_{1,18}$**	**RSE**	**R^2**

(A)											
H_33S_49W	Nov	Juvenile	Male	Intercept	39.589	6.023	6.573	<0.001	6.438	0.780	0.263
				meanT	-0.737	0.290	-2.537	0.021			
H_33S_51W	Nov	Juvenile	Male	Intercept	38.177	5.454	7.000	<0.001	6.468	0.780	0.264
				meanT	-0.685	0.269	-2.543	0.02			
H_33S_51W	Dec	Juvenile	Female	Intercept	52.902	8.207	6.446	<0.001	10.098	0.990	0.359
				meanT	-1.173	0.369	-3.178	0.005			
H_33S_51W	Dec	Juvenile	Male	Intercept	42.002	6.280	6.688	<0.001	7.940	0.757	0.306
				meanT	-0.796	0.282	-2.818	0.011			
H_35S_51W	Dec	Juvenile	Male	Intercept	39.979	6.894	5.799	<0.001	5.168	0.801	0.223
				meanT	-0.723	0.318	-2.273	0.035			
H_33S_51W	Jan	Feeding	Male	Intercept	42.350	7.354	5.759	<0.001	6.019	0.787	0.251
				meanT	-0.742	0.302	-2.453	0.025			
H_33S_49W	Jan	Feeding	Female	Intercept	55.357	10.940	5.060	<0.001	6.802	1.053	0.274
				meanT	-1.194	0.458	-2.608	0.018			
H_33S_49W	Jan	Feeding	Male	Intercept	45.167	8.061	5.603	<0.001	6.697	0.776	0.271
				meanT	-0.873	0.337	-2.588	0.019			
H_35S_51W	Mar	Feeding	Female	Intercept	44.807	8.529	5.253	<0.001	4.445	1.107	0.198
				meanT	-0.759	0.360	-2.108	0.049			
(B)											
J_35S_53W	Nov	Juvenile	Female	Intercept	38.498	5.162	7.458	<0.001	5.119	1.091	0.221
				meanT	-0.644	0.285	-2.262	0.036			

J_35S_53W	Nov	Juvenile	Male	Intercept	34.363	3.589	9.575	<0.001	7.861	0.758	0.304
				meanT	-0.555	0.198	-2.804	0.012			
(C)											
MO_38S_55W	Oct	Juvenile	Male	Intercept	31.548	3.132	10.074	<0.001	5.355	0.798	0.229
				meanT	-0.641	0.277	-2.314	0.033			
MO_40S_57W	Jan	Feeding	Female	Intercept	16.134	4.786	3.371	0.003	5.008	1.094	0.218
				meanT	0.610	0.272	2.238	0.038			
MO_44S_61W	Feb	Feeding	Female	Intercept	15.436	5.332	2.895	0.01	4.578	1.104	0.203
				meanT	0.717	0.335	2.140	0.046			
MO_46S_61W	Feb	Feeding	Female	Intercept	16.920	4.547	3.721	0.002	4.766	1.099	0.209
				meanT	0.687	0.315	2.183	0.043			
MO_48S_62W	Feb	Feeding	Female	Intercept	17.266	4.172	4.138	0.001	5.275	1.087	0.227
				meanT	0.717	0.312	2.297	0.034			
MO_50S_61W	Feb	Feeding	Female	Intercept	16.956	3.853	4.400	<0.001	6.594	1.058	0.268
				meanT	0.871	0.339	2.568	0.019			
MO_50S_63W	Feb	Feeding	Female	Intercept	17.584	3.975	4.424	<0.001	5.433	1.084	0.232
				meanT	0.785	0.337	2.331	0.032			

Appendix B.3. Model coefficients, standard errors and significance tests for linear models of mean DML as a function of mean temperature in the inshore feeding grounds during the feeding period. DML, dorsal mantle length; SE, standard error; RSE, residual standard error; MI, migration inshore; MO, migration offshore.

Box	**Month**	**Sex**	**Period**		**Slope**	**SE**	**t value**	**p-value**	**$F_{1,18}$**	**RSE**	**R^2**
MI_46S_65W	Jan	Female	Feeding	Intercept	23.260	5.682	4.094	0.001	0.396	1.223	0.022

				meanT	0.234	0.371	0.629	0.537			
MI_46S_65W	Jan	Male	Feeding	Intercept	21.265	4.162	5.110	<0.001	0.537	0.896	0.029
				meanT	0.199	0.272	0.733	0.473			
MI_46S_65W	Feb	Female	Feeding	Intercept	24.012	4.819	4.983	<0.001	0.344	1.225	0.019
				meanT	0.177	0.302	0.586	0.565			
MI_46S_65W	Feb	Male	Feeding	Intercept	22.943	3.562	6.441	<0.001	0.148	0.905	0.008
				meanT	0.086	0.223	0.385	0.705			
MI_46S_65W	Mar	Female	Feeding	Intercept	29.023	5.434	5.340	<0.001	0.163	1.231	0.009
				meanT	-0.146	0.361	-0.404	0.691			
MI_46S_65W	Mar	Male	Feeding	Intercept	25.112	4.009	6.263	<0.001	0.040	0.908	0.002
				meanT	-0.053	0.266	-0.200	0.844			
MI_46S_65W	Apr	Female	Feeding	Intercept	31.703	6.295	5.037	<0.001	0.600	1.216	0.032
				meanT	-0.374	0.483	-0.774	0.449			
MI_46S_65W	Apr	Male	Feeding	Intercept	25.444	4.697	5.417	<0.001	0.058	0.908	0.003
				meanT	-0.087	0.360	-0.241	0.812			
MI_46S_67W	Jan	Female	Feeding	Intercept	24.719	5.719	4.322	<0.001	0.137	1.232	0.008
				meanT	0.138	0.372	0.370	0.716			
MI_46S_67W	Jan	Male	Feeding	Intercept	21.513	4.169	5.160	<0.001	0.452	0.898	0.024
				meanT	0.182	0.271	0.672	0.51			
MI_46S_67W	Feb	Female	Feeding	Intercept	23.771	4.705	5.052	<0.001	0.425	1.222	0.023
				meanT	0.192	0.294	0.652	0.523			
MI_46S_67W	Feb	Male	Feeding	Intercept	22.471	3.473	6.470	<0.001	0.282	0.902	0.015

				meanT	0.115	0.217	0.531	0.602			
MI_46S_67W	Mar	Female	Feeding	Intercept	26.940	5.868	4.591	<0.001	0.000	1.236	0.000
				meanT	-0.007	0.389	-0.018	0.985			
MI_46S_67W	Mar	Male	Feeding	Intercept	23.320	4.308	5.413	<0.001	0.053	0.908	0.003
				meanT	0.066	0.285	0.231	0.82			
MI_46S_67W	Apr	Female	Feeding	Intercept	31.506	7.197	4.377	<0.001	0.422	1.222	0.023
				meanT	-0.354	0.546	-0.650	0.524			
MI_46S_67W	Apr	Male	Feeding	Intercept	25.531	5.346	4.776	<0.001	0.052	0.908	0.003
				meanT	-0.092	0.405	-0.228	0.822			
MI_40S_59W	Jan	Female	Feeding	Intercept	7.124	9.903	0.719	0.481	3.963	1.119	0.180
				meanT	0.964	0.484	1.991	0.062			
MI_40S_59W	Jan	Male	Feeding	Intercept	10.611	7.366	1.441	0.167	3.462	0.833	0.161
				meanT	0.670	0.360	1.861	0.079			
MI_40S_59W	Feb	Female	Feeding	Intercept	16.311	10.609	1.538	0.142	0.984	1.204	0.052
				meanT	0.501	0.505	0.992	0.334			
MI_40S_59W	Feb	Male	Feeding	Intercept	18.721	7.901	2.369	0.029	0.501	0.897	0.027
				meanT	0.266	0.376	0.708	0.488			
MI_40S_59W	Mar	Female	Feeding	Intercept	31.665	9.966	3.177	0.005	0.235	1.229	0.013
				meanT	-0.242	0.498	-0.485	0.633			
MI_40S_59W	Mar	Male	Feeding	Intercept	26.751	7.353	3.638	0.002	0.110	0.906	0.006
				meanT	-0.122	0.368	-0.332	0.744			
MI_40S_59W	Apr	Female	Feeding	Intercept	36.458	7.579	4.811	<0.001	1.615	1.185	0.082

				meanT	-0.547	0.430	-1.271	0.22			
MI_40S_59W	Apr	Male	Feeding	Intercept	29.531	5.685	5.194	<0.001	0.844	0.889	0.045
				meanT	-0.296	0.323	-0.919	0.37			
MI_40S_61W	Jan	Female	Feeding	Intercept	14.564	6.424	2.267	0.036	3.653	1.127	0.169
				meanT	0.658	0.344	1.911	0.072			
MI_40S_61W	Jan	Male	Feeding	Intercept	16.568	4.848	3.418	0.003	2.556	0.851	0.124
				meanT	0.415	0.260	1.599	0.127			
MI_40S_61W	Feb	Female	Feeding	Intercept	17.747	7.825	2.268	0.036	1.349	1.193	0.070
				meanT	0.470	0.404	1.162	0.261			
MI_40S_61W	Feb	Male	Feeding	Intercept	19.195	5.842	3.286	0.004	0.768	0.890	0.041
				meanT	0.265	0.302	0.876	0.392			
MI_40S_61W	Mar	Female	Feeding	Intercept	29.275	7.677	3.813	0.001	0.101	1.233	0.006
				meanT	-0.132	0.415	-0.318	0.754			
MI_40S_61W	Mar	Male	Feeding	Intercept	25.532	5.653	4.516	<0.001	0.047	0.908	0.003
				meanT	-0.066	0.306	-0.216	0.832			
MI_40S_61W	Apr	Female	Feeding	Intercept	32.839	6.343	5.177	<0.001	0.898	1.207	0.048
				meanT	-0.366	0.387	-0.948	0.356			
MI_40S_61W	Apr	Male	Feeding	Intercept	26.796	4.742	5.650	<0.001	0.275	0.902	0.015
				meanT	-0.151	0.289	-0.524	0.607			
MI_44S_65W	Jan	Female	Feeding	Intercept	16.956	7.468	2.271	0.036	1.751	1.180	0.089
				meanT	0.579	0.438	1.323	0.202			
MI_44S_65W	Jan	Male	Feeding	Intercept	17.421	5.517	3.158	0.005	1.562	0.872	0.080

				meanT	0.404	0.323	1.250	0.227			
MI_44S_65W	Feb	Female	Feeding	Intercept	19.659	6.829	2.879	0.01	1.105	1.200	0.058
				meanT	0.405	0.386	1.051	0.307			
MI_44S_65W	Feb	Male	Feeding	Intercept	20.625	5.099	4.045	0.001	0.524	0.896	0.028
				meanT	0.208	0.288	0.724	0.479			
MI_44S_65W	Mar	Female	Feeding	Intercept	29.139	7.605	3.831	0.001	0.092	1.233	0.005
				meanT	-0.137	0.450	-0.303	0.765			
MI_44S_65W	Mar	Male	Feeding	Intercept	24.999	5.604	4.461	<0.001	0.015	0.909	0.001
				meanT	-0.041	0.332	-0.123	0.904			
MI_44S_65W	Apr	Female	Feeding	Intercept	32.171	7.698	4.179	0.001	0.482	1.220	0.026
				meanT	-0.357	0.515	-0.694	0.497			
MI_44S_65W	Apr	Male	Feeding	Intercept	26.422	5.714	4.624	<0.001	0.136	0.906	0.008
				meanT	-0.141	0.382	-0.369	0.716			
MI_44S_63W	Jan	Female	Feeding	Intercept	18.360	5.758	3.189	0.005	2.170	1.168	0.108
				meanT	0.520	0.353	1.473	0.158			
MI_44S_63W	Jan	Male	Feeding	Intercept	17.983	4.225	4.256	<0.001	2.249	0.857	0.111
				meanT	0.389	0.259	1.500	0.151			
MI_44S_63W	Feb	Female	Feeding	Intercept	17.283	6.310	2.739	0.013	2.294	1.165	0.113
				meanT	0.564	0.372	1.515	0.147			
MI_44S_63W	Feb	Male	Feeding	Intercept	18.453	4.728	3.903	0.001	1.538	0.873	0.079
				meanT	0.346	0.279	1.240	0.231			
MI_44S_63W	Mar	Female	Feeding	Intercept	27.357	5.728	4.776	<0.001	0.008	1.236	0.000

				meanT	-0.033	0.355	-0.092	0.928			
MI_44S_63W	Mar	Male	Feeding	Intercept	23.546	4.208	5.595	<0.001	0.033	0.908	0.002
				meanT	0.048	0.261	0.182	0.857			
MI_44S_63W	Apr	Female	Feeding	Intercept	31.967	6.441	4.963	<0.001	0.637	1.215	0.034
				meanT	-0.363	0.455	-0.798	0.435			
MI_44S_63W	Apr	Male	Feeding	Intercept	25.561	4.809	5.315	<0.001	0.068	0.907	0.004
				meanT	-0.088	0.340	-0.260	0.798			
MI_48S_64W	Jan	Female	Feeding	Intercept	18.656	5.207	3.583	0.002	2.471	1.159	0.121
				meanT	0.610	0.388	1.572	0.133			
MI_48S_64W	Jan	Male	Feeding	Intercept	18.663	3.859	4.836	<0.001	2.148	0.859	0.107
				meanT	0.422	0.288	1.466	0.16			
MI_48S_64W	Feb	Female	Feeding	Intercept	19.296	4.717	4.091	0.001	2.560	1.157	0.125
				meanT	0.540	0.337	1.600	0.127			
MI_48S_64W	Feb	Male	Feeding	Intercept	20.045	3.567	5.619	<0.001	1.435	0.875	0.074
				meanT	0.306	0.255	1.198	0.246			
MI_48S_64W	Mar	Female	Feeding	Intercept	25.178	4.721	5.333	<0.001	0.123	1.232	0.007
				meanT	0.125	0.357	0.351	0.73			
MI_48S_64W	Mar	Male	Feeding	Intercept	22.774	3.464	6.575	<0.001	0.198	0.904	0.011
				meanT	0.116	0.262	0.445	0.662			
MI_48S_64W	Apr	Female	Feeding	Intercept	26.923	5.638	4.775	<0.001	0.000	1.236	0.000
				meanT	-0.008	0.496	-0.016	0.987			
MI_48S_64W	Apr	Male	Feeding	Intercept	23.160	4.137	5.599	<0.001	0.078	0.907	0.004

				meanT	0.101	0.364	0.279	0.784			
MI_48S_66W	Jan	Female	Feeding	Intercept	19.311	5.208	3.708	0.002	2.091	1.170	0.104
				meanT	0.555	0.384	1.446	0.165			
MI_48S_66W	Jan	Male	Feeding	Intercept	18.493	3.805	4.860	<0.001	2.345	0.855	0.115
				meanT	0.430	0.281	1.531	0.143			
MI_48S_66W	Feb	Female	Feeding	Intercept	19.392	4.944	3.922	0.001	2.271	1.165	0.112
				meanT	0.527	0.350	1.507	0.149			
MI_48S_66W	Feb	Male	Feeding	Intercept	19.542	3.690	5.296	<0.001	1.677	0.870	0.085
				meanT	0.338	0.261	1.295	0.212			
MI_48S_66W	Mar	Female	Feeding	Intercept	25.623	4.641	5.521	<0.001	0.068	1.234	0.004
				meanT	0.091	0.349	0.261	0.797			
MI_48S_66W	Mar	Male	Feeding	Intercept	22.701	3.397	6.682	<0.001	0.226	0.903	0.012
				meanT	0.121	0.255	0.475	0.64			
MI_48S_66W	Apr	Female	Feeding	Intercept	24.650	5.926	4.160	0.001	0.136	1.232	0.007
				meanT	0.192	0.521	0.369	0.717			
MI_48S_66W	Apr	Male	Feeding	Intercept	21.195	4.311	4.916	<0.001	0.524	0.896	0.028
				meanT	0.274	0.379	0.724	0.478			
MI_42S_60W	Jan	Female	Feeding	Intercept	17.834	5.691	3.134	0.006	2.506	1.159	0.122
				meanT	0.523	0.331	1.583	0.131			
MI_42S_60W	Jan	Male	Feeding	Intercept	18.059	4.215	4.285	<0.001	2.205	0.858	0.109
				meanT	0.364	0.245	1.485	0.155			
MI_42S_60W	Feb	Female	Feeding	Intercept	15.580	6.667	2.337	0.031	2.852	1.149	0.137

				meanT	0.628	0.372	1.689	0.108			
MI_42S_60W	Feb	Male	Feeding	Intercept	17.132	4.997	3.428	0.003	2.068	0.861	0.103
				meanT	0.401	0.279	1.438	0.168			
MI_42S_60W	Mar	Female	Feeding	Intercept	27.752	5.716	4.855	<0.001	0.026	1.236	0.001
				meanT	-0.054	0.334	-0.161	0.874			
MI_42S_60W	Mar	Male	Feeding	Intercept	24.228	4.206	5.761	<0.001	0.000	0.909	0.000
				meanT	0.005	0.246	0.020	0.984			
MI_42S_60W	Apr	Female	Feeding	Intercept	32.967	5.875	5.612	<0.001	1.093	1.201	0.057
				meanT	-0.408	0.390	-1.045	0.31			
MI_42S_60W	Apr	Male	Feeding	Intercept	26.614	4.415	6.028	<0.001	0.272	0.902	0.015
				meanT	-0.153	0.293	-0.522	0.608			
MI_42S_62W	Jan	Female	Feeding	Intercept	17.844	7.245	2.463	0.024	1.541	1.187	0.079
				meanT	0.493	0.397	1.241	0.23			
MI_42S_62W	Jan	Male	Feeding	Intercept	17.676	5.325	3.320	0.004	1.555	0.872	0.080
				meanT	0.364	0.292	1.247	0.228			
MI_42S_62W	Feb	Female	Feeding	Intercept	17.132	8.545	2.005	0.06	1.290	1.194	0.067
				meanT	0.515	0.453	1.136	0.271			
MI_42S_62W	Feb	Male	Feeding	Intercept	19.389	6.400	3.030	0.007	0.592	0.895	0.032
				meanT	0.261	0.339	0.770	0.451			
MI_42S_62W	Mar	Female	Feeding	Intercept	31.828	7.541	4.221	0.001	0.439	1.222	0.024
				meanT	-0.278	0.419	-0.663	0.516			
MI_42S_62W	Mar	Male	Feeding	Intercept	26.679	5.584	4.778	<0.001	0.180	0.905	0.010

				meanT	-0.132	0.310	-0.424	0.677			
MI_42S_62W	Apr	Female	Feeding	Intercept	36.675	7.623	4.811	<0.001	1.669	1.183	0.085
				meanT	-0.618	0.478	-1.292	0.213			
MI_42S_62W	Apr	Male	Feeding	Intercept	28.895	5.758	5.018	<0.001	0.634	0.894	0.034
				meanT	-0.288	0.361	-0.796	0.436			
MI_42W_64W	Jan	Female	Feeding	Intercept	12.522	9.477	1.321	0.203	2.282	1.165	0.113
				meanT	0.761	0.504	1.511	0.148			
MI_42W_64W	Jan	Male	Feeding	Intercept	14.193	7.001	2.027	0.058	2.091	0.861	0.104
				meanT	0.538	0.372	1.446	0.165			
MI_42W_64W	Feb	Female	Feeding	Intercept	10.887	10.075	1.081	0.294	2.506	1.158	0.122
				meanT	0.825	0.521	1.583	0.131			
MI_42W_64W	Feb	Male	Feeding	Intercept	16.571	7.693	2.154	0.045	1.013	0.885	0.053
				meanT	0.400	0.398	1.007	0.327			
MI_42W_64W	Mar	Female	Feeding	Intercept	29.357	10.840	2.708	0.014	0.054	1.235	0.003
				meanT	-0.136	0.585	-0.233	0.818			
MI_42W_64W	Mar	Male	Feeding	Intercept	25.518	7.977	3.199	0.005	0.023	0.909	0.001
				meanT	-0.065	0.431	-0.151	0.882			
MI_42W_64W	Apr	Female	Feeding	Intercept	36.129	11.918	3.031	0.007	0.609	1.216	0.033
				meanT	-0.558	0.716	-0.780	0.445			
MI_42W_64W	Apr	Male	Feeding	Intercept	29.075	8.839	3.289	0.004	0.291	0.902	0.016
				meanT	-0.286	0.531	-0.539	0.596			
MI_46S_63W	Jan	Female	Feeding	Intercept	20.167	5.428	3.715	0.002	1.511	1.188	0.077

				meanT	0.448	0.365	1.229	0.235			
MI_46S_63W	Jan	Male	Feeding	Intercept	19.491	3.996	4.877	<0.001	1.459	0.874	0.075
				meanT	0.324	0.269	1.208	0.243			
MI_46S_63W	Feb	Female	Feeding	Intercept	20.636	5.276	3.911	0.001	1.383	1.192	0.071
				meanT	0.399	0.339	1.176	0.255			
MI_46S_63W	Feb	Male	Feeding	Intercept	20.624	3.930	5.248	<0.001	0.883	0.888	0.047
				meanT	0.237	0.253	0.940	0.36			
MI_46S_63W	Mar	Female	Feeding	Intercept	27.434	5.008	5.478	<0.001	0.015	1.236	0.001
				meanT	-0.041	0.337	-0.120	0.905			
MI_46S_63W	Mar	Male	Feeding	Intercept	24.050	3.683	6.530	<0.001	0.005	0.909	0.000
				meanT	0.018	0.248	0.071	0.944			
MI_46S_63W	Apr	Female	Feeding	Intercept	29.955	5.782	5.180	<0.001	0.292	1.227	0.016
				meanT	-0.242	0.447	-0.541	0.595			
MI_46S_63W	Apr	Male	Feeding	Intercept	24.657	4.285	5.754	<0.001	0.006	0.909	0.000
				meanT	-0.027	0.332	-0.081	0.937			

Appendix B.4. Model coefficients, standard errors and significance tests for generalised linear models of proportion of mature females as a function of mean temperature in the (A) Hatching grounds, (B) Juvenile grounds, and the (C) Migration paths. SE, standard error; RD, residual deviance; ED, explained deviance; H, hatching grounds; J, juvenile grounds; MI, migration inshore; MO, migration offshore.

(A)									
Box	**Month**	**Period**		**Slope**	**SE**	**t value**	**p-value**	**RD**	**ED**
H_33S_49W	Jul	Hatching	Intercept	11.095	2.842	3.904	0.001	1730.600	0.436

			meanT	-0.550	0.147	-3.734	0.002		
H_33S_49W	Aug	Hatching	Intercept	10.117	3.559	2.843	0.011	2185.768	0.287
			meanT	-0.506	0.187	-2.706	0.014		
H_35S_51W	Jul	Hatching	Intercept	8.313	3.493	2.380	0.029	2410.316	0.214
			meanT	-0.426	0.190	-2.238	0.038		
H_35S_51W	Aug	Hatching	Intercept	7.166	2.527	2.835	0.011	2208.458	0.280
			meanT	-0.375	0.142	-2.644	0.016		
H_33S_51W	Oct	Juvenile	Intercept	7.995	3.439	2.325	0.032	2439.637	0.205
			meanT	-0.409	0.187	-2.181	0.043		
H_33S_49W	Oct	Juvenile	Intercept	11.122	4.971	2.237	0.038	2458.836	0.198
			meanT	-0.541	0.253	-2.138	0.047		
H_35S_51W	Oct	Juvenile	Intercept	8.507	2.541	3.348	0.004	1989.573	0.351
			meanT	-0.425	0.135	-3.159	0.005		
(B)									
J_37S_53W	Oct	Juvenile	Intercept	3.793	1.403	2.703	0.015	2364.992	0.229
			meanT	-0.199	0.084	-2.36	0.03		
(C)									
MI_42S_64W	Feb	Feeding	Intercept	-11.376	5.385	-2.112	0.049	2435.646	0.206
			meanT	0.614	0.278	2.206	0.041		
MI_46S_63W	Feb	Feeding	Intercept	-6.109	3.030	-2.016	0.059	2423.513	0.210
			meanT	0.427	0.196	2.184	0.042		
MI_48S_64W	Feb	Feeding	Intercept	-5.674	2.791	-2.033	0.057	2412.869	0.213
			meanT	0.443	0.200	2.215	0.040		

MO_46S_61W	Feb	Feeding	Intercept	-5.889	2.699	-2.182	0.043	2346.911	0.235
			meanT	0.442	0.187	2.369	0.029		
MO_48S_62W	Feb	Feeding	Intercept	-5.201	2.554	-2.037	0.057	2408.409	0.215
			meanT	0.427	0.191	2.235	0.038		

Appendix B.5. Model coefficients, standard errors and significance tests for generalised linear models of proportion of mature females as a function of mean temperature in the (A) Hatching grounds and (B) Juvenile grounds during the feeding period. SE, standard error; RD, residual deviance; ED, explained deviance; H, hatching grounds; J, juvenile grounds.

Box	**Month**	**Period**		**Slope**	**SE**	**t value**	**p-value**	**RD**	**ED**
(A)									
H_33S_51W	Jan	Feeding	Intercept	11.269	6.039	1.866	0.078	2621.859	0.145
			meanT	-0.441	0.247	-1.783	0.091		
H_33S_51W	Feb	Feeding	Intercept	7.807	6.040	1.292	0.213	2845.517	0.072
			meanT	-0.290	0.240	-1.209	0.242		
H_33S_51W	Mar	Feeding	Intercept	-0.814	6.574	-0.124	0.903	3060.882	0.002
			meanT	0.054	0.269	0.202	0.842		
H_33S_51W	Apr	Feeding	Intercept	7.554	5.711	1.323	0.202	2838.933	0.074
			meanT	-0.308	0.250	-1.234	0.233		
H_33S_49W	Jan	Feeding	Intercept	7.703	5.973	1.290	0.213	2845.596	0.072
			meanT	-0.300	0.249	-1.205	0.244		
H_33S_49W	Feb	Feeding	Intercept	0.729	4.574	0.159	0.875	3066.999	0.000
			meanT	-0.009	0.186	-0.047	0.963		
H_33S_49W	Mar	Feeding	Intercept	-2.809	5.747	-0.489	0.631	3014.750	0.017

			meanT	0.136	0.235	0.578	0.571		
H_33S_49W	Apr	Feeding	Intercept	-0.137	6.535	-0.021	0.984	3065.788	0.001
			meanT	0.028	0.282	0.099	0.922		
H_35S_51W	Jan	Feeding	Intercept	8.323	6.592	1.263	0.223	2852.092	0.070
			meanT	-0.332	0.280	-1.186	0.251		
H_35S_51W	Feb	Feeding	Intercept	12.627	6.400	1.973	0.064	2559.323	0.166
			meanT	-0.498	0.263	-1.895	0.074		
H_35S_51W	Mar	Feeding	Intercept	1.052	5.114	0.206	0.839	3065.580	0.001
			meanT	-0.023	0.215	-0.106	0.917		
H_35S_51W	Apr	Feeding	Intercept	5.504	5.486	1.003	0.329	2938.206	0.042
			meanT	-0.221	0.243	-0.911	0.374		
(B)									
J_35S_53W	Jan	Feeding	Intercept	2.644	5.871	0.450	0.658	3046.378	0.007
			meanT	-0.093	0.255	-0.363	0.721		
J_35S_53W	Feb	Feeding	Intercept	0.120	6.683	0.018	0.986	3066.811	0.000
			meanT	0.016	0.281	0.059	0.954		
J_35S_53W	Mar	Feeding	Intercept	0.682	5.337	0.128	0.900	3067.197	0.000
			meanT	-0.007	0.233	-0.032	0.975		
J_35S_53W	Apr	Feeding	Intercept	2.911	4.829	0.603	0.554	3028.106	0.013
			meanT	-0.115	0.231	-0.497	0.625		

Appendix B.6. Model coefficients, standard errors and significance tests for linear models of abundance as a function of mean temperature in the hatching grounds during the hatching period. SE, standard error; RSE, residual standard error. H, hatching grounds; J, juvenile grounds.

Box	Month	Period		Slope	SE	t value	p-value	$F_{1,18}$	RSE	R^2
(A)										
H_33S_51W	Jul	Hatching	Intercept	-1.082	6.015	-0.180	0.859	0.227	1.361	0.012
			meanT	0.175	0.366	0.477	0.639			
H_33S_51W	Aug	Hatching	Intercept	3.813	3.562	1.070	0.299	0.327	1.358	0.018
			meanT	-0.130	0.226	-0.572	0.574			
H_33S_49W	Jul	Hatching	Intercept	-4.242	7.211	-0.588	0.564	0.699	1.344	0.037
			meanT	0.314	0.375	0.836	0.414			
H_33S_49W	Aug	Hatching	Intercept	-6.155	7.880	-0.781	0.445	1.016	1.333	0.053
			meanT	0.420	0.417	1.008	0.327			
H_35S_51W	Jul	Hatching	Intercept	5.707	6.498	0.878	0.391	0.366	1.356	0.020
			meanT	-0.216	0.357	-0.605	0.553			
H_35S_51W	Aug	Hatching	Intercept	3.754	4.672	0.804	0.432	0.179	1.363	0.010
			meanT	-0.113	0.267	-0.423	0.677			
(B)										
J_35S_53W	Jul	Hatching	Intercept	0.989	5.272	0.188	0.853	0.023	1.369	0.001
			meanT	0.057	0.380	0.151	0.882			
J_35S_53W	Aug	Hatching	Intercept	3.500	3.023	1.158	0.262	0.326	1.358	0.018
			meanT	-0.130	0.227	-0.571	0.575			

Appendix B.7. Model coefficients, standard errors and significance tests for linear models of abundance as a function of mean temperature in the (A) Hatching grounds (B) Juvenile grounds, and (C) Migration paths. SE, standard error; RSE, residual standard error; H, hatching grounds; J, juvenile grounds; MI, migration inshore; MO, migration offshore.

Box	**Month**	**Period**		**Slope**	**SE**	**t value**	**p-value**	**$F_{1,18}$**	**RSE**	**R^2**
(A)										
H_33S_51W	Dec	Juvenile	Intercept	-22.048	9.872	-2.233	0.038	5.831	1.191	0.245
			meanT	1.072	0.444	2.415	0.027			
H_33S_49W	Jan	Feeding	Intercept	-33.336	11.568	-2.882	0.010	9.220	1.114	0.339
			meanT	1.470	0.484	3.036	0.007			
(B)										
J_35S_53W	Dec	Juvenile	Intercept	-16.031	7.038	-2.278	0.035	6.415	1.176	0.263
			meanT	0.870	0.343	2.533	0.021			
J_36S_55W	Dec	Juvenile	Intercept	-12.797	6.283	-2.037	0.057	38.414	5.401	0.231
			meanT	0.737	0.317	2.324	0.032			
J_36S_55W	Mar	Feeding	Intercept	-16.99	6.37	-2.667	0.016	35.959	8.705	0.326
			meanT	0.862	0.292	2.95	0.009			
J_35S_53W	Mar	Feeding	Intercept	-24.998	8.303	-3.011	0.008	10.411	1.090	0.366
			meanT	1.173	0.363	3.227	0.005			
(C)										
MI_44S_65W	Jan	Feeding	Intercept	18.447	7.723	2.388	0.028	4.661	1.221	0.206
			meanT	-0.978	0.453	-2.159	0.045			

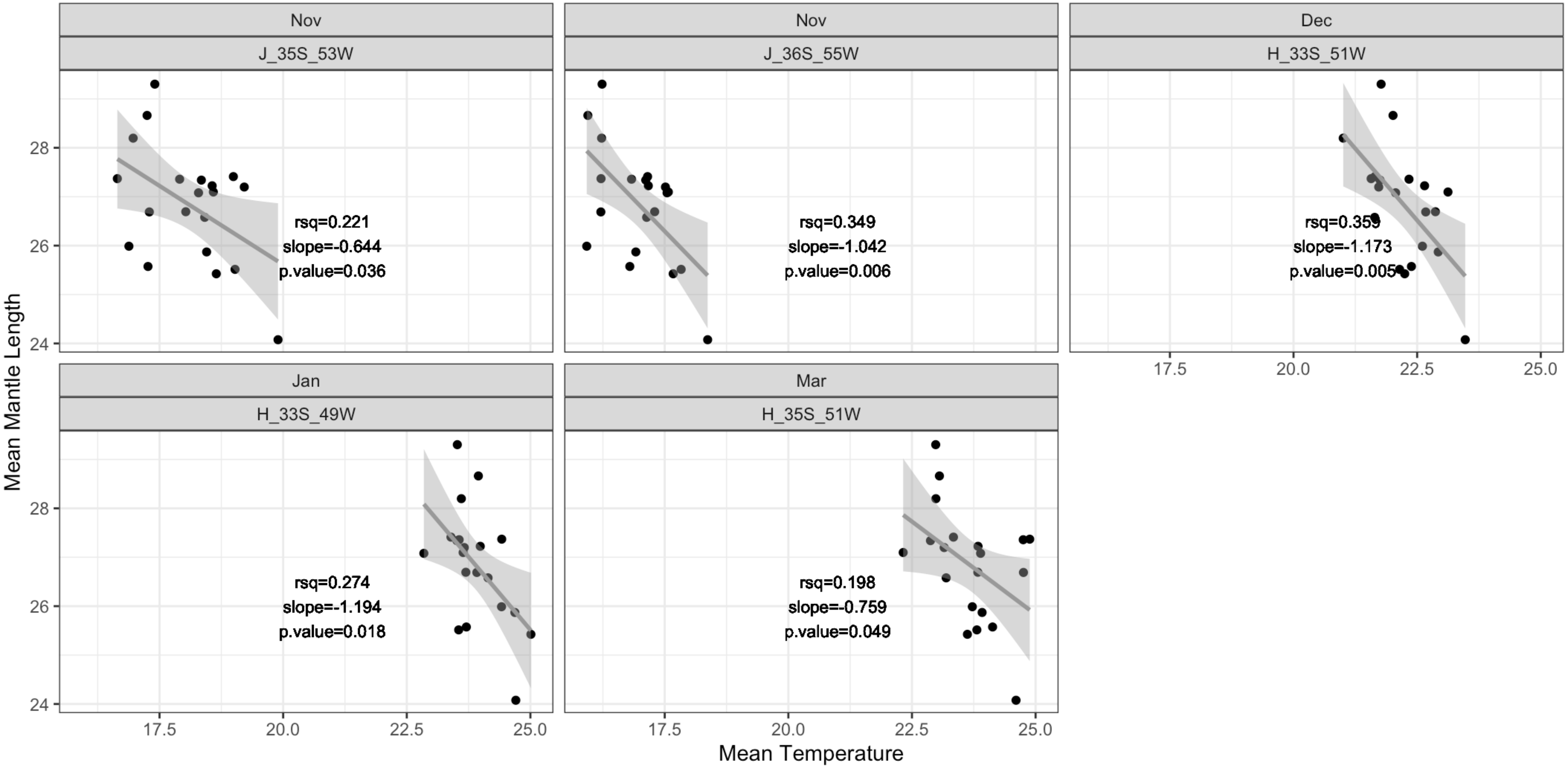
MI_40S_59W	Feb	Feeding	Intercept	27.965	10.371	2.696	0.015	6.377	1.177	0.262
			meanT	-1.246	0.493	-2.525	0.021			
MI_40S_61W	Feb	Feeding	Intercept	20.290	7.857	2.582	0.019	5.555	1.197	0.236
			meanT	-0.957	0.406	-2.357	0.030			
MI_42S_60W	Feb	Feeding	Intercept	18.331	6.925	2.647	0.016	5.718	1.193	0.241
			meanT	-0.924	0.386	-2.391	0.028			
MO_40S_57W	Feb	Feeding	Intercept	16.174	6.176	2.619	0.017	5.439	1.200	0.232
			meanT	-0.790	0.339	-2.332	0.032			
MO_42S_58W	Feb	Feeding	Intercept	14.190	5.559	2.552	0.020	4.992	1.212	0.217
			meanT	-0.753	0.337	-2.234	0.038			
MO_44S_61W	Feb	Feeding	Intercept	14.626	5.880	2.487	0.023	4.781	1.218	0.210
			meanT	-0.808	0.370	-2.187	0.042			
MO_46S_61W	Feb	Feeding	Intercept	12.546	5.064	2.478	0.023	4.532	1.224	0.201
			meanT	-0.746	0.350	-2.129	0.047			

Appendix B.8. Model coefficients, standard errors and significance tests for linear models of CPUE as a function of mean DML. CPUE, catch per unit effort; DML, dorsal mantle length.

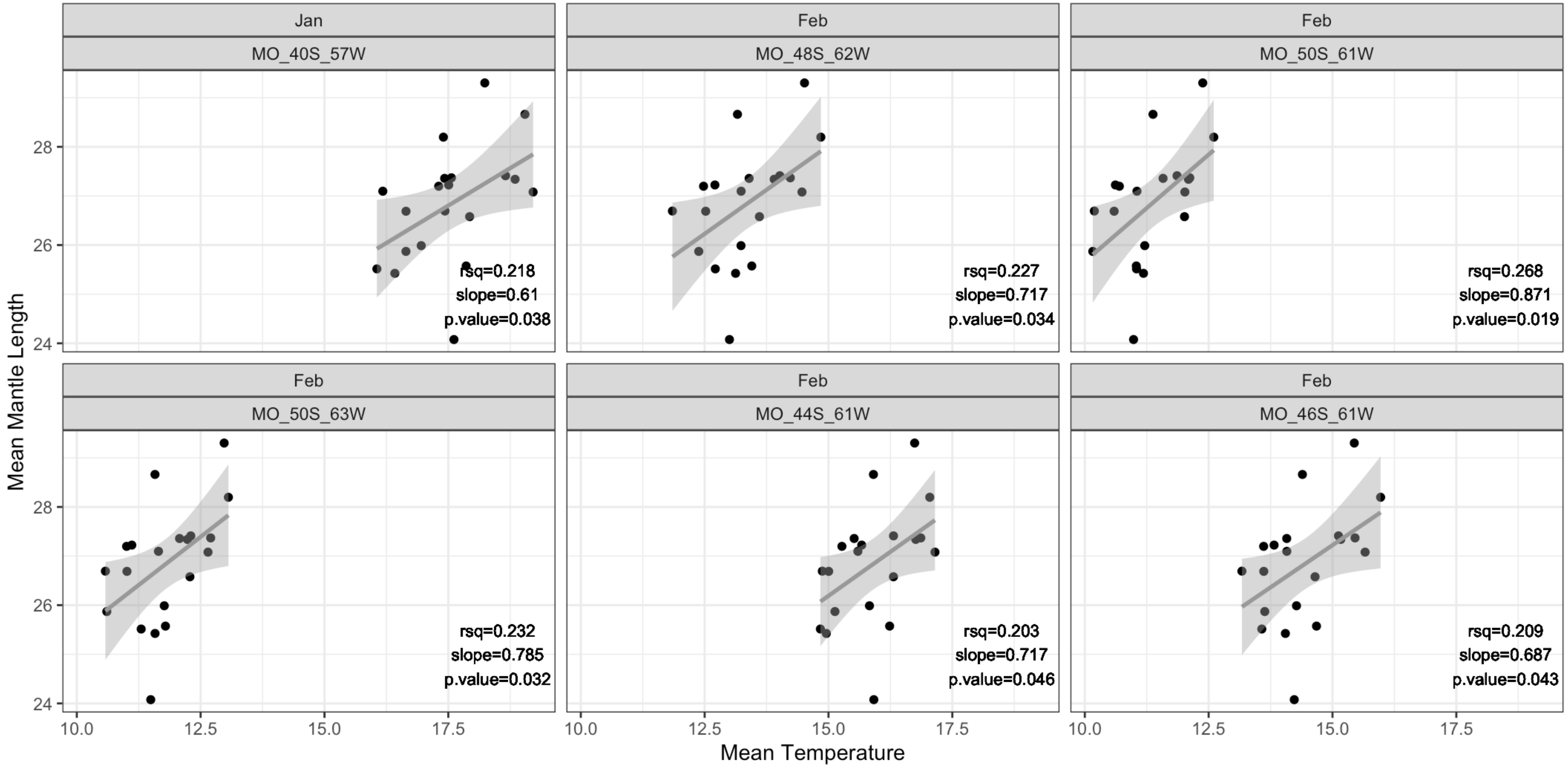
Sex		**Slope**	**Std. error**	**t value**	**p-value**	**R^2**
Female	Intercept	27.603	0.412	66.994	<0.001	0.229
	CPUE_mt_h	-0.432	0.187	-2.315	0.033	
Male	Intercept	24.748	0.321	77.116	<0.001	0.135
	CPUE_mt_h	-0.244	0.145	-1.678	0.111	

Appendix C

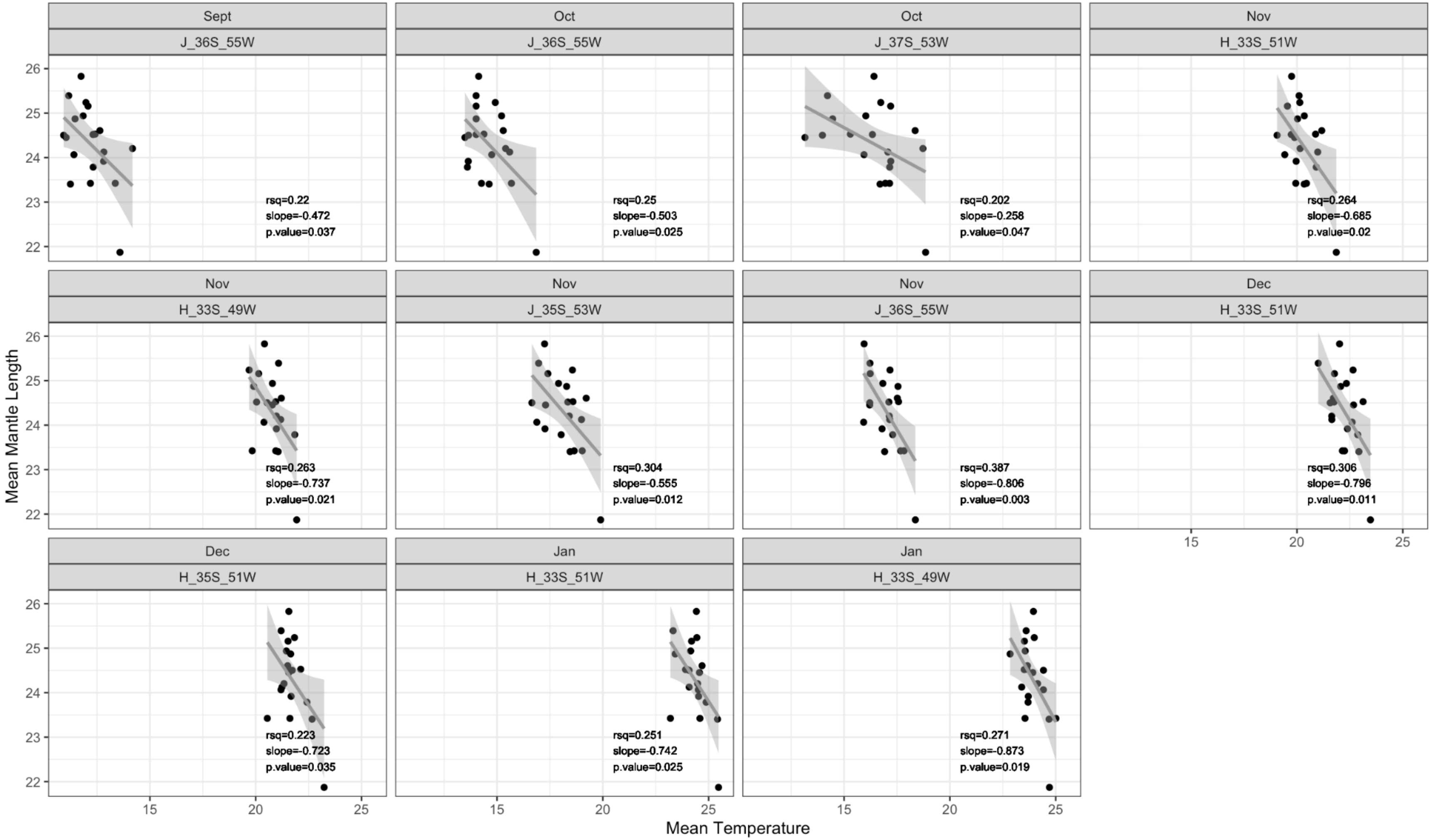
Appendix C.1 Mean DML of females as a function of mean temperature for the hatching and juvenile grounds boxes. Significant models are presented.



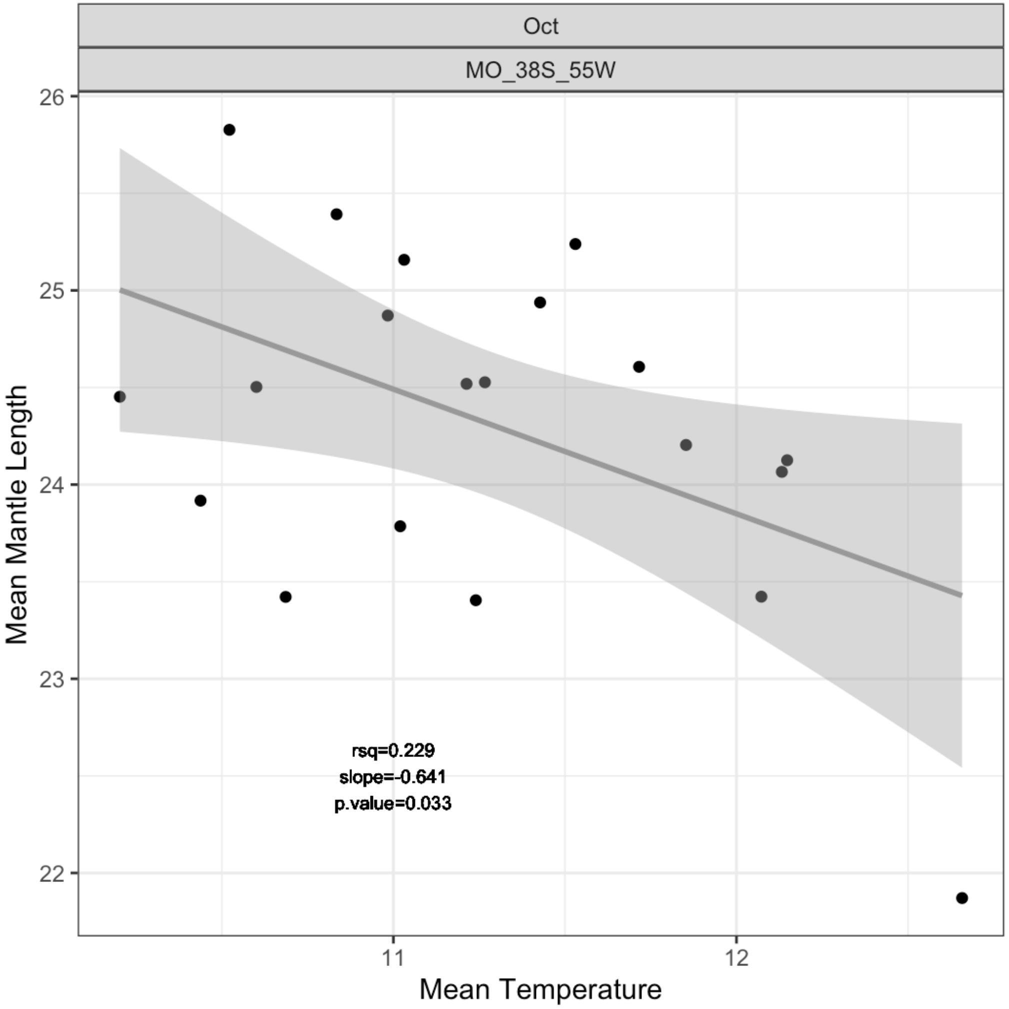
Appendix C.2 Mean DML of females as a function of mean temperature for outer migration route boxes. Significant models are presented.



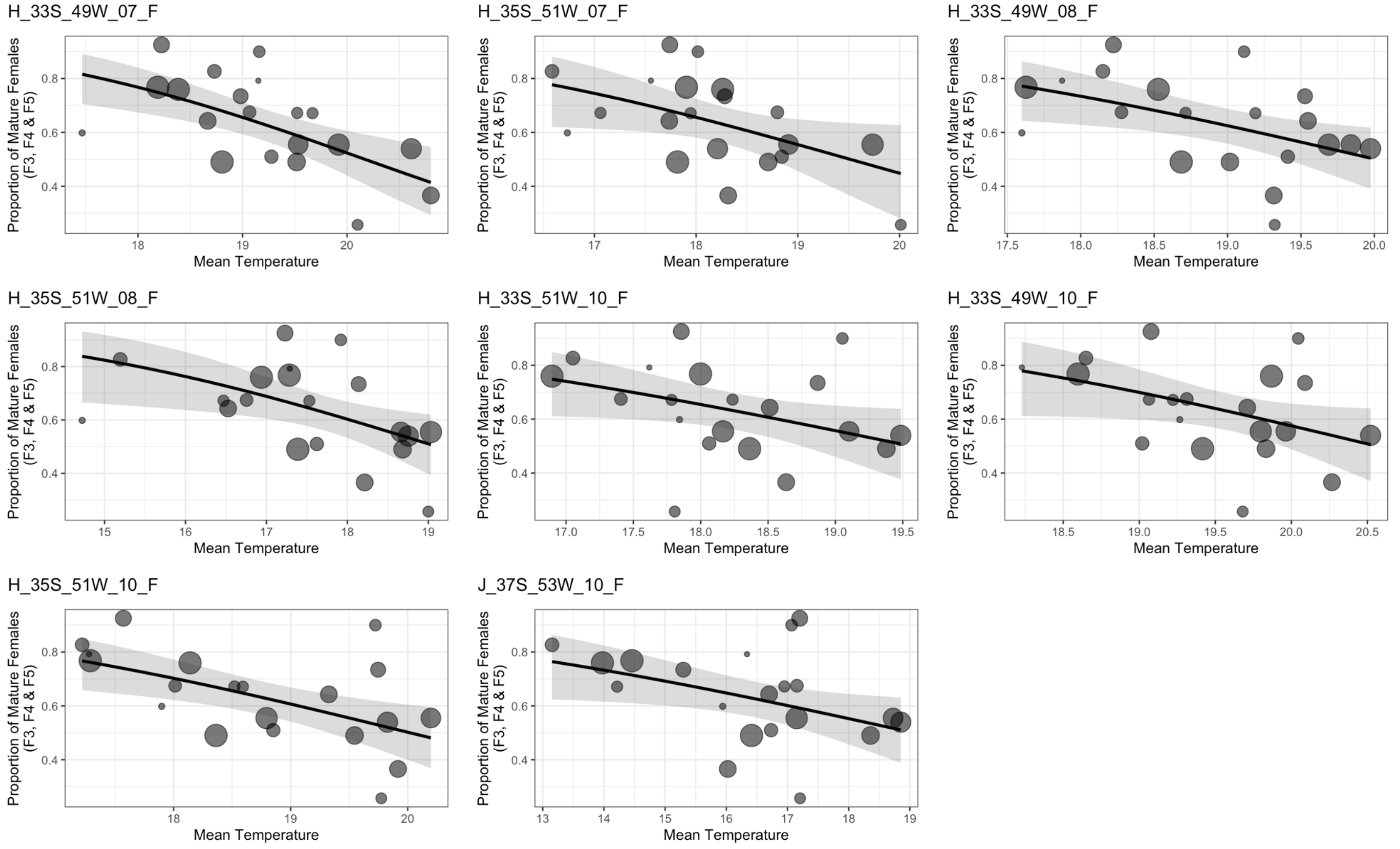
Appendix C.3 Mean DML of males as a function of mean temperature for the hatching and juvenile grounds boxes. Significant models are presented.



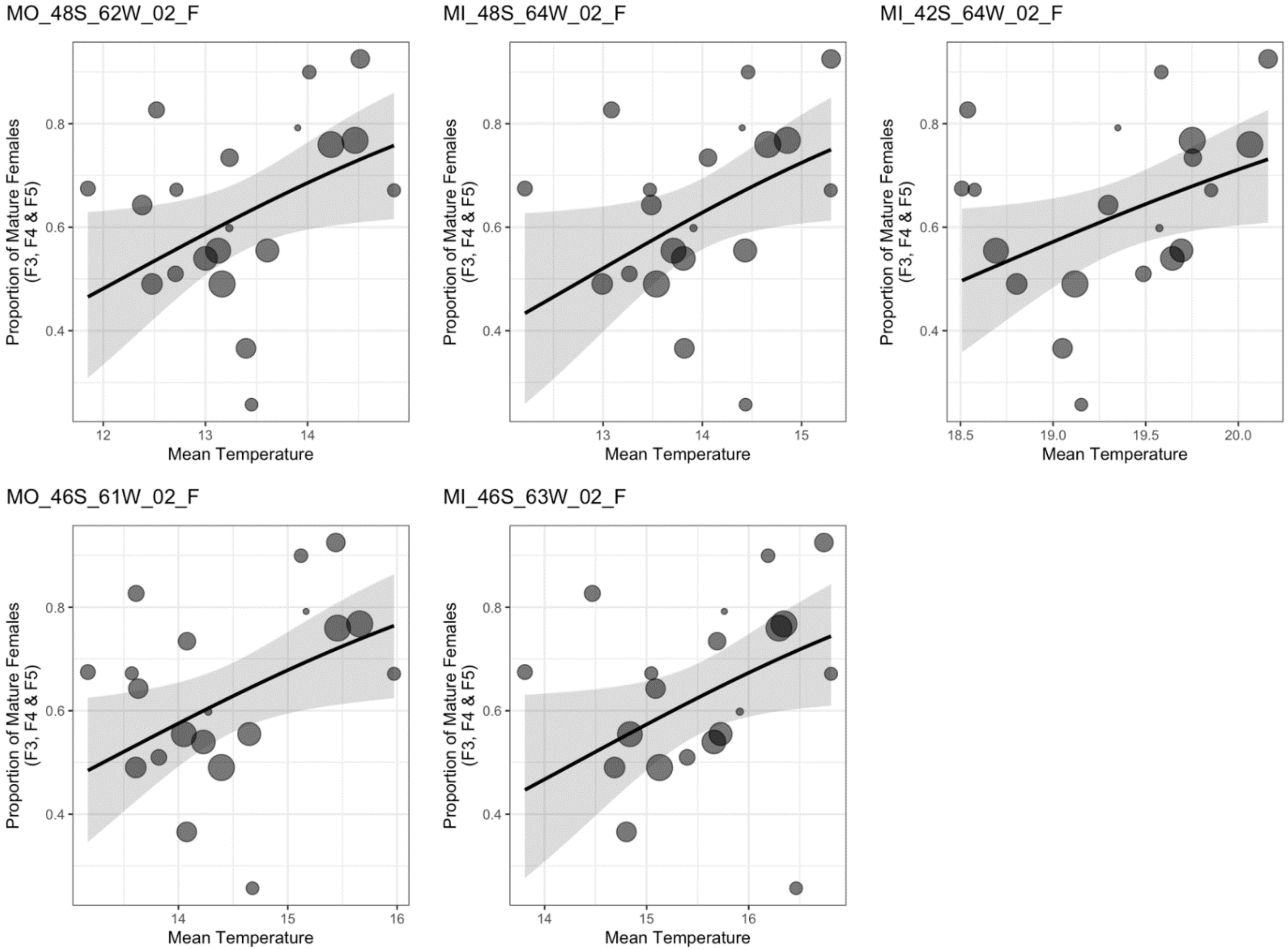
Appendix C.4 Mean DML of males as a function of mean temperature for outer migration route boxes. Significant model is presented.



Appendix C.5 Proportion of mature females (F3, F4 & F5) as a function of mean temperature for the juvenile and hatching grounds. Significant models are presented.



Appendix C.6 Proportion of mature females (F3, F4 & F5) as a function of mean temperature for the outer and inner migration paths. Significant models are presented.



MO_48S_62W_02_F

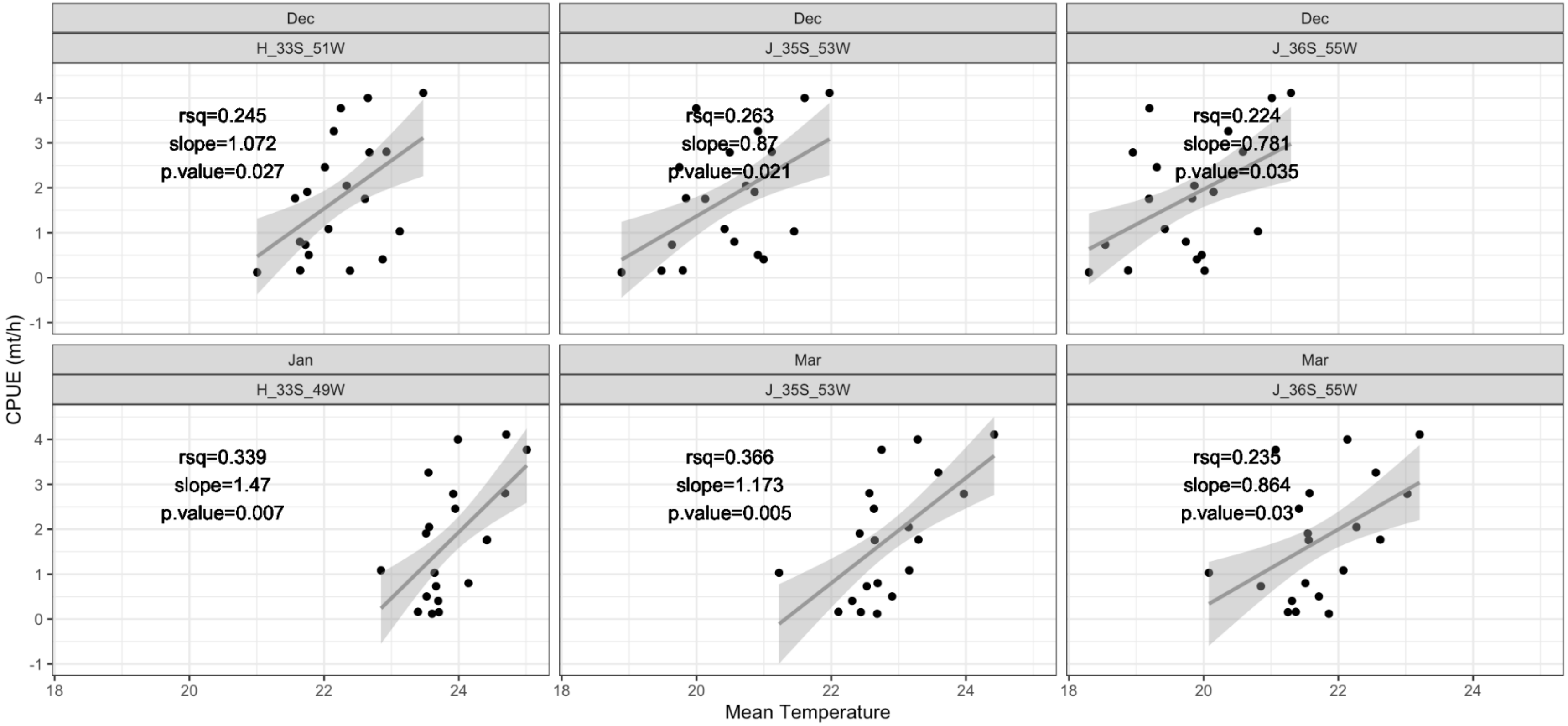
MI_48S_64W_02_F

MI_42S_64W_02_F

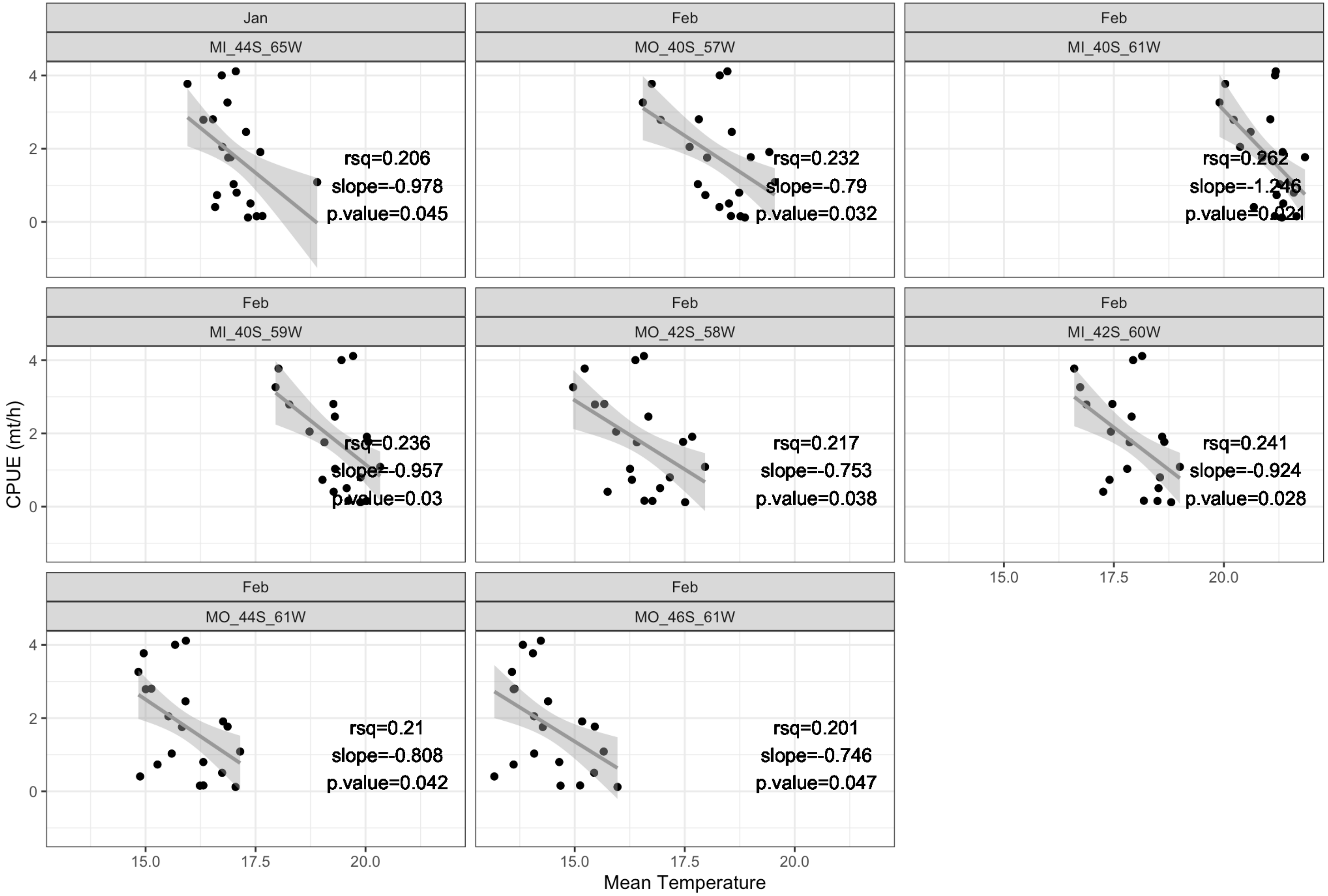
MO_46S_61W_02_F

MI_46S_63W_02_F

Appendix C.7 Abundance as a function of mean temperature for the hatching and juvenile grounds. Significant models are presented.



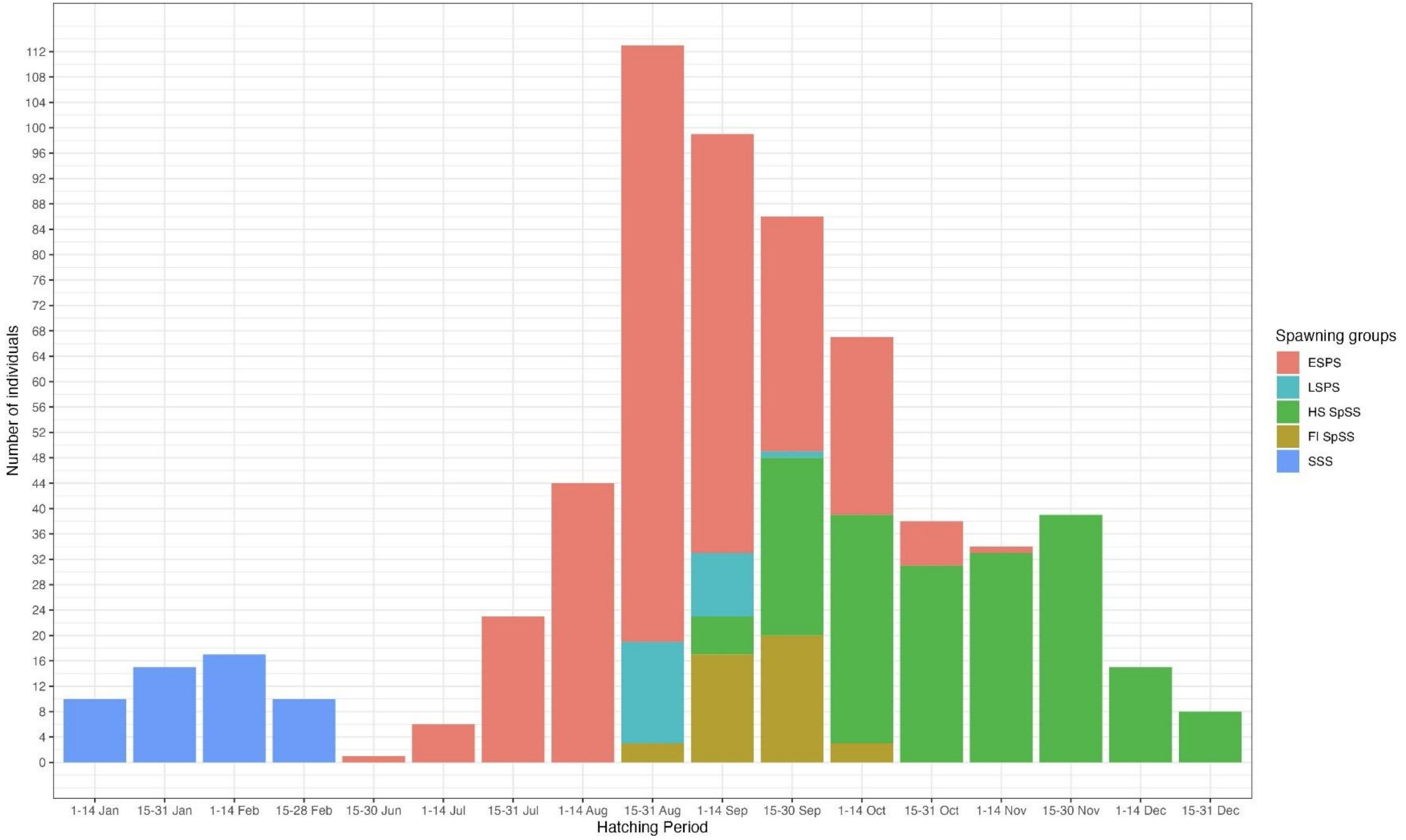
Appendix C.8 Abundance as a function of mean temperature for the outer and inner migration paths. Significant models are presented.



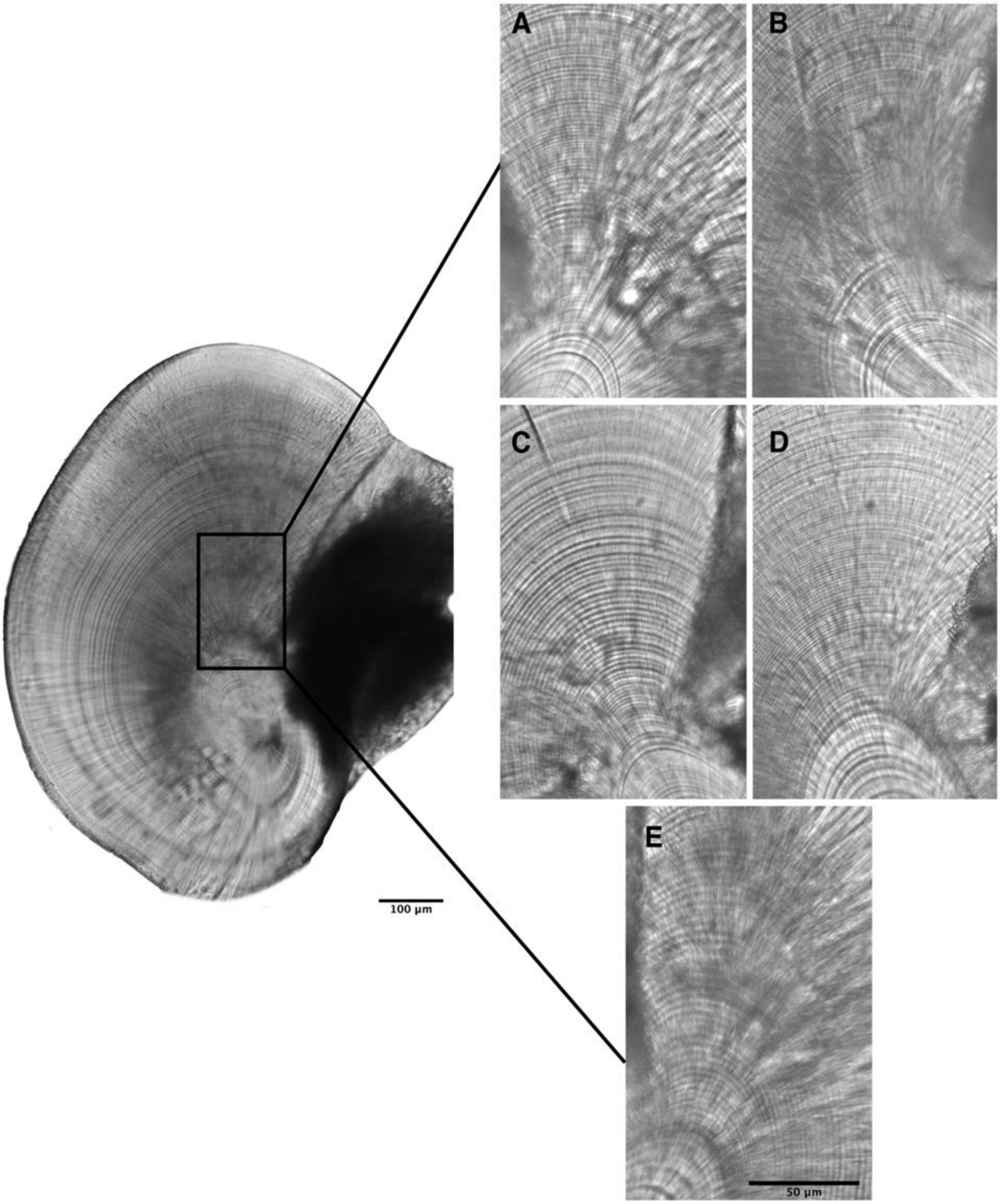
Chapter III: Appendices

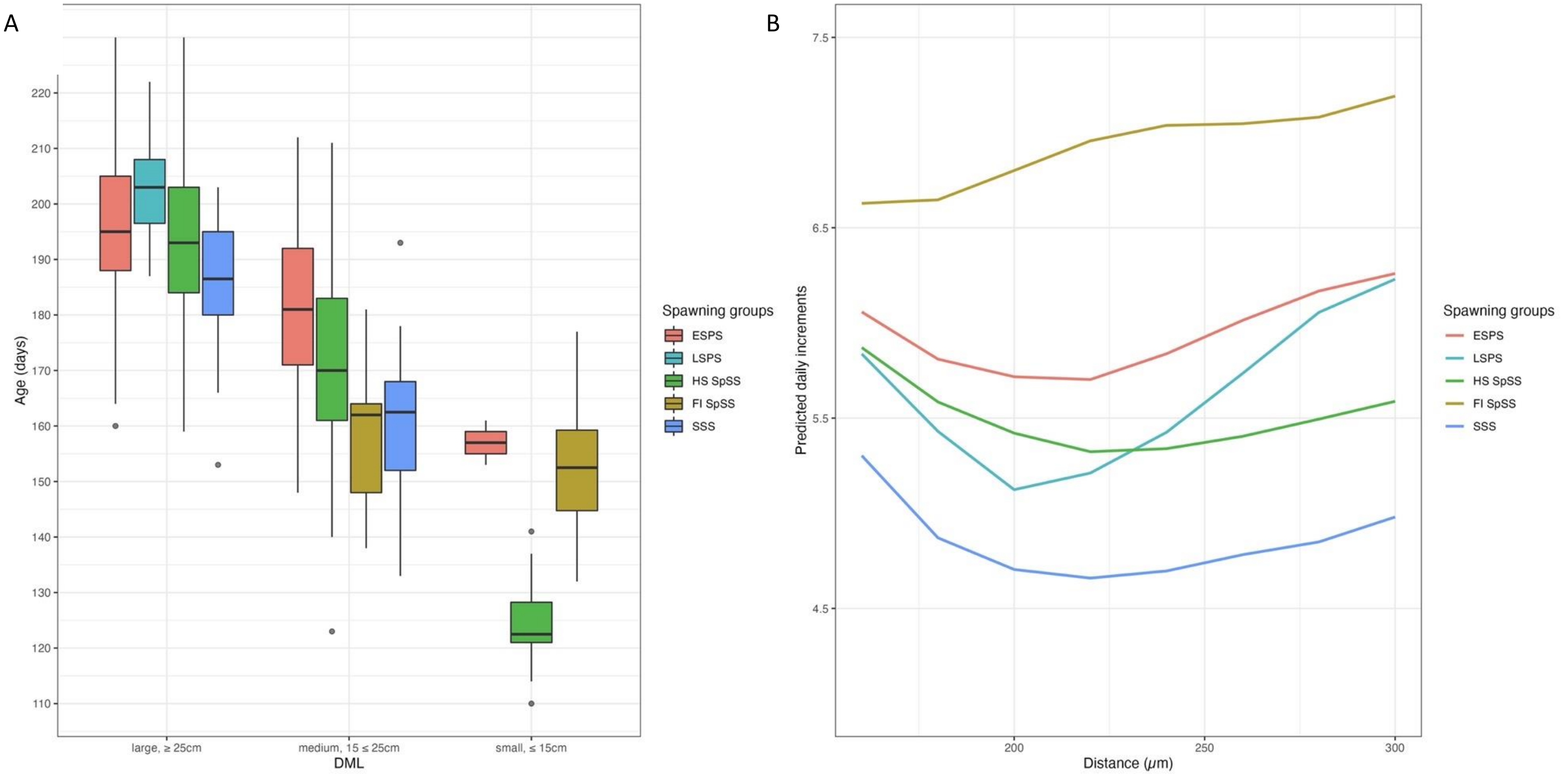
Appendix A

Distribution of hatching dates of different spawning groups



Statolith microstructure differences between the identified spawning groups: (A) early-maturing South Patagonian Stock (B) late-maturing South Patagonian Stock; (C) High Seas Spring Spawning Stock; (D) Falkland Islands Spring Spawning Stock; (E) Summer Spawning Stock.

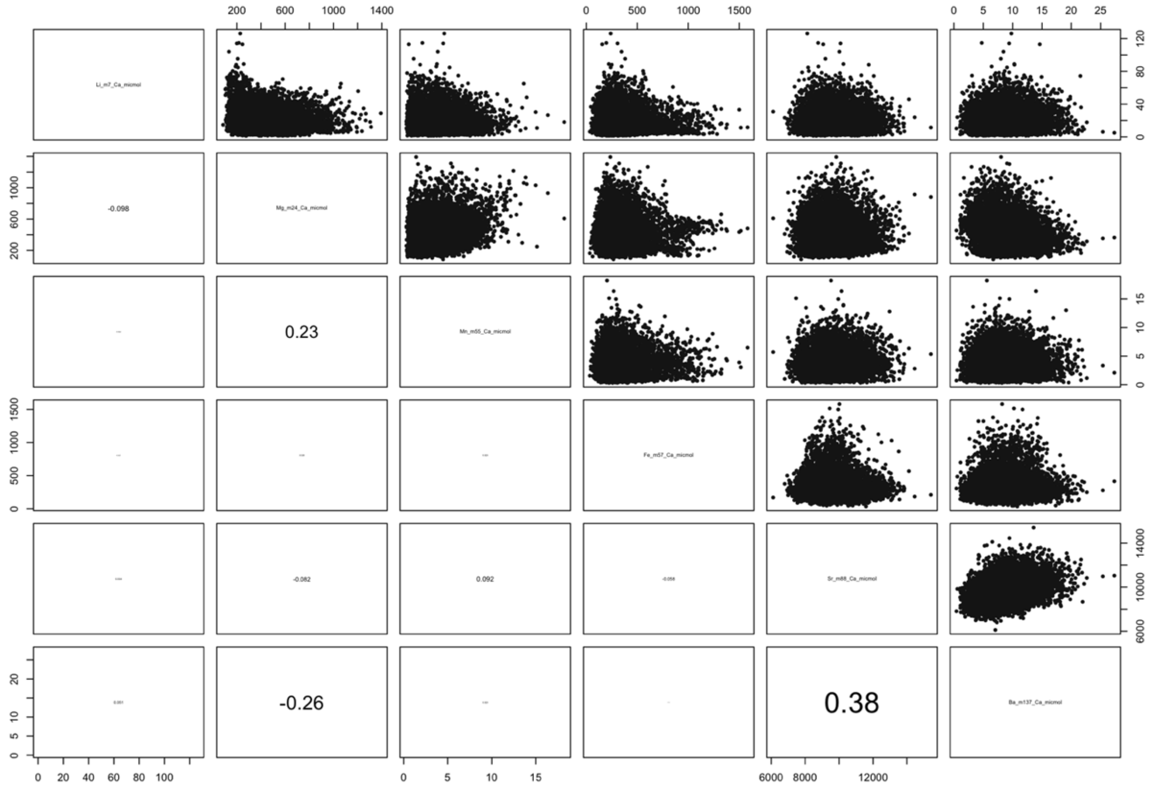




(A) Age range per spawning group as a function of DML groups (B) Predicted number of daily growth increments using loess smoothing (span=0.4) in a subset of the dark zone of the statolith as a function of distance for the spawning groups identified. ESPS, early-maturing South Patagonian Stock; LSPS,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

Appendix B

Pair plots and correlation coefficients of all elements used in the present study.



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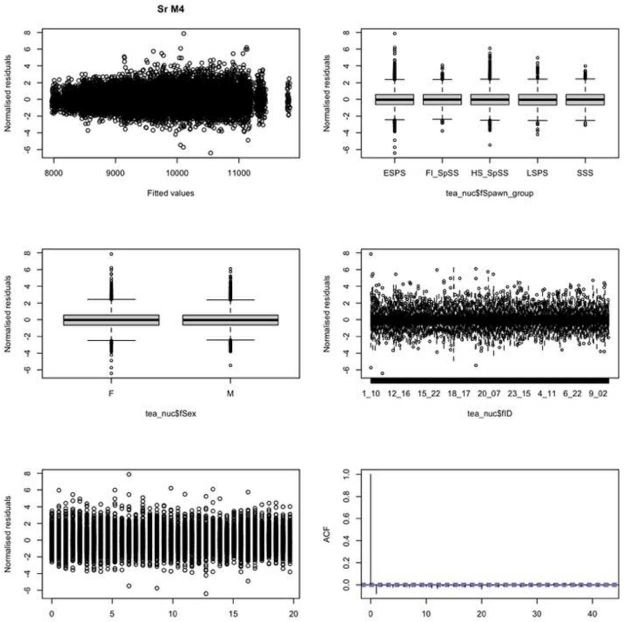
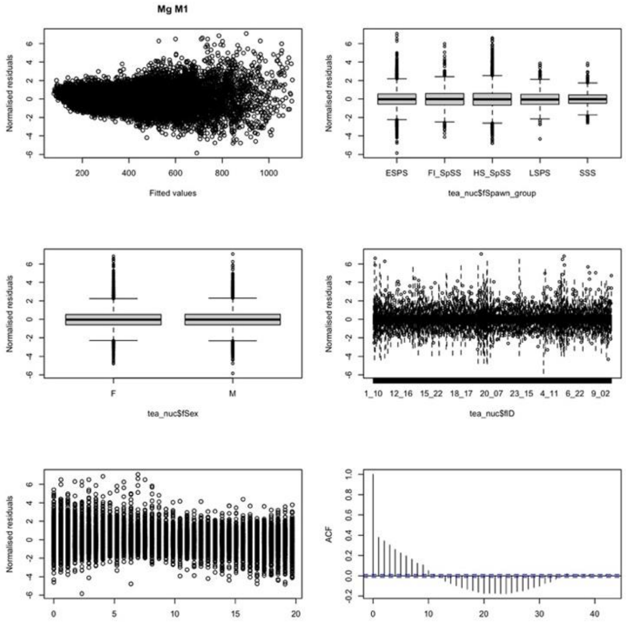
Model selection univariate GAMMs. Df, model degrees of freedom; DE deviance explained, AIC, Akaike's Information Criterion.

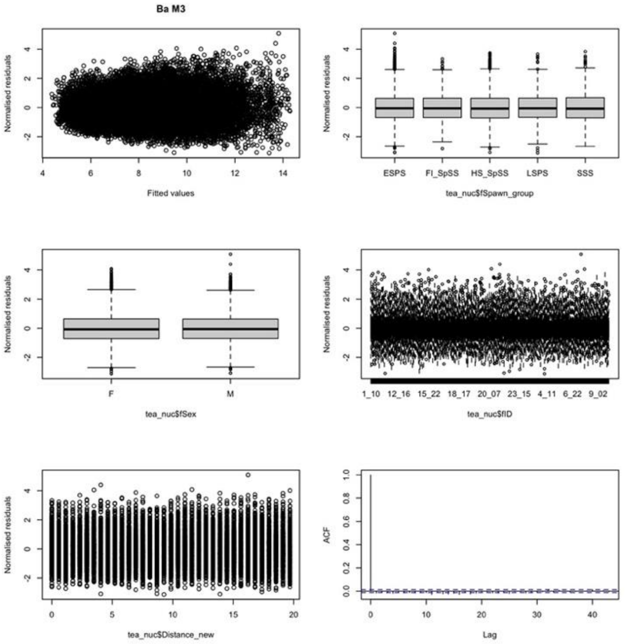
Model No.	Model specification	Df	DE (%)	AIC
Sr M1	Sr:Ca ~ f(Distance:Spawning group) + Spawning group + Sex	8	53.3	349,918.40
Sr M2	Sr:Ca ~ f(Distance:Sex) + Spawning group + Sex	9	53.4	349,904.30
Sr M3	Sr:Ca ~ f(Distance) + Spawning group + Sex	9	53.3	349,906.40
Sr M4	**Sr:Ca ~ f(Distance) + Spawning group**	**7**	**53.3**	**349,906.30**

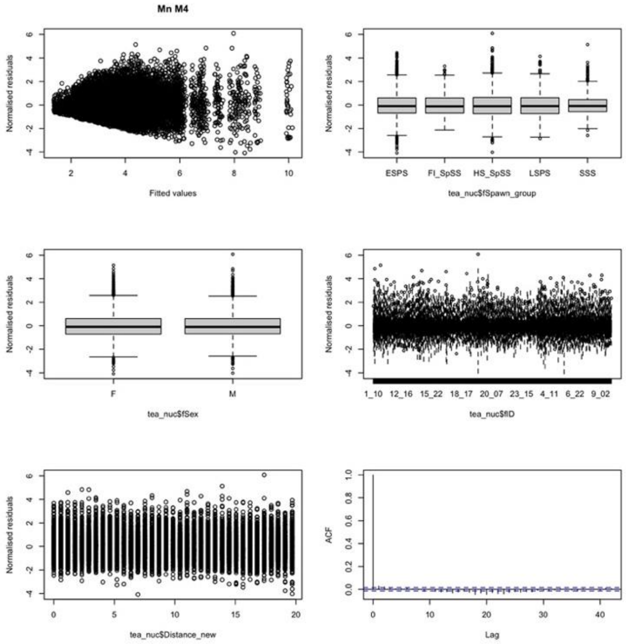
Model No.	Model specification	Df	DE (%)	AIC
Ba M1	Ba:Ca~ f(Distance:Spawning group) + Spawning group + Sex	14	36.0	105,641.20
Ba M2	Ba:Ca~ f(Distance:Sex) + Spawning group + Sex	9	36.0	105,655.40
Ba M3	**Ba:Ca~ f(Distance:Spawning group) + Spawning group**	**13**	**36.0**	**105,641.20**
Ba M4	Ba:Ca~ f(Distance) + Spawning group + Sex	8	35.9	105,655.10
Ba M5	Ba:Ca~ f(Distance) + Spawning group	7	35.9	105,655.10
Mg M1	**Mg:Ca ~ f(Distance:Spawning group) + Spawning group + Sex**	**17**	**90.2**	**238,909.90**
Mg M2	Mg:Ca ~ f(Distance:Sex) + Spawning group + Sex	12	90.2	239,205.50
Mg M3	Mg:Ca ~ f(Distance) + Spawning group + Sex	10	90.0	239,222.30
Mn M1	Mn:Ca ~ f(Distance:Spawning group) + Spawning group + Sex	12	33.3	74,142.86
Mn M2	Mn:Ca ~ f(Distance:Sex) + Spawning group + Sex	11	33.3	74,144.96
Mn M3	Mn:Ca ~ f(Distance) + Spawning group + Sex	9	33.3	74,139.23
Mn M4	**Mn:Ca ~ f(Distance) + Spawning group**	**8**	**33.3**	**74,139.08**

GAMM model validation





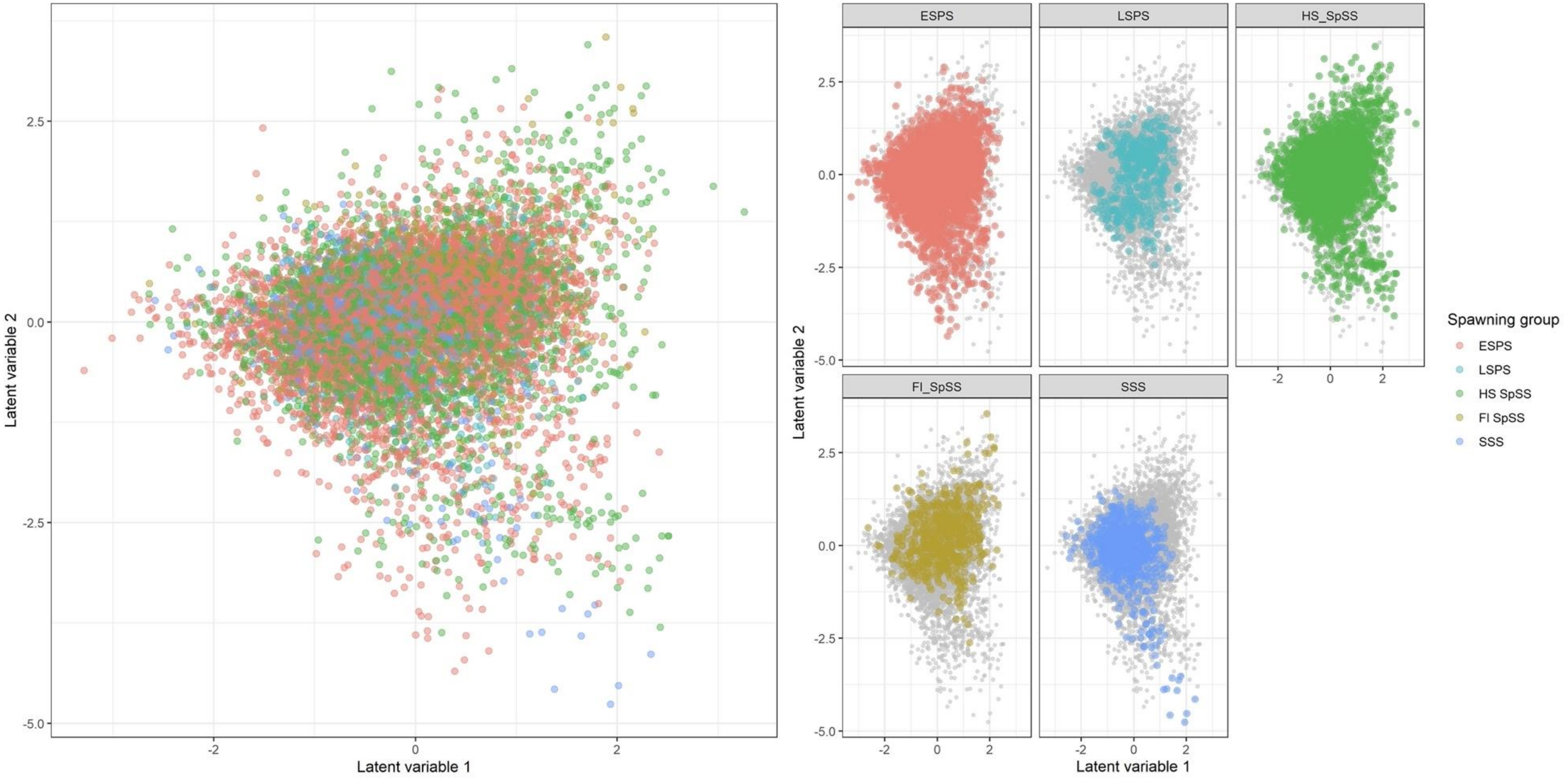




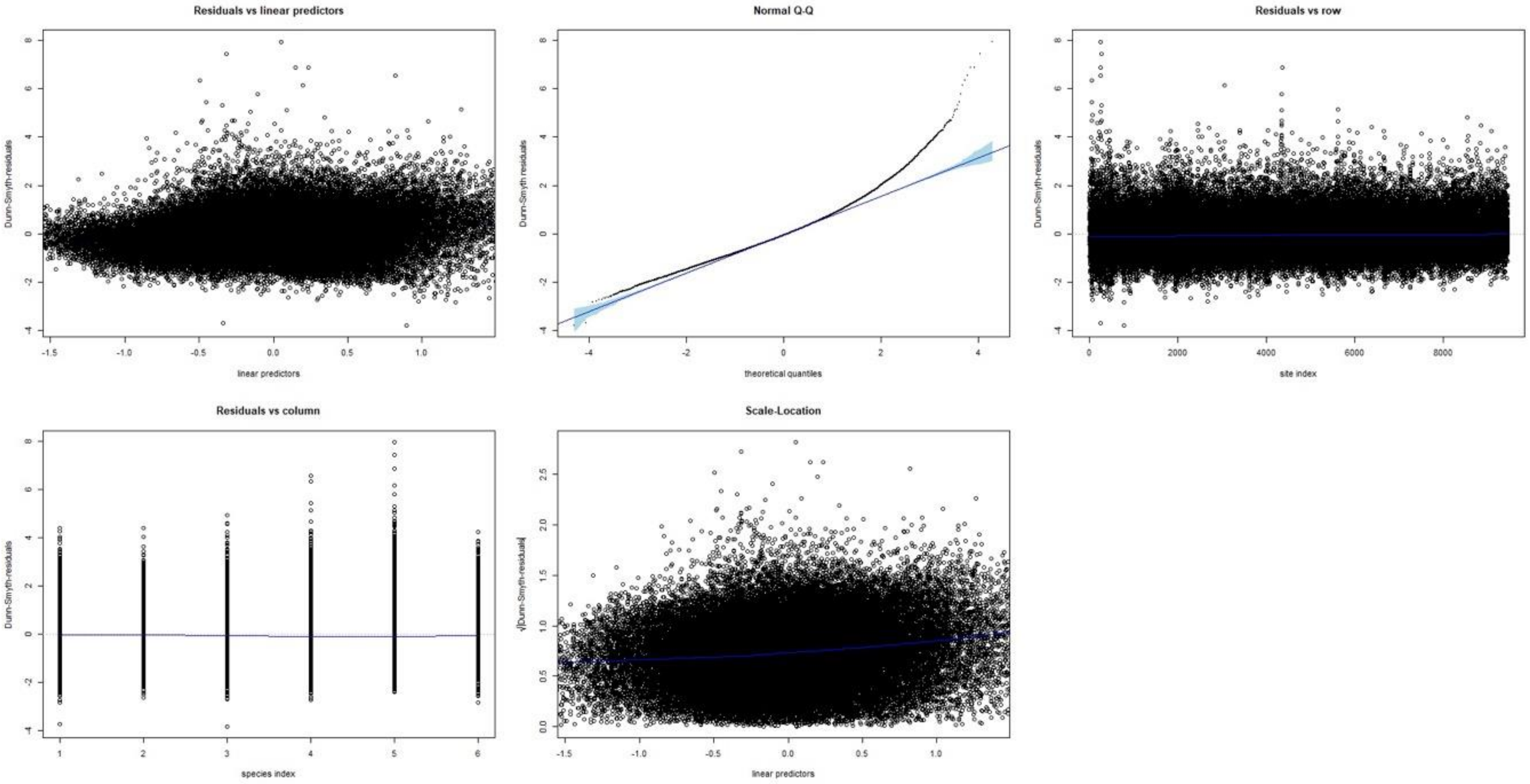
GLLVM Model selection. Final model highlighted in bold text. LV, latent variable

Dataset used	Model No.	Model specification	Df	AIC
Complete	M1.1	Y ~ 1 + 1LV	19	153,287.40
	M1.2	Y ~ 1 + 2LV	24	151,528.40
	M2.1	Y ~ 1 + Distance + 1LV	25	151,931.50
	M2.2	**Y ~ 1 + Distance + 2LV**	**30**	**150,320.80**
Subsampled	M1.1	Y ~ 1 + 1LV	19	34,851.64
	M1.2	Y ~ 1 + 2LV	24	34,329.82
	M2.1	Y ~ 1 + Distance + 1LV	25	34,472.24
	M2.2	**Y ~ 1 + Distance + 2LV**	**30**	**34,024.34**
	M3.1	Y ~ 1 + Distance + Distance2 + 1LV	31	34,478.97
	M3.2	Y ~ 1 + Distance + Distance2 + 2LV	36	34,031.54

MGLLVM ordination of LVs from the complete dataset (n=514)



Model validation subsampled dataset (n=115), M2.2



Coefficients calculated for the elemental ratios and the standard error for the Gaussian multivariate generalised linear latent variable model performed on the subsampled dataset.

Coefficient	Estimate	Std. error	z value	p-value
Sr:Ca	-0.002	0.004	-0.558	0.5771
Ba:Ca	0.009	0.004	1.973	<0.05*
Mg:Ca	-0.035	0.002	-14.927	<0.0001***
Mn:Ca	0.004	0.004	0.938	0.3481
Li:Ca	-0.005	0.004	-1.092	0.2747
Fe:Ca	0.004	0.003	1.068	0.2855

Appendix C

Spawning group contribution to clusters identified using hierarchical clustering. 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.

Spawning group	Cluster	Contribution (%)
ESPS	1	21.8
	2	16.9
	3	2
LSPS	1	19
	2	23.7
	3	4
HS SpSS	1	20.1
	2	20.9
	3	34
FI SpSS	1	15.4
	2	26.7
	3	6
SSS	1	23.7
	2	11.8
	3	54

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Sasha, thank you for believing in me and supporting me throughout this journey, and boy does it feel like a long one! Thank you for always being calm and reassuring me every time I panicked out about something. Thank you for teaching me with patience and kindness. Thank you for our morning coffee chats, they were always interesting and helpful. Henk-Jan, thank you for guiding and advising me throughout the writing of this thesis, your comments and feedback were always appreciated. Thank you for sacrificing your evenings, weekends and holidays to answer my emails and send me comments. Even though the distance between Stanley and the Kiel is huge, I always felt that you were there when I needed reassurance that I'm on the right track.

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Finally, thank you to the people of the Falkland Islands, I did not expect to travel more than 13,000 km away from home and be made to feel welcome like this.

PS I would never forget to include the little dog in this section, not that she can read…but thank you **Ninja** for always being cute and silly in a way that always makes me laugh.

Declaration

I, Irina Chemshirova, hereby declare that the dissertation submitted, entitled: "**Population structure of the Argentine shortfin squid *Illex argentinus* (Castellanos 1960, Family Ommastrephidae)**" was written independently by me and only using the sources listed. The content and design of the thesis, apart from the supervisor's guidance, is my own work. The thesis has not been submitted either partially or wholly as a part of a doctoral degree to another body. This is my first and only doctoral procedure. Chapter I of this dissertation has been published in a peer reviewed journal in 2021, whereas Chapter II has been submitted to a peer reviewed journal and is currently under review, Chapter III is in preparation to be submitted. The contributions to the manuscripts made by myself and my co-authors are explained in the section "Author Contributions". This work was performed respecting the Rules of Good Scientific Practice of the German Research Foundation. I have not been deprived of an academic degree.

Kiel, 08.03.2023

Irina Chemshirova