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Life histories and evolution: insights from notothenioids (Teleostei, Perciformes)

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RIASSUNTO

Gli organismi presentano un’enorme diversità nelle caratteristiche del loro ciclo biologico, sia a livello intra- che interspecifico, mostrando differenze tra popolazioni che vivono in habitat diversi. Tale variabilità fenotipica nel ciclo biologico, è stata messa in relazione all’influenza dei fattori ambientali, e rappresenta il risultato della selezione naturale. Uno degli aspetti chiave dello studio dei processi che hanno portato all’evolversi di queste differenze è cercare di comprendere quali siano le variabili ambientali che influenzano la variabilità dei tratti del ciclo biologico e le relazioni che tra di loro intercorrono. Diversi studi hanno messo in luce come la temperatura e la disponibilità trofica sembrano essere i parametri che meglio spiegano l’andamento dei diversi tratti come i tassi di crescita, l’età alla maturità sessuale, la fecondità, l’investimento riproduttivo, le dimensioni delle uova e delle larve. I pesci del Sottordine Notothenioidea rappresentano un esempio unico di radiazione adattativa in ambiente marino, dominano le acque che circondano il continente antartico sia nel numero di specie, superando le 120 (47%), che in biomassa (90-95%). Durante la loro evoluzione, i nototenioidei hanno subito una profonda diversificazione di alcuni caratteri, adattandosi alle nicchie ecologiche disponibili. Le rigide condizioni dell’ambiente antartico hanno contribuito a differenziare i pesci antartici dalle altre specie ittiche, rallentandone i ritmi di crescita, modificando gli aspetti riproduttivi, lo sviluppo larvale e l’ecologia. I nototenioidei sono distribuiti esclusivamente nell’Oceano Meridionale e in aree sub-antartiche quali Sud America, Nuova Zelanda e le coste più a sud-est dell’Australia. È stata descritta una notevole variabilità dei tratti del ciclo biologico tra specie e tra popolazioni della stessa specie, legata alle diverse condizioni ambientali a cui sono sottoposti gli individui. Con queste premesse, pur essendo disponibili filogenesi recenti ed aggiornate dei pesci nototenioidei, non sono mai stati applicati metodi comparativi che, tenendo conto delle relazioni filogenetiche, permettano di valutare il peso dei diversi fattori ambientali sull’evoluzione delle diverse strategie riproduttive.

L’obiettivo del progetto di dottorato è stato di indagare la variabilità di alcuni tratti del ciclo biologico di specie di nototenioidei in relazione alle variabili ambientali, quali la temperatura dell’acqua, la produttività primaria e la copertura del ghiaccio. Il metodo dei contrasti indipendenti, utilizzato per controllare per le relazioni filogenetiche, rappresenta un punto di forza poiché permette di eliminare la dipendenza dei dati ed evitare quindi possibili relazioni dovute alla storia evolutiva delle specie piuttosto che a reali adattamenti evolutivi. La ricerca ha previsto lo studio della biologia delle specie campionate e la raccolta di dati da letteratura, prendendo in esame lo sviluppo dei gameti, l’investimento gonadico, la fecondità e le dimensioni degli ovociti. I risvolti del presente lavoro sono interessanti sia da un punto di vista della biologia evoluzionistica, per il peculiare adattamento mostrato dai nototenioidei nel loro ambiente, che conservazionistico, poiché la conoscenza dei tratti riproduttivi e della loro risposta ai cambiamenti ambientali è fondamentale per una efficace gestione sostenibile delle specie sfruttate dalla pesca e per valutare la loro capacità di resilienza a superare i cambiamenti climatici in corso. Infatti, alcune specie tra i

nototenioidei, sono state soggette in passato a forte pressione di pesca che, in pochi anni, ha portato al collasso degli stock ittici.

Nella presente ricerca sono stati studiati i tratti del ciclo biologico di 17 specie appartenenti a tutte le otto famiglie di nototenioidei (*Papers* I, II, III, IV, V e VI), fornendo informazioni nuove sulla biologia delle specie e rafforzando quelle disponibili in letteratura. La variabilità intraspecifica nei tratti del ciclo biologico è stata valutata in tre specie grazie alla disponibilità di campioni provenienti da popolazioni di aree diverse (*Papers* I, III e IV). I dati ottenuti dall'analisi delle singole specie, sono stati quindi utilizzati per il confronto interspecifico (*Paper* VI). I campioni disponibili sono stati raccolti durante due spedizioni antartiche a bordo della nave tedesca *R/V Polarstern* (ANT-XXVIII/4, 2012 e ANT-XXIX/9, 2014), lungo la Penisola Antartica e nel Mare di Weddell. Ulteriori campioni di quattro specie, provenienti da diverse aree (South Orkney, South Georgia, Burdwood Bank e versante ovest della Penisola Antartica) sono stati forniti da collaboratori di ricerca. I risultati ottenuti dallo studio sulle singole specie permettono di evidenziare delle caratteristiche comuni tra i nototenioidei.

In tutte le specie analizzate, le femmine hanno presentato un ovario sincrono per gruppi in cui è possibile distinguere nettamente due classi dimensionali di ovociti. Le femmine sessualmente mature hanno mostrato ovociti in fase avanzata di vitellogenesi, che verranno deposti nella stagione riproduttiva in corso, e ovociti in stadio di alveoli corticali o previtellogenetici, che matureranno per l'evento riproduttivo dell'anno successivo. La presenza contemporanea di ovociti in stadio avanzato di vitellogenesi, insieme ad altri allo stadio di alveoli corticali, evidenzia un'ovogenesi di lunga durata, verosimilmente, superiore ad un anno. I testicoli sono stati descritti come di tipo lobulare *unrestricted* e, in individui sessualmente maturi, hanno mostrato la presenza di spermatozoi nei dotti e spermatogoni localizzati soprattutto nella parte distale. Inoltre, è emerso come il processo di maturazione degli spermatozoi sia completo prima dell'inizio del periodo di riproduzione. È possibile quindi affermare che anche la spermatogenesi, come l'ovogenesi, è un processo molto lungo e, in generale, la lunga e lenta gametogenesi osservata è probabilmente correlata alla bassa temperatura e all'alto investimento riproduttivo dei nototenioidei. Lo studio della biologia delle specie ha fornito nuove informazioni sulla fecondità (assoluta e relativa), sulle dimensioni degli ovociti e sull'indice gonadosomatico (IGS), informazioni poi utilizzate per le analisi comparative (*Paper* VI). La stima dell'età, attraverso la lettura degli otoliti, ha permesso di valutare il tasso di crescita, la relazione tra la taglia e l'età, l'età massima e quella alla maturità sessuale (*Papers* II, III, IV, V). I risultati seguono le attese, infatti, come descritto in specie di acque fredde, i nototenioidei mostrano lento accrescimento, raggiungimento tardivo della maturità sessuale (circa 50-87% rispetto alla lunghezza massima) e longevità media (fino a 24 anni).

Dal confronto intraspecifico emerge come la variabilità osservata nei tratti del ciclo biologico, possa essere attribuita a fattori ambientali che agiscono su piccola scala, come ad esempio correnti fredde dovute allo scioglimento del ghiaccio e/o a caratteristiche biologiche tipiche della specie. Infatti, il tipo di uova (pelagiche o demerse), la presenza o meno di cure parentali, la distribuzione degli individui adulti e la mobilità della specie, possono avere un ruolo chiave nell'influenzare l'areale di distribuzione e di dispersione di una specie.

Il confronto tra le due popolazioni dell'*icefish Chaenocephalus aceratus*, una specie bentonica a bassa mobilità, con uova demersali e presenza di cure parentali, ha dimostrato una fecondità più alta nell'area con temperatura dell'acqua più elevata e che non risente delle correnti fredde che si originano nel Mare di Weddell.

Non emergono, invece, differenze intraspecifiche nei tratti riproduttivi in *Pleuragramma antarctica*, una specie pelagica, con uova cryopelagiche (cioè deposte sotto lo strato di ghiaccio stagionale) e in *Notothenia rossii*, dove i lunghi tempi di incubazione delle uova pelagiche e della fase larvale, sembrano assicurare connettività tra le aree investigate. In generale le discrepanze emerse in termini di dimensioni degli ovociti e IGS, sono probabilmente legate a differenze temporali nei periodi di campionamento.

Le analisi comparative a livello interspecifico hanno evidenziato relazioni tra fattori ambientali e tratti del ciclo biologico. La taglia massima, a differenza degli altri tratti considerati, non ha presentato nessuna relazione con le variabili ambientali. Sembra quindi che la regola di Bergmann, che descrive la relazione positiva tra taglia massima e latitudine, con individui più grandi in ambienti più freddi (latitudini più alte), non sia applicabile alle alte latitudini.

Nei nototenioidei è emersa una tendenza significativa a produrre ovociti più grandi al diminuire della produttività primaria, che raggiunge i valori minimi procedendo verso il Polo, sebbene presenti variabilità stagionale. A livello interspecifico, quindi, si osservano ovociti di maggiori dimensioni in specie che occupano alte latitudini, a conferma di quanto precedentemente previsto dai modelli teorici generali e dimostrato in diversi studi sperimentali. I principali fattori ambientali individuati come possibili responsabili del generale aumento delle dimensioni degli ovociti con la latitudine sono la disponibilità di cibo e la temperatura. In questo caso, nei nototenioidei, sembra avere maggior influenza la disponibilità di cibo, considerando che la produttività primaria può esserne una misura. Inoltre, comunemente, da uova più grandi schiudono larve di maggiori dimensioni, che dovrebbero mostrare quindi migliori abilità competitive, capacità di nutrirsi di prede di diverse dimensioni e una maggiore resistenza alla carenza di cibo. Complessivamente queste caratteristiche dovrebbero garantire un'elevata probabilità di sopravvivenza delle larve in condizioni di risorse alimentari limitanti. Inoltre, la dimensione degli ovociti aumenta al crescere della taglia massima. In termini generali, però, i pesci di grande taglia non mostrano una tendenza a produrre necessariamente uova grandi, basti pensare a pesci di notevoli dimensioni, come il tonno e il pesce luna, che depongono uova molto piccole. Sembra quindi che la taglia corporea finisca più per limitare il range di dimensioni delle uova in pesci di piccola taglia, piuttosto che determinare, la dimensione massima delle uova che una specie può produrre. Questo emerge anche dai risultati ottenuti per i nototenioidei, dove le specie di maggiori dimensioni, hanno a disposizione una maggior gamma di combinazioni per ripartire lo sforzo riproduttivo, tra qualità in termini di dimensioni e quantità degli ovociti. Le specie distribuite alle basse latitudini antartiche producono ovociti sia piccoli che grandi, mentre le specie di alte latitudini presentano esclusivamente ovociti di grandi dimensioni. In considerazione del compromesso esistente tra numero e dimensione degli ovociti che una femmina può produrre per un dato evento riproduttivo, la relazione negativa tra taglia degli ovociti e produttività primaria dovrebbe comportare un aumento della fecondità relativa, ovvero del numero di ovociti prodotti in relazione al peso della femmina, in condizioni alimentari favorevoli, tuttavia questo risultato non è stato osservato. Invece, la fecondità relativa sembra essere maggiormente influenzata dalla temperatura con, mediamente, un maggior numero di ovociti prodotti a maggiori temperature (basse latitudini antartiche), aspetto questo che necessita di ulteriori approfondimenti.

L'indice gonadosomatico, considerato una misura dell'investimento riproduttivo, risulta positivamente legato alla copertura di ghiaccio, mostrando valori molto alti in corrispondenza delle alte latitudini. A medie latitudini antartiche, durante l'estate australe, lo scioglimento stagionale del ghiaccio innesca la proliferazione del fitoplancton, portando ad un importante aumento locale della produttività primaria il cui effetto a cascata si estende a tutta la rete trofica. Alle alte latitudini, invece, l'inverno è lungo e buio, la copertura di ghiaccio è permanente e la produttività primaria, di conseguenza, rimane attorno a valori bassi per tutto l'anno. Queste condizioni difficili possono aver portato ad un generale maggior investimento energetico nella riproduzione per le popolazioni di queste aree, che si riflette anche nell'investimento in ovociti (dimensioni e/o quantità), rispecchiato appunto dall'indice gonadosomatico.

Il risultato generale che emerge da questo studio mette in risalto quelle che sembrano essere le principali forze evolutive che agiscono in ambiente antartico. È possibile inoltre affermare che il metodo comparativo si è dimostrato uno strumento valido e robusto per studiare la risposta adattativa alle condizioni ambientali. I nototenioidei si sono confermati essere un eccellente taxon per studi in ambito evolutivo, e ulteriori indagini potrebbero portare ad una più completa ed esaustiva comprensione dei processi evolutivi. È auspicabile quindi aumentare il numero delle specie in esame, allargando la ricerca anche ad altri taxa, e includere altri tratti del ciclo biologico, come la taglia/età alla maturità sessuale e l'età massima.

Da un punto di vista conservazionistico, prendendo in considerazione le caratteristiche comuni a tutti i nototenioidei, la produzione di pochi ovociti di grandi dimensioni, l'alto investimento nello sviluppo delle gonadi e, in alcuni casi, la presenza di cure parentali, i bassi tassi di crescita e una maturità sessuale tardiva, complessivamente è possibile inquadrare il sottordine come un taxon altamente vulnerabile, specialmente in uno scenario di cambiamenti climatici e di potenziale riapertura della pesca.

ABSTRACT

Organisms show an extraordinary variation in their life history traits, both at inter- and intraspecific level, exhibiting phenotypic variations among populations inhabiting different habitat. The variability in life history traits has been related to environmental conditions, as a result of selective processes or phenotypic response to them. In addition to the huge diversity in life history traits, several trade-offs among them have been recognized. The variability in life history traits and in their trade-offs related to environmental conditions represents a key aspect in the study of evolutionary processes. A wide variation in life history traits is documented among teleost fishes, both among and within species, reflecting the effects of evolutionary forces acting on them over time and across environmental conditions. Several life history traits, such as growth rate, age at sexual maturity, fecundity, reproductive investment, egg size, hatching size, are strongly influenced by temperature and food availability. Notothenioid fish represent a unique example of fish adaptive radiation in marine environment. They dominate the waters surrounding the Antarctic continent both by species number, with over 120 species (47%), and biomass (90-95%). Notothenioids distribution is limited exclusively to the Antarctic and sub-Antarctic regions (South America, New Zealand and South East Australia). Variability in their life history traits at inter- and intra-specific levels has been described, with a latitudinal trends in some reproductive traits. In this framework, despite the availability of recent phylogenies of notothenioids, a comparative analysis aimed at studying habitat dependent evolution of reproductive strategies has not yet been performed. Given the particular characteristics of their habitats and the uniqueness of notothenioid fishes, they can be considered an excellent taxon model to investigate the evolution of life history traits in relation to environmental factors.

The aim of this PhD project was to investigate the evolution of life history traits of notothenioid species in relation to environmental variables (such as sea water temperature, primary productivity and sea ice cover), controlling for phylogenetic relationships. The applied methodology (Independent Contrast Method) represents a key aspect in this study since its robustness can prevent the attribution of correlations between life history traits and environmental variables to evolutionary processes, being instead a consequence of phylogenetic relationships among considered species. Using life history traits estimated from collected species and reported in data available in literature, the study focused the attention on gametogenesis and, gonadal investment, such as fecundity and egg size. Such a comparative study of habitat dependent variation of life history traits is interesting for evolutionary biology, given the extreme adaptations shown by Antarctic fishes to their peculiar environment, as well as for conservation biology, because the knowledge of reproductive traits and of their sensitiveness to environmental changes is recognized as a crucial information for the sustainable management of exploited species and their resilience capacity to overcome current climate change.

In the present research, life history traits of 17 species, belonging the eight notothenioid families were investigated (Papers I, II, III, IV, V, VI), providing original information on their

biology and strengthening the reliability of the available literature. Intraspecific variability in life history traits was examined in three species, including in the analyses specimens sampled in different areas (Papers I, III and IV). Unpublished life history traits data of 15 species were included in the interspecific comparison (Paper VI). Species included in this study were collected during two cruises carried out onboard the German *R/V Polarstern* (ANT-XXVIII/4, 2012 and ANT-XXIX/9, 2014) off the Antarctic Peninsula and in the Weddell Sea. Samples, belonging to four notothenioid species, were provided by partner scientists during past Antarctic expeditions (2009, 2010 and 2011 during the austral summer) in different areas (i.e. South Orkney, South Georgia, Burdwood Bank and Western Antarctic Peninsula).

Comparing the results obtained in studies dealing with individual species, the histological analyses generally indicated some similarities among notothenioids. In all the analysed species, females presented a group synchronous ovarian type, in which two clearly distinct groups of oocytes were visible. The more advanced stage of maturity in maturing or mature females were oocytes at late stage of vitellogenesis, constituting the batch of the current season, while the other group included oocytes at the cortical alveoli or previtellogenic stages, representing the next year batch. The occurrence of late vitellogenic oocytes together with others at the cortical alveoli stage supports a prolonged oogenesis, likely lasting more than one year. Testes were of the unrestricted spermatogonial testicular type. In mature males, testes were completely filled with sperm with few cysts of early-stages of spermatogenesis, indicating that sperm maturation completes before the beginning of the breeding season. Long lasting gametogenesis is likely related to the low temperature and to the high reproductive investment.

The study on individual species provided new data on absolute and relative fecundity, egg size, gonadosomatic index (GSI) and age estimates and therefore information useful for the comparative analyses (Paper VI). By means of age estimates through otolith reading, growth rate, age/length relationship, maximum age and age at sexual maturity were assessed (Papers II, III, IV, V). As expected for cold water fish species, notothenioids exhibit slow growth rate, late sexual maturity (at about 50-87% of maximum size) and long life span (up to 24 years).

The intraspecific comparison highlighted that the observed variation in life history traits could be addressed to local conditions (i.e. local prevailing currents linked to cold water masses and melting ice) and/or be a consequence of the species reproductive habits, including egg type, presence/absence of parental care, adult distribution and mobility. In *Chaenocephalus aceratus*, a benthic species with demersal eggs and parental care, fecundity was higher in the warmer study area, that is not influenced by cold water masses coming from Weddell Sea, respect to the colder area. In the Antarctic silverfish (*Pleuragramma antarctica*), the only species lying cryopelagic eggs (i.e. eggs laid to the lower surface of platelet pack ice), and in *Notothenia rossii*, in which individuals shift from benthic to bentho-pelagic habits achieving the adult phase, no differences emerged between populations, with the exception of some differences in oocyte size and GSI, likely related to temporal differences in sampling periods.

The results of the interspecific comparative analyses highlighted some correlation between environmental factors and life history traits, revealing potential evolutionary forces. Differently from the other biological traits considered in this study, the maximum size did not correlate with any environmental variable. These results support the observation that the Bergmann's rule, describing the occurrence of a positive relationship between maximum size and latitude, with larger specimens found in colder environments (higher latitudes), does not apply at high latitudes.

In notothenioids egg size was negatively related to primary production. Primary production, in turn, is negatively related to latitude, and general theoretical models and relevant studies indicate a positive relationship between egg size and latitude. Food availability and temperature have been claimed as factors driving the general increasing trend in egg size with latitude. Primary production can be considered a proxy of food availability, therefore in notothenioids food availability appears to be the main factor influencing egg size. A positive relationship between egg and larval size is common, and larger larvae are expected to show enhanced competitive abilities, improved capacity to feed on a wide size range of prey items and enhanced starvation resistance, and ultimately higher survival probabilities in extreme conditions. Egg size is also positively related to the maximum parental body size. Generally, large fishes, as for instance tunas or the Ocean sunfish *Mola mola*, are not more likely to have large eggs than small ones, nonetheless fish size may indeed constrain, rather than determine, the range of possible egg sizes, as suggested also by the results on notothenioids. Large notothenioids could therefore have a wider range of tactics in the partition of reproductive effort between fecundity and egg size. While Low Antarctic species produce small and large eggs, High Antarctic species present only large eggs. Considering the trade-off between egg size and number, a positive relationship between relative fecundity (i.e. the number of eggs in relation to body weight) and primary production was expected but it was not found. Conversely a positive relationship was observed between relative fecundity and mean water temperature, although it remains an unsolved issue which need to be further investigated. Energetic investment in female reproductive effort, i.e. maximum female GSI, showed a positive relationship with sea ice cover. Seasonal pack ice melting, occurring in summer at intermediate Antarctic latitudes, triggers phytoplankton blooms, resulting in an increased primary production with a cascading effect on the pelagic food web. At higher latitudes, where maximum values of GSI have been detected, the sea ice cover is permanent through the year and the primary production is likely to remain low, being also influenced by the long and dark Antarctic winter. These extreme conditions may therefore trigger a higher investment in reproduction, including the investment in eggs (size and/or number) represented by the female GSI.

The insights provided by this study shed light on the major factors that appear to drive the evolutionary processes occurring in the Antarctic environment. The comparative method proved to be a robust tool in investigating adaptive response to different environmental conditions. Despite notothenioids demonstrated to be an excellent model group to study evolutionary process, further investigations, extending to other taxa and species geographical distributions, are necessary to trace more general and comprehensive patterns in the evolution of life history traits. In any case, shared reproductive features such as low fecundity, large egg size, high reproductive investment in gonads and, in some cases, in parental care, low growth rate and late sexual maturity, depict notothenioids as a taxon highly vulnerable to climate change and fishery re-opening scenarios.

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INTRODUCTION

1. The evolution of life history traits

The life history includes several biological traits of life cycle of organisms: their size at birth, how they develop and their growth rate, the age and size at sexual maturity, how many offspring and of which size and sex ratio they produce, their maximum age and size (Stearns 1992).

Life history theory attempts to explain how the evolutionary forces shape organisms to increase their survival and reproductive success (Darwin 1871), and therefore their fitness. The optimization of life history traits is influenced by the so called "boundary conditions" (Stearns 2000): life history traits are constrained by both selective factors in the environment, like as the extrinsic mortality (the variance in the probability of death that is not accounted for by mating or parenting effort) and factors intrinsic to the organism (i.e., trade-offs among traits, constraints) (Stearns 1992; Roff 1992; Stearns 2000). Once these conditions have been understood and defined, life history models can be used to predict size at birth, growth pattern, age and size at sexual maturity, number, size, and sex-ratio of offspring, age-, stage- or size-specific reproductive effort, age-, stage- or size-specific rates of survival, and lifespan.

Optimization and quantitative genetics are two approaches used to investigate the construction of life-history trait predictions (Roff 1992; Stearns 1992). Optimization criteria are usually used in theoretical evolutionary studies where the predictions identify the optimal phenotype that is expected to evolve under specified constraints and trade-offs (Roff 1992; Stearns 1992). Quantitative genetic models predicts life history traits combining information on selective pressures and heritability of phenotypic traits (Falconer and Mackay 1996; Roff 1997; Lynch and Walsh 1998).

The fitness of an individual organism can be defined as “the average number of offspring left by an individual relative to the number of offspring left by an average member of the population” (Ridley 2004). To maximize the fitness, survival and reproduction should be maximized at all ages/sizes of an organism. The life history traits of an hypothetic organism should be therefore selected as to allow the organism to start reproducing as soon as it is born, produce an infinite number of offspring, and live forever. These so called "Darwinian demons" (Law 1979) organisms do not exist in the real world because of constrains, resources’ limitations and trade-offs in life history traits and indeed natural selection cannot maximize life history traits, and thus fitness, beyond certain limits. Life history traits are often traded-off among each other (Stearns 1992; Roff 1992). As stated by Stearns (1989), “trade-offs represent the costs paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another. If there were no trade-offs, then selection would drive all traits correlated with fitness to limits imposed by history and design”. However, several life history traits are maintained well within those limits. “Therefore, trade-offs must exist”. Starting from the Seventies, trade-offs in life history traits have been investigated in numerous studies through field observations, experimental manipulations, phenotypic correlations in the laboratory and genetic correlations, therefore playing a central role in the development of life history theory (Stearns 1989). Three main levels of trade-offs have been recognized: phenotypic trade-offs, genetic trade-offs (genetic covariance), and an intermediate level, involving physiological and developmental mechanisms leading to the allocation of the available resources among different functions, like reproduction (current and future), growth, survival, etc. (Stearns 1989). Among the most studied trade-off, the ones between current reproduction and survival and between current and future reproduction have been more extensively investigated. Since organisms have a limited amount of resources, they have to partition them between reproductive and non-reproductive activities. Reproductive effort is the proportion of parental resources devoted to the different activities related to reproduction, from gametes production, to several other activities that depend on reproductive sites, mating system, the occurrence of parental care, etc., in the study species. Therefore reproductive effort may include mate search and attraction, migration towards the breeding sites, nest building, parental care on eggs/offspring and, in general, any activity that increase offspring production and survival. The investment in the current reproduction decreases the individual's survival probability, growth rate, and therefore the future reproduction (Roff 1992; Stearns 1992). The attainment of sexual maturity is indeed related, in several species, to a consistent decrease (or stop) in growth rates. Since individual size is positively related, in several species, to fecundity (see for instance in fish Jennings *et al.* 2001), higher capacity to attract mates, better parental care (Andersson 1994), a decrease in growth rates, due to the attainment of sexual maturity and/or the investment in current reproduction, implies a decrease in the future reproductive success. On the other hand, the current investment in reproduction increases the offspring production and survival. The reproductive effort appears indeed regulated by trade-offs between the advantages of high investment in reproduction (Stearns 1989) and the associated costs (Lindén and Møller 1989). Individuals can modulate their current reproductive effort in response, for instance, to ecological factors, like predation or food competition (Julliard *et al.* 1997; Both 1998), condition, ability to raise the juvenile phase of their offspring (Lack 1947; Pettifor *et al.* 1988), the quality (Wedell 1996; Kolm 2001) and the attractiveness of their mate (De Lope and Møller 1993; Petrie and Williams 1993).

Considering a given investment in reproduction, again several traits may be traded-off. In the trade-off between fecundity and egg/offspring size, an increase in egg/offspring size implies a decrease in fecundity (Bell 1980). This trade-off constitutes an essential component of several evolutionary models predicting optimal reproductive allocation in vertebrates (Smith and Fretwell 1974; Charnov 1997). The evolutionary basis of trade-off correlations are generally genetic based (Roff 2002), the negative genetic correlation between the number and size/quality of offspring has been reported in many taxa, such as fish (Snyder 1991), reptiles (Sinervo 2000), birds (Krist 2011) and mammals (Oksanen *et al*. 2002; Mappes and Koskela 2004).

Organisms differ enormously in their life history traits, both among and within species, showing phenotypic variations among populations inhabiting different habitats (Roff 2002; Begon *et al*. 1990). Life history variation, at intra-specific level, is often strongly influenced by the environmental conditions (e.g. temperature, nutrition, predators), this phenomenon is known as phenotypic plasticity, recognised as the capacity of a single genotype to generate a variety of phenotypes across different environments (Stearns 1992; Roff 1997). The reaction norm is the mathematical function describing the phenotypic plasticity of a specific genotype, representing the phenotypes expressed by a genotype in different environments. For a given genotype, adaptive reaction norms are expected to evolve across environmental gradients providing the expressed phenotypes (Schmalhausen 1949; Stearns and Koella 1986), so that optimal values of fitness-related traits should change under different conditions. Theoretically, if a genotype experiences highly variable environmental conditions, it often achieves an higher fitness if it has phenotypic plasticity (environmental variability hypothesis) (Stearns 1992; Hutchings 1996). Many life history traits (e.g. age at maturity, fecundity) exhibit a high degree of plasticity, and natural populations show genetic variation for plasticity.

There is general consensus that selection on life history traits acts through maximization of global fitness measures (Roff 1992; Stearns 1992; Charlesworth 1994) and that aspects of fecundity and survival are important parts of these measures. Indeed it is important to define two different kinds of fitness measures: local fitness and global fitness. The first focuses on a specific component (e.g. number of eggs, hatching success, larval survival, etc.) and implies that the maximization of a fitness component will also maximize the overall fitness; the global fitness is the overall fitness of an organism and measures every life history constituents and their inter-relationships. An example of global fitness measures are the net reproductive rate (R_0) (the mean number of offspring that a female produces in her life) and the instantaneous rate of natural increase (r). It includes the product of all age classes (x) specific fecundity (m_x, expected number of offspring for a female in age class x) and survival (l_x), the probability of surviving from beginning to age class x:

$$1 = \sum_{x\,=\,a}^{\omega} e^{-rx}\, l_x m_x$$

$$R_0 = \sum_{x\,=\,a}^{\omega} l_x\, m_x$$

where *a* is age at maturity and *w* is the expected age at death.

The evolution of life history traits is highly correlated to environmental conditions. In this context, three main environmental scenarios have been taken into considerations when studying life history traits: predictable environments, stochastic environments, and density-dependent conditions.

Life history evolution in stable, predictable environments

In order to simplify the analyses on the evolution of life history traits, most studies have assumed a constant/stable environment and neither density-dependent nor frequency-dependent selection (Charlesworth 1994; Roff 2002). These assumptions have been considered a realistic scenario in several case studies (Roff 1992). When the propagation of genotypes in a population is density independent and occurs in a stable environment, the optimal phenotypes are expected to remain constant (Roff 1992; Stearns 1992).

Assuming stable age structures, density independence and equilibrium conditions, the optimization models predict that an increase in adult mortality rate relative to the juvenile mortality rate selects for a decrease in age at maturity and an increase in reproductive effort (Reznick 1982; Hutchings 1993; Reznick *et al.* 1996). Focusing on fish, starting from the von Bertalanffy growth function (that predicts the length of a fish as a function of its age) a general model for optimal maturation age (α) in fish can be estimated as:

$$\alpha = \frac{1}{k}\ln\left(\frac{3k}{M} + 1\right)$$

where M and k are constants, representing natural mortality and growth respectively. This model predicts accurately the optimal age at maturity from empirical data (Roff 1984; Stearns 1992). Taking into account the assumptions of maximizing global fitness measure (r or R_0) the estimation of the optimal ages at maturity has to consider its costs (increased mortality, reduced growth, reduced future fecundity) and benefits (survival and reproduction). Changes in different costs and benefits relationship should be taken into account to analyse and estimate the global fitness (Roff, 1992; Stearns, 1992).

Life history evolution in stochastic environments

Temporal variability in the environment can change demographic parameters (i.e. survival, fecundity, etc.) and can lead to the evolution of life history strategies differing from those that are optimal in constant environments (Tuljapurkar 1990; Orzack and Tuljapurkar 2001; Metcalf and Koons 2007). Demography of the populations is affected by the variability of environmental conditions (Charlesworth 1994). While spatial variation impacts on a proportion of the population, time variation has influence on the entire population (Roff 1992). The capacity of individuals to spread the risk (related to predators, disease, or other environmental hazards), across time is the best solution in variable, uncertain environments (Cole 1954; Murphy 1968; Gadgil and Bossert 1970; Bell 1980; Schaffer 1974; Orzack 1985; Roff 1992). Different traits are involved in risk-spreading like iteroparity, variable age at maturity (Wilbur and Rudolf 2006) and delayed seed germination (Cohen 1966; Rees *et al.* 2006).

To predict optimal phenotypic values of life history traits in stochastic environments, other equations than those applied in stable systems have to be used, considering the variation in demographic rates (Orzack and Tuljapurkar 1989; Metz *et al.* 1992;

Charlesworth 1994). Indeed the direction of selection may vary in time and space in a stochastic environment (Via and Lande 1985; Hutchings 1996). Period-specific finite rates of increase may be estimated by dividing the population growth history into different periods.

Life history evolution under density-dependence conditions

Life history traits as growth, survival and reproduction are density dependent if their rates change as a function of the density or number of individuals in a population. The main factor causing density dependence is the occurrence of limited food resources. In these cases, competitive interactions among individuals are expected to occur. Density-dependent factors are known to influence the optimal outcome of the trade-off egg/offspring size vs fecundity. In several oviparous species, including fish, there is a positive correlation between egg size and hatched larvae (Roff 1992). Larger offspring have enhanced competitive abilities, improved capacity to feed on a wide size range of prey items and better starvation resistance, and therefore higher survival probabilities (Conover and Schultz 1997). Therefore, in a density-dependent scenario a reduction in egg/offspring number and an increase in egg/offspring size is expected (Smith and Fretwell 1974; Sibly and Calow 1983).

The influences of resource abundance have been experimentally tested on fishes, e.g. on salmonids where females' reproductive investment, in terms of offspring (large eggs, nest digging, nest guarding), is very high and where larger offspring and juveniles have better capabilities to access and defend optimal feeding areas; the dominant status, attained by larger offspring, ensures an enhanced access to preys and, given the stress-induced behaviour in subordinates, this is retained even when resources are not limited (Kalleberg 1958; Abbott *et al.* 1985; Abbott and Dill 1989). Since increased egg size is balanced by reduced fecundity (trade-off), the optimal egg size is constrained by the costs related to the decrease in egg number (Bell 1980). Furthermore abiotic factors, like incubation temperature and the oxygen availability can constraint the increase in eggs size (Chapman 1988).

In addition to the three main environmental scenarios mentioned above, some general patterns in life history traits have been highlighted at geographical scales. A positive relationship between maximum size and latitude has been observed (Bergmann 1847). Although firstly proposed and described for endothermic species, this pattern has been observed also in ectothermic ones, at both inter- and intraspecific levels (this phenomenon has also been called James's rule; Blackburn *et al.* 1999). Even if some authors disagree with the occurrence of this latitudinal cline (Blackburn *et al.* 1999), nonetheless it has been documented in a variety of species (Ashton 2001; Meiri and Dayan 2003; Huston and Wolverton 2011), including fish (Fisher *et al.* 2010). This pattern in size variation with latitude has been put in relation with two main factors that may act as causes: temperature, influencing the metabolic rates (Bergmann 1847; Angilletta and Dunham 2003) and net primary production as a proxy of food quantity and quality

(Huston and Wolverton 2011). At geographical scale both factors correlate negatively with latitude. The general pattern of size vs latitude, however, seems not to apply in two contexts: the tropics and above 60° of latitude (Huston and Wolverton 2011). Similarly to body size, a latitudinal cline in egg/offspring size has been documented among closely related species in different taxa of marine animals (Rass 1941, 1986) and the production of larger eggs/offspring at higher latitudes has been mainly attributed to poleward decreasing temperature (Rass 1986; Laptikhovsky 2006).

Teleost fish life histories

Teleost fishes display a considerable inter- and intraspecific variation in life history traits, reflecting the effects of evolutionary forces acting on them over time and across environmental conditions in their worldwide distribution. Species may be annual or show quite long lives, up to several decades, they can mature as soon as they are born, as in the case of the Embiotocidae *Micrometrus minimus* or at several years of age, most fishes spawn repeatedly, while some are semelparous, spawning only once during their lives. Most fishes remain one gender throughout adult life but many species change sex, and some are parthenogenetic, producing young from unfertilized eggs. Most Teleost fish species are oviparous, with larvae hatching from eggs, however some show internal gestation and the newborn are juveniles. Fertilization is mostly external but internal one occurs in some families. Many marine fishes produce pelagic eggs, laid in the water column and drift by water current. Eggs are laid on the bottom in most fresh water fishes, but also in some marine species. Eggs can be left on the bottom or laid inside nests, that can be simple depressions in the bottom or more elaborate structures usually made by male. Parental care, mainly including egg guarding and in some cases also young guarding, is present in some demersal spawners and is mainly paternal (Helfman *et al*. 1997) . Considering the life history traits of teleost fish, Adams (1980) recognized two types of species, r- and K-selected, and stated that adult size, maximum age, and age at maturity should be all positively correlated. The limit of this prediction is that r and K-selection hypothesis does not take into account the size effects and the age structure, as well as the fluctuations in mortality and fecundity rates (Stearns 1976; Stearns 1992). Many authors highlighted three endpoints of a triangular continuum describing life history strategies in fish: the *opportunistic strategists*, including small, rapidly maturing, short lived fishes; the *periodic strategists*, including larger, highly fecund and longer living fishes; the *equilibrium strategists*, including intermediate size fishes that often exhibit parental care producing fewer but larger eggs (Winemiller and Taphorn 1989; Winemiller and Rose1992). Winnemiller and Rose (1992), in their large comparative study of life history traits among North American fishes, highlighted a positive relationship between longevity and age at maturity in marine fishes. Many authors found that size at maturity is negatively correlated with the slope (b value) of the fecundity-length relationship and that steep slopes are linked to reduced growth rate after maturation (Schopka and Hempel, 1973; Wootton 1979;

Rochet 2000). Short-lived species tend to have lower values of b than longer-lived ones. It is possible to hypothesize that the more growth takes place after maturity, the less energy is available for reproduction, resulting in a slower increase of fecundity with size (Pease and Bull 1988; Stearns 1992; Frank and Leggett 1994). Fecundity and egg size show a wide range of variation among fish species and represent key features of a reproductive strategy. To a given reproductive effort allocated, a trade-off between few large (quality) or many small (quantity) eggs exists and may produce highly different recruitment to juveniles (Smith and Fretwell 1974; Ware 1975). The variability in the allocation of reproductive effort to egg size and fecundity seems not to be influenced by body size (Ware 1975; Duarte and Alkaraz 1989), in fact large fish are not more likely to produce large eggs than small fish (Miller 1984; Hislop 1984; Duarte and Alcaraz 1989), as testified by many very large fish species spawning small eggs. For example, the ocean sunfish *Mola mola* (up to 2300 kg of weight and 310 cm of length) and sprat *Sprattus sprattus* (maximum 27 g and 16 cm) both produce eggs that are around 1mm in diameter (Froese and Pauly 2008). Duarte and Alcaraz (1989), highlighted that the range of egg sizes possible for fishes of a given size is constrained by a lower limit to mature egg size (ca. 0.3 mm of diameter) and a size-dependent upper limit to egg size (Fig. 1). Therefore, fish size appears to constrain, rather than to determine, the range of egg sizes possible for fishes of any one size. The greater spectrum of egg sizes possible for large fish imply that a greater range of tactics in the partition of reproductive effort between fecundity and egg size is available to large fish (Duarte and Alcaraz 1989).

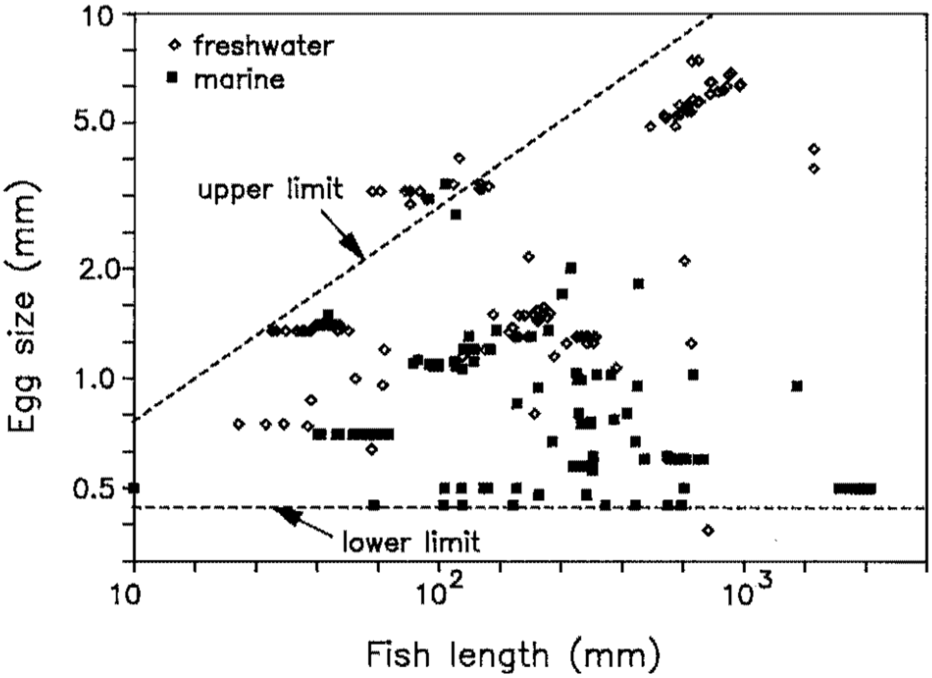

Figure 1. The relationship between fish length and egg size. Dashed lines represent the hypothesized upper and lower limits to egg size (modified from Duarte and Alcaraz 1989).

Regarding egg size, in general large eggs are typically demersal and produced by species inhabiting estuaries, littoral environment or Antarctic environments (Dando 1984; Kock and Kellermann 1991). In the marine context, pelagic eggs develop in a peculiar environment, often characterized by temporal stochastic oceanic circulation and patchy structure, that influence the location and the trophic conditions of early fish stages. While there is no or little parental control on the pelagic eggs, in demersal eggs there is a greater control on the environmental characteristics that embryos will experience. As a consequence, the variability in the growing conditions experienced by offspring could be reduced. Indeed, larval mortality of pelagic eggs seems influenced mostly by the stochasticity of the habitat where hatching occurs, while larval mortality from demersal eggs seems influenced by a greater extent by the potential survival of the individual larvae. In this context the optimal trade-off increasing the fitness in pelagic spawners seems toward maximizing eggs quantity (that imply small eggs) whereas in demersal eggs producing larger eggs (that imply fewer eggs) maximizing the larval survival. Small pelagic eggs have a relative faster hatching time, which should reduce the distance between spawning and hatching locations, thus reducing, in some cases, the possibility to reach unsuitable environments (Duarte and Alcaraz 1989).

Considering demersal eggs, some studies highlighted a positive relation between egg size and parental care (Balon 1984; Kolm and Ahnesjö 2005) and laying demersal eggs has been considered as a preliminary step towards the increase of parental care (Potts 1984; Duarte and Alcaraz 1989). Many fish species produce eggs of similar size, despite large variation in other traits points toward some shared ecological mechanisms that underlie the evolution of offspring size. Moreover, Duarte and Alcaraz (1989), studying the allocation of reproductive effort between fecundity and egg size in marine and fresh water fishes, concluded that it is mostly independent of their phylogenetic linkage or proximity, but depends on the specific characteristics of the habitat they occupy. In fish, as in several other taxa, many models predict that offspring should be as small or as large as possible but not in between (Shine 1978; Christiansen and Fenchel 1979). Empirical observations showed a bimodal distribution in egg size among fish species, with eggs being either relatively small or large, and few cases of intermediate sizes (Parker and Begon 1986; Duarte and Alcaraz 1989; Kock 1992; Laptikhovsky, 2006). Moreover, some geographical variation has been highlighted in the size frequency distribution of eggs, indeed in the tropics and at temperate latitudes fishes are represented by species with both small and large eggs, whereas in polar regions only by species with large eggs (Laptikhovsky, 2006). In general, in several marine animal taxa, including fish, it has been found a trend of increasing egg size with the decrease of temperature towards high latitudes and higher depths (Laptikhovsky 2006). This trend called “Rass–Thorson– Marshall’s rule” (Mileykovsky 1971; Alekseev 1981) has reached a paradigm status through time and is now accepted by marine biologists (Levin and Bridges 2001).

Egg size is generally species-specific, although within species it may vary between populations and even within a population. Intraspecific variation in egg size could be related to female size (bigger females are likely to produce larger eggs; reviewed by Johnstone 1997), time of spawning (eggs spawned early in a season by multiple spawners tend to be larger; Chambers 1997), food availability (larger eggs/offspring should be preferred where/when food is scanty, as predicted by Parker and Begon 1986) or to different spawning events (interannual fluctuations; Johnstone and Leggett 2002). Undeniably, a single rule cannot describe changes in egg size and, regardless general rules, differences in egg size between closely related populations, species and higher taxa could be a 'hallmark' of habitat dependent evolutionary patterns of reproductive strategies (Laptikhovsky 2006).

Species life history values do not provide independent aspects for comparative analyses because species share characteristics through descendent from common ancestors (Harvey and Purvis 1991). The similarity in life histories between closely related species may be attributed to the phylogenies, in particular when the environments experienced by the populations considered are not markedly different (Rochet 2000). But when closely related populations, species or higher taxa, show differences in a life history trait under different environmental and ecological conditions, an eventual habitat dependent evolutionary pattern should be investigated.

2. Antarctic marine environment

Antarctica is the largest ecosystem on Earth and has a very long geological evolution history (Knox and Lowry 1977). The Antarctic marine fauna is linked to past climate changes and it is the result of a series of evolutionary events, proceeded through the Cenozoic, first in Gondwana coastal waters (~ 55 Million years ago, Mya) and later, after the breakup of Gondwana, on the Antarctic shelf (Clarke and Johnston 1996, Eastman 2005).

The Southern Ocean represents a unique natural laboratory to study the evolutionary processes that shape biological diversity in extreme environments. Antarctic organisms evolved under the influence of several geological and climatic factors, such as geographic isolation of the landmass and continental shelves, extreme low temperature and intense seasonality. Indeed, polar marine environments are subjected to large seasonal variations in sea ice cover and sun light irradiation, greatly affecting the biology and distribution of organisms (Moline *et al.* 2008).

The opening of the Drake Passage in the early Oligocene (~ 40 Mya) has enabled the formation of the Antarctic Polar Front (APF, established ~ 38 Mya; Eastman 1993) and of the Antarctic Circumpolar Current (ACC, also called West Wind Drift, established ~ 25 Mya). The APF represents the main circulation pattern and encircles the continent, making the Southern Ocean a semi-enclosed sea (Fig. 2). The ACC is from 200 to 1200 km wide, is positioned at 45° - 60°S, depending from the longitude, and flows around the continent in a clockwise direction, driven by persistent westerly winds and extends to the bottom. The surface current velocity is between 25 and 30 cm/s (Gordon 1971, 1988) depending on its location (Foster 1984). The origin and the establishment of the APF, the ACC and the cooling of the Southern Ocean resulted in a natural oceanographic barrier to the migration of organisms in either directions, becoming a key factor in the evolution of Antarctic fauna and leading to the diversification and high rate of endemism in different taxonomic groups (Di Prisco *et al.* 2012; Eastman 2000; Eastman and Clarke 1998; Ekau 1991). Antarctica hosts the most isolated fish populations among Oceans and may be considered an “evolutionary incubator” (Turner *et al.* 2009).

The APF and the ACC not only forms a physical barrier to the northerly dispersion of eggs and larvae of the Antarctic marine organisms, but also drives their transport around the continent (Knox 1970). Furthermore, the great distance from other shelf areas, the high water depth and the subzero temperatures strengthen the isolation of Antarctica. Nevertheless, some Antarctic organisms have migrated and crossed the ACC, colonizing the shallow water habitats of the sub-Antarctic islands and the waters around the tip of South America.

The Antarctic Costal Current (AC) is an East-Wind Drift coastal flow current, westward flowing, that does not encircle the continent. The interaction of AC and the ACC creates upwelling and large clockwise gyres in the Ross, Weddell, and Bellingshausen Seas (Fig. 2). The upwelling moves relatively warm and rich in nutrient waters near the edge of the continental shelf, flowing off the continental slopes (Gordon 1971), playing a key role for primary production in the continental shelf break region (Knox 2006).

The Southern Ocean shows the lowest seawater temperatures (-1.9°C) and the lowest salinity (34.8 ppt) among oceans (Legg *et al.* 2009). The majority of the Southern Ocean shows temperature below 0°C, and winter and summer surface seawater temperatures differ around only 2°C (Deacon 1984). In large portions of the Southern Ocean, temperature of surface waters differs from that of deep waters of only 4-5°C (Knox 1970). Temperature directly influences physical parameters like gas solubility and water density. Since oxygen solubility is inversely proportional to temperature, Antarctic species are never exposed to hypoxic conditions.

South of 60°S, the Southern Ocean is almost completely ice-covered during winter, whereas in summer the coverage is reduced by 75% (Foster 1984). The extension of the ice cover is not homogeneous around the continent depending on the longitude.

High Antarctic latitudes are subjected in the winter season to long periods of darkness and during summer to long period of daylight. This strong seasonality in light results in a strong seasonality in primary production, with the highest values during summer. The intense and long summer irradiation leads, indeed, to high levels of primary production in the surface waters over the continental shelf and northward beyond the shelf break. Primary production during summer is linked also to the ice melting and the algae release in the seawater (Smith and Sakshaug 1990). The melted water from the pack ice contributes to stabilize the surface waters, keeping the phytoplankton in the euphotic region. In these layers, the predominant zooplankton are copepods and the Antarctic krill *Euphausia* spp. (Smith and Schnack-Schiel 1990). These zooplanktonic organisms are important prey for several fish species and, in some cases, they are the exclusive prey of notothenioid fishes inhabiting the continental shelf of the Antarctic Peninsula region (Kock, 1985).

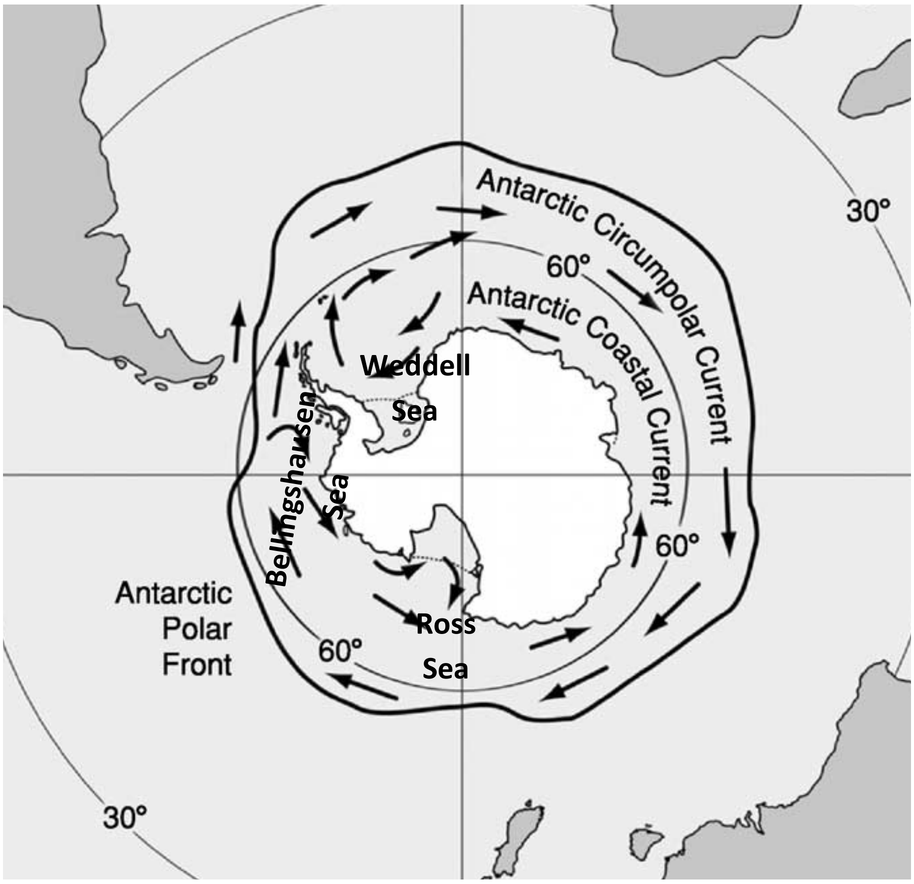

Figure 2. Antarctic Circumpolar Current and Antarctic Coastal Current. The thick line represents the mean position of the Antarctic Polar Front (modified from DeVries and Steffensen 2009).

Seawater temperature close to the freezing point, ice coverage and seasonal variability in the ice thickness and the intermittent primary production make Antarctica a unique environment and are the main forces that led Antarctic fish evolution.

In the present study, three ecological zones were considered in the species distribution: High, Low and Sub Antarctic (see Fig. 1 Paper VI). They are based on oceanographic frontal zones and ice cover extension: the High Antarctic zone is the area close to the continent which is covered by ice most of the year; the Low Antarctic zone is between the northern limit of the pack ice in summer-autumn and the ACC; the Sub Antarctic zone is located north of the ACC up to about 35° S of latitude (modified from Kock 1992).

3. The notothenioids diversity

The fish fauna of the Southern Ocean is quite well-known from a taxonomic point of view, although new species are regularly described (Eastman 2005; Eakin *et al*. 2015). Among the Antarctic fish, notothenioids are the most speciose taxon, dominating the

Antarctic fish fauna in terms of diversity and abundance. This dominance of a single taxonomic group is unique among shelf faunas of the world (Eastman 2005).

Notothenioids were subjected to radiation in the sub-zero waters of the Antarctic continental shelf (Eastman 1993; Clarke and Johnston 1996; Eastman 2000), constituting a special type of adaptive radiation (Eastman and McCune 2000; Eastman and Clarke 1998): a disproportionately high number of closely related species that have evolved rapidly within a delimited area where most species are endemic. Eastman and Clarke (1998) have suggested that five Antarctic notothenioid families exhibit a high speciosity and endemism characteristic of a species flocks. Further examples of species flock are found elsewhere only in isolated environments such as the African lakes (Greenwood 1975; Ekau 1991). The high latitude shelf waters encircling Antarctica form a unique evolutionary hot spot (Eastman 1993, 2005). It is not the number of species that distinguishes the Antarctic fish from all other in the world, but the nature of fish biodiversity (Eastman 2005). Notothenioids are the endemic southern hemisphere component of the fauna that evolved *in situ* (Andriashev 1965), endemism is three times higher than in fauna from other isolated areas, accounting 70–90% of the fish biomass in most parts of the Southern Ocean (Kock 2005). The number of notothenioid species evolved in the Antarctic shelf waters exceeds the rather small number of other Antarctic species (Eastman and Eakin 2000).

In the early Miocene (25–22 million years ago) the Antarctic shelf was subjected to a series of tectonic and oceanographic events that modified the geography and the faunal composition leading to the gradual isolation and cooling of the Antarctica (Anderson 1999). Changes of habitat and trophic structure of the Antarctic ecosystem led to the extinction of many fish present in Eocene. Thus the diversity of the fauna was reduced and new ecological niches became available to other fish that were diversifying (notothenioids) or immigrating (liparids and zoarcids). From an ecological perspective and in a circumstance of reduced competition, notothenioids filled a broad variety of ecological niches (Eastman and Eakin 2000). The evolutionary adaptation of the fish fauna to the Southern Ocean is the consequence of the unique physical characteristics of the environment.

The perciform suborder Notothenioidea diversified into about 139 species belonging to eight families (Eastman and Eakin 2015). Three phylogenetically ancestral families, Bovichtidae, Pseudaphritidae and Eleginopidae, comprise eleven primarily non-Antarctic species, distributed around the southern South America, the Falkland Islands, the southern New Zealand and the southern eastern Australia (Eastman 1993). The remaining families, Artedidraconidae, Bathydraconidae, Channichthyidae, Harpagiferidae and Nototheniidae are, with few exceptions, endemic of Antarctic waters and are referred as the 'Antarctic clade' (Eastman 1993).

The Antarctic notothenioids evolved many anatomical and physiological adaptations to the Antarctic environment. Among them, the evolution of antifreeze glycopeptides has been highly investigated (Chen *et al.* 1997; Cheng and DeVries 2002). Moreover they lost traits no longer “necessary” in permanently cold water (Rutschmann *et al.* 2011). Some notothenioids lost the heat-shock protein response (Place and Hofmann 2005; Clark *et al.* 2008), and the family Channichthyidae represents the only known vertebrate group that lacks erythrocytes and haemoglobin in the adults (Ruud 1954; Near *et al.* 2006). In addition, all notothenioids lack a functional swim bladder, though several pelagic species evolved neutral buoyancy by a reduced calcification of the skeleton and accumulation of lipid deposits (De Vries and Eastman 1981; Eastman 1993; Klingenberg and Ekau 1996). Compared to fish from other areas, notothenioids present some distinctive characteristics: high reproductive investment, prolonged gametogenesis, low fecundity and relatively large eggs, slow growth rate (Andriashev 1965; Kock and Kellermann 1991).

Age at sexual maturity and age at first spawning are unknown for several species. Nonetheless, in the species in which these traits have been investigated, sexual maturity appears to be attained at about 55-80% of the maximum size (Kock and Kellermann 1991). Many species that may live at least 13-20 years do not spawn before they are 6-8 years old (Kock *et al.* 1985).

The high reproductive investment is documented by high gonadosomatic indices (GSI, the percentage of body weight represented by gonad weight) between 15 and more than 50% in females. Males GSI, except for a few species (e.g. *Notothenia rossii*, *N. coriiceps*), is generally much lower; however, prolonged male parental care is a rather common trait and considerably increases reproductive effort in males. In many species, egg development requires about two years to complete the vitellogenesis (Kock and Kellermann 1991; Everson 1994; Everson *et al.* 1996; Kock and Everson 1997). Egg diameter after fertilization ranges from 2.0 mm in *Lepidonotothen larseni* and *Pleuragramma antarctica* to 5.0 mm, or more, in *Notothenia rossii*, many icefish and some other species (Camus and Duhamel 1985; Hubold 1990; Konecki and Targett 1989). Potential fecundity is often quite low, if compared to temperate fishes, ranging from few tens in artedidraconid family, few thousands in channichthyids, to the highest value of hundreds of thousands found in *Dissostichus* species (Kock and Kellermann 1991). In the Antarctic notothenioids, maturing ovaries are typically filled with oocytes in two stages of maturation, large yolky oocytes, forming the batch that will be released in the current season, and pre-vitellogenic oocytes representing next year spawning (Kock and Kellermann 1991). Intermittent spawning has not been observed and eggs are probably released in one batch, as can be inferred by the presence of only one group of mature oocytes with homogeneous size.

A general trend was observed in notothenioids in producing less but larger eggs towards higher latitudes, in particular in the family Nototheniidae. In fact, notothenioids exhibit two main reproductive strategies in the Seasonal Pack-ice Zone, producing either small and large eggs, whereas they have almost exclusively a relatively few large eggs in the High Antarctic Zone close to the continent (Fig. 3) (Ekau 1991; Kock 1992; Kock and Wilhelms 2003).

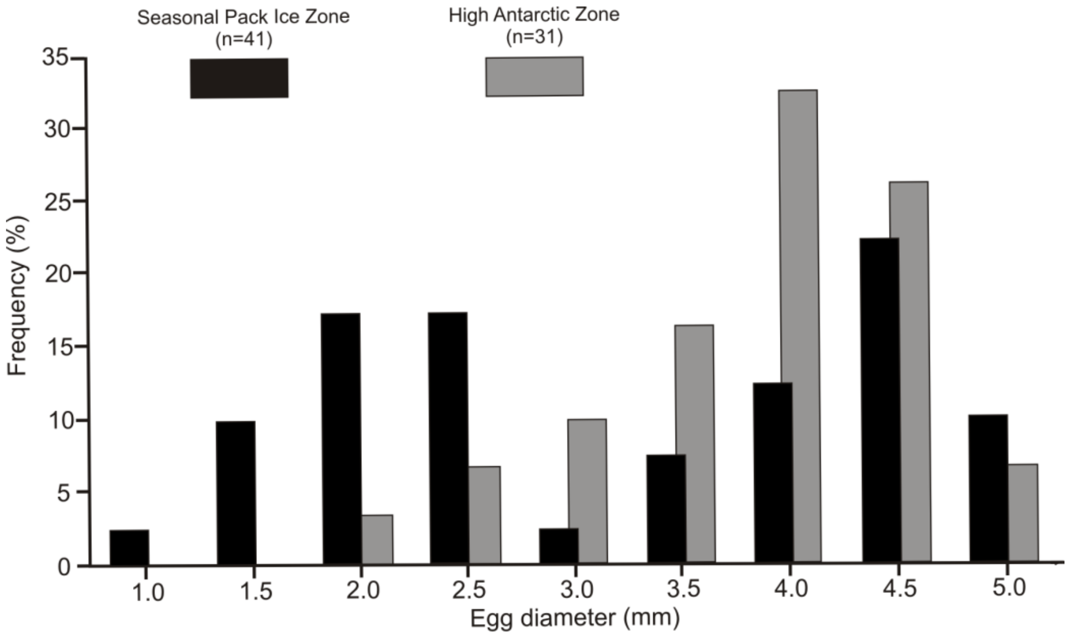

Figure 3. Frequency distribution of egg size in 46 populations of 38 notothenioid species in the Seasonal Pack-ice Zone (including northern islands), and in the High-Antarctic Zone (modified from Kock and Kellermann 1991.).

Species with large eggs are also characterized by long embryonic development duration (up to six months). In fish egg size determines larval characteristics, showing a positive relationship with larval size at hatching and, in general, with the duration of pelagic larval stages (Duarte and Alcarez 1989). Larvae and juveniles are pelagic in notothenioids and may last for 2-4 years, resulting in a high potential for dispersal along the continental shelf, but also in considerable vulnerability of early life history stages (Kock 2005a).

Notothenioids have diversified into a number of ecologically diverse life history types (Eastman 2000), therefore despite some common features, they also display variability in life history traits at inter- and intra-specific levels, often linked to their different spatial, temporal and habitat niches.

3. Notothenioids phylogeny

Notothenioids have undergone extraordinary evolutionary episodes since the onset of widespread glaciation in Antarctica, approximately 34 million years ago, when the Southern Ocean cooled to the freezing point of seawater (-1.9°C) (Cheng 1998; DeVries 1988). The modern Antarctic fish fauna is exclusively marine and different in composition from previous one (Eastman 1991). Although sharing a benthic common ancestor, Antarctic notothenioids actually inhabit all ecological niches in the Southern Ocean, from benthic to epibenthic, semipelagic, cryopelagic, and pelagic habitats (Eastman 1993).

First phylogenetic investigations on notothenioids were based on morphological characters and the subsequent molecular phylogenies were generally congruent (Near *et al.* 2004). The phylogeny of notothenioids has been an issue highly investigated in the last decades, nevertheless several critical areas of the notothenioids tree remain to be completely clarified.There is a gap of 38 My between Oligocene and Eocene, as no Antarctic fish fossil records were found (Cheng 1998; DeVries 1988). Despite this lack, notothenioid species diversity and phylogeny, at family level, is sufficiently well known and allows to recognize macro evolutionary events and reconstruct the notothenioid adaptive radiation in the Southern Ocean (Fig. 4) (Bargelloni *et al.* 2000; Eastman 1991; Eastman 2005; Kock 1992; Near *et al.* 2004, 2012).

The phylogenetic position of notothenioids among major lineages of perciform fishes was recently solved; the subtropical and temperate fish, *Percophis brasiliensis*, distributed along the southern coast of South America, is now recognised the notothenioid sister lineage (Near *et al*. 2015). The main problems that still remains to be solved concern the mono- or paraphyletic origin of the Nototheniidae and Bathydraconidae families (Bargelloni *et al.* 1997; Derome *et al.* 2002; Near *et al.* 2004, 2006; Near and Cheng 2008).

Generally the Nototheniidae are considered paraphyletic, and the position of several nototheniid genera is not yet resolved (e.g. *Dissostichus*, *Aethotaxis*, and *Pleuragramma*). The basal position of *Dissostichus mawsoni* and the sister species relationships of *Gobionotothen gibberifrons* and *Pleuragramma antarctica* remains unstable and dependent on the markers or analysis approach employed (Sanchez *et al.* 2007; Near and Cheng 2008; Rutschmann *et al.* 2011; Dettai *et al.* 2012; Near *et al.* 2012). Many authors suggested that *Dissostichus* species form a clade, but their association with *Aethotaxis* is quite weak (Near *et al.* 2004; Near and Cheng 2008; Sanchez *et al.* 2007). The position of *P. antarctica* cannot be considered resolved until now; it is rarely, and only with mitochondrial datasets, associated with the two other genera *Dissostichus* and *Aethotaxis* (Near and Cheng 2008; Dettai *et al.* 2012). The clade including the genera *Trematomus*, *Lepidonotothen* and *Patagonotothen* has been highlighted by many authors and analyses (Near and Cheng 2008; Sanchez *et al.* 2007; Rutschmann *et al.* 2011; Dettai *et al*. 2012). The *Patagonotothen* genus forms a clade, while the monophyly of the *Trematomus* genus has been recently debated (Kuhn and Near 2009; Janko *et al.* 2011; Dettai *et al.* 2012). *Notothenia* and *Gobionotothen* species seem to be more closely related to the High Antarctic clade than to the rest of nototheniids (Dettai *et al.* 2012).

The High Antarctic clade, including the Artedidraconidae and Channichthyidae, is considered monophyletic, but the position of the Bathydraconidae is in some cases contradictory (Near and Cheng 2008; Rutschmann *et al.* 2011; Dettai *et al.* 2012).

In this framework, the necessity to use a notothenioid cladogram for comparative methods purpose needs to find a compromise.

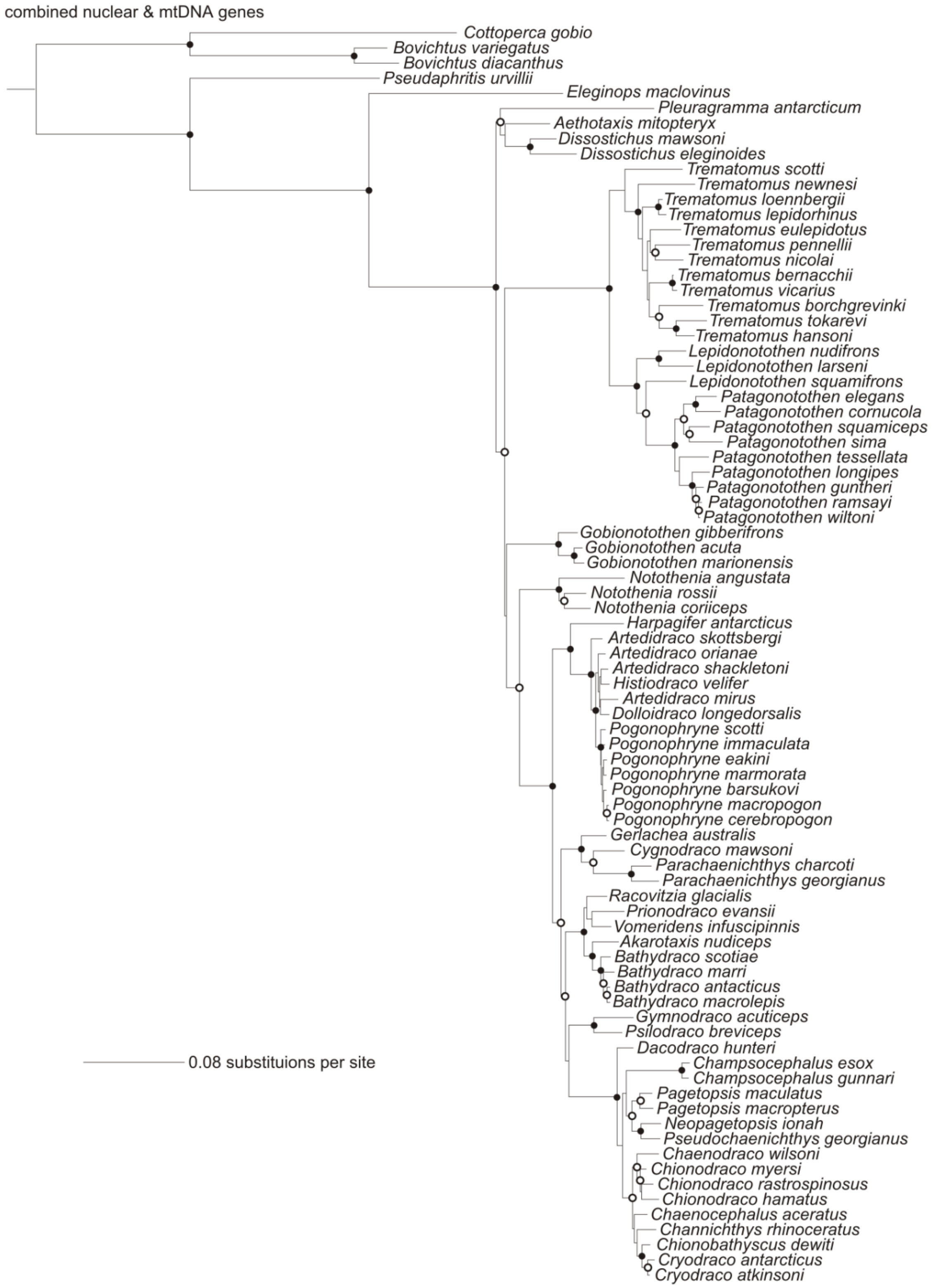

Figure 4. Maximum likelihood phylogeny of 83 notothenioid species based on combined five nuclear and two mitochondrial DNA genes. Branch lengths are scaled to the estimated number of nucleotide substitutions (modified from Near *et al.* 2014).

5. Comparative methods

The comparative method, comparing populations, species or higher taxa, is the most common tool for understanding past evolutionary processes and examining how organisms are adapted to their environments (Harvey and Pagel 1991; Brooks and McClennan 2002). Comparative methods have been used extensively to infer evolutionary adaptations, i.e. the changes in response to natural selection (Rochet 2000; Garland and Adolph 1994; Sanford *et al.* 2002), and in particular have been widely used in the evolutionary studies on sexual selection (e.g. Hosken *et al.* 2001; Nunn 2002; Smith and Cheverud 2002; Aparicio *et al.* 2003; Cox *et al.* 2003).

To compare species or taxa it is fundamental to take into consideration the phylogenetic relationships among them. Indeed traits cannot be assumed to be independent among species/higher taxa for statistical purposes (Harvey and Purvis 1991; Garland *et al.* 2005), because of the so-called "phylogenetic inertia": more closely related the species are, more similar they tend to be (Harvey and Purvis 1991). The relationship between two characteristics or between a trait and an environmental variable can therefore be the result of common evolutionary history. Several life history traits are similar among taxonomic groups, and this tendency is called lineage-specific effect (Lynch 1991; Freckleton *et al.* 2002).

Three mechanisms were identified to explain the similarity among closely related species (Harvey and Pagel 1991; Harvey and Purvis 1991):

- *phylogenetic niche conservatism*, that takes place when close relatives occupy similar niches, retaining their niche-related traits through speciation events, whereas distant relatives are more dissimilar for those traits;
- *time lag*, the evolution of adaptations takes time and the appropriate genetic variance, the phenotypic state of some traits can therefore be the results of past environmental conditions;
- *phenotype-dependent responses to selection*, organisms with similar phenotypes may express similar traits if exposed to the same selective pressures.

It is possible to distinguish two different approaches among comparative analyses: the directional and non-directional comparisons. The directional comparison relates the character states among different taxa levels, following the evolutionary changes between ancestral and descendants. The non-directional comparison analyses evolutionary trends across nodes at the same phylogenetic level (Harvey and Pagel 1991).

The more traditional approach of comparative studies involves non-directional analyses of contemporary taxa, particularly useful for detecting and describing the current relationships between traits and environments across taxa. Coupled with careful biological and phylogenetic arguments, these analyses enable to make inferences about adaptations (Harvey and Pagel 1991).

Different comparative methods have been developed and in the past two decades the approach of comparative methods has been changed and updated over (Garland *et al.*

2005). The applied method depends on the type of variables, if they are a) continuous (quantitative) or b) discrete (qualitative). Moreover, the different methods assume different theoretical models of character evolution and may require different sample size and/or different information on the phylogenetic tree, for instance some methods require phylogenetic distances, while others require only phylogenetic relationships (Harvey and Pagel 1991). In any case, every comparative analysis requires a completely resolved phylogenetic tree in addition to the value for each trait/environmental variable that has to be analysed. In this study only the method of independent contrasts is presented.

The independent contrasts' method is a non-directional method based on the phylogenetically standardized differences in traits among species sharing a common ancestor. In details, to take into account phylogenetic relationships, the differences in the analysed traits are calculated between two related species as showed below:

Standardize contrast: $d_1/\sqrt{(L_1+L_2)}$

where d_1 is the difference in the trait value between the two species, and L_1 and L_2 are the distances of each species from their common ancestor. In this way, contrasts between more closely related species will weigh more than contrasts between less closely related ones in the analysis. In addition to the standardized contrast between species, the same contrasts are calculated also between nodes, following the same approach. The values of each trait at nodes are calculated starting from the traits' values of the species. The standardized contrasts, being differences between related species/nodes, assure the independence of the data. If a relation between two traits exists, it is expected that it will also be present between the contrasts, preserving the same trend (Felsenstein 1985; Harvey and Pagel 1991). The independent contrasts' method assumes the Brownian model as model of evolution, however it is robust if this assumption is not fully met (Roff 1992). Moreover it does not require wide sample size. Although several methods have been developed in the last decades, the independent contrasts' one is still the most adopted method.

6. Aims of the study

The aim of this Ph.D. project is to investigate the variability and evolution in relation to environment of some life history traits, and the trade-off between them, using notothenioids as model taxon. Particular attention has been focused on reproductive traits and their relationships with environmental characteristics, controlling for phylogenetic relationships. In particular, the study focused on: egg size and type (demersal or pelagic), fecundity, gametogenesis, reproductive period and reproductive effort (e.g. gonadosomatic Index, presence of parental care), size/age at first maturity relative to maximum size.

For these purposes, several notothenioid species were analysed starting from sampled species and including literature data to build a complete dataset of the life history traits mentioned above. In order to compare the life history traits with environmental characteristics, environmental data available for the distribution range of each species were added to the dataset.

Despite the availability of recent, up to date and resolved phylogenies of notothenioids (Bargelloni *et al.* 1994; Rutschmann *et al.* 2011; Dettai *et al.* 2012; Near *et al.* 2012; Colombo *et al.* 2015), a comparative method, that takes into account phylogenetic relationships among species (Harvey and Pagel 1991; Roff 2002), has not yet been applied to study the evolution of life history traits in relation to environment in this taxon. Such a comparative study of life history traits is interesting for evolutionary biology, given the adaptations shown by Antarctic fishes to their peculiar environment (Kock and Kellermann 1991), as well as for conservation biology, since the knowledge of reproductive traits of exploited species is recognized crucial for their sustainable management.

REFERENCES

Abbott J.C., Dubrack R.L., Orr C.D. (1985). The interaction between size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). Behaviour 92: 241-253.

Abbott J.C., Dill L.M. (1989). The relative growth of dominant and subordinate juvenile steelhead trout (*Salmo gairdneri*) fed equal rations. Behaviour 108(1/2): 104-113.

Adams P.B. (1980). Life history patterns in marine fishes and their consequences for fisheries management. Fishery Bulletin of the U.S. Fish and Wildlife 78: 1-12.

Alekseev F.E. (1981). Rass–Thorson–Marshall rule and biological structure of marine communities. In: G.G. Vinberg. 4th Congress of All-Union Hydrobiological Society. Theses of reports. Part I. Naukova Dumka, Kiev 4-6.

Andersson M. (1994). Sexual selection. Princeton University Press, Princeton, NJ, 624 pp.

Anderson J.B. (1999). Antarctic Marine Geology. Cambridge University Press, Cambridge, 289 pp.

Andriashev P. (1965). A general review of the Antarctic fish fauna. In: Van Oye P., Van Menghem J. (eds) Biogeography and Ecology in Antarctica. Monographiae Biologicae 15: 491-550.

Angilletta M.J., Dunham A.E. (2003). The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. The American Naturalist 162(3): 332-342.

Aparicio J.M., Bonal R., Cordero P.J. (2003). Evolution of the structure of tail feathers: implications for the theory of sexual selection. Evolution 57: 397-405.

Ashton K.G. (2001). Are ecological and evolutionary rules being dismissed prematurely? Diversity and Distribution 7(6): 289–295.

Balon E.K. (1984). Reflections on some decisive events in the early life of fishes. Transactions of the American Fisheries Society 113: 178-185.

Bargelloni L., Ritchie P.A., Patarnello T., Battaglia B., Lambert D.M., Meyer A. (1994). Molecular evolution at subzero temperatures: mitochondrial and nuclear phylogenies of fishes from Antarctica (suborder Notothenioidei), and the evolution of antifreeze glycopeptides. Molecular Biology and Evolution 6: 854-863.

Bargelloni L., Patarnello T., Ritchie P.A., Battaglia B., Meyer A. (1997). Molecular phylogeny and evolution of notothenioid fish based on partial sequences of 12S and 16S ribosomal RNA mitochondrial genes. In: Battaglia B., Valencia J., Walton D. (eds) Antarctic communities: species, structure and survival. Cambridge University Press, Cambridge, 45-50.

Bargelloni L., Marcato S., Zane L., Patarnello T. (2000). Mitochondrial phylogeny of Notothenioids: a molecular approach to Antarctic fish evolution and biogeography. Systematic Biology 49(1): 114-129.

Begon M., Harper J.L., Townsend C.R. (1990). Ecology – Individuals, Populations, Communities. Blackwell Scientific Publications, Oxford, England, 1068 pp.

Bell G. (1980). The costs of reproduction and their consequences. The American Naturalist 116(1): 45-76.

Bergmann C. (1847). Ueber die verhä ltnisse der wärmeö konomie der thiere zu ihrer grö sse. Gottinger studien 3: 595-708.

Blackburn T.M., Gaston K.J., Loder N. (1999). Geographic gradients in body size: a clarification of Bergmann's rule. Diversity and Distributions 5: 165-174.

Both C. 1998. Density dependence of clutch size: habitat heterogeneity or individual adjustment? Journal of Animal Ecology 67: 659-666.

Brooks D.R., McLennan D.A. (2002). The nature of diversity: an evolutionary voyage of discovery. University of Chicago Press, Chicago, 676 pp.

Camus P., Duhamel G. (1985). Ponte et développement embryonnaire de *Notothenia rossii rossii* (Richardson, 1844) Nototheniidae des îles Kerguelen. Cybium 9(3): 283-293.

Chambers R.C. (1997). Environmental influences on egg and propagule sizes in marine fishes. In: Chambers R.C., Trippel E.A. (eds) Early life history and recruitment in fish populations. Springer Netherlands 63-102.

Chapman D.W. (1988). Critical review of variables used to define effects of fines in redds of large salmonids. Transactions of the American Fisheries Society 117: 1-21.

Charlesworth B. (1994). Evolution in age structured populations, 2nd ed. Cambridge University Press, Cambridge, 324 pp.

Charnov E.L. (1997). Trade-off-invariant rules for evolutionary stable life histories. Nature 387: 393–394.

Cheng C.-H.C. (1998). Origin and mechanism of evolution of antifreeze glycoproteins in polar fishes. In: Di Prisco G., Pisano E., Clarke A. (eds) Fishes of Antarctica: a biological overview (2012). Springer Science & Business Media, 311-328.

Cheng L., DeVries A.L., Cheng C.-H.C. (1997). Evolution of antifreeze glycoprotein gene from a trypsinogen gene in Antarctic notothenioid fish. Proceedings of the National Academy of Sciences of the USA 94: 3811-3816.

Cheng C.H.C., DeVries A.L. (2002). Origin and evolution of fish antifreeze proteins. In: Ewart K.V., Hew C.L. (eds). Fish antifreeze proteins. World Scientific, Singapore, 83-107 pp.

Christiansen F.B. (1974). Sufficient conditions for protected polymorphism in a subdivided populations. The American Naturalist 108: 157-166.

Clarke A., Johnston I.A. (1996). Evolution and adaptive radiation of Antarctic fishes. Trends in Ecology & Evolution 11(5): 212-218.

Clark M.S., Fraser K.P.P., Burns G., Peck L.S. (2008). The HSP70 heat shock response in the Antarctic fish *Harpagifer antarcticus*. Polar Biology 31: 171-180.

Cohen J.E. (1966). A model of simple competition. Harvard University Press, Cambridge, 138 pp.

Cole L.C. (1954). The population consequence of life history phenomena. Quarterly Review Biology 19: 103-137.

Colombo M., Damerau M., Hanel R., Salzburger W., Matschiner M. (2015). Diversity and disparity through time in the adaptive radiation of Antarctic notothenioid fishes. Journal of Evolutionary Biology 28: 376-394.

Conover D.O., Schultz E.T. (1997). Natural selection and the evolution of growth rate in the early life history: what are the trade-offs? In: Chambers R.C., Trippel E.A. (eds) Early life history and recruitment in fish populations. Springer Netherlands 305-332.

Cox R.M., Skelly S.L., John-Alder H.B. (2003). A comparative test of adaptive hypotheses for sexual dimorphism in lizards. Evolution 57: 1653-1669.

Dando P.R. (1984). Reproduction in estuarine fish. In: Potts G.W., Wooton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, Oxford, 155-170.

Darwin C.R. (1871). The descent of man and selection in relation to sex. Murray, London.

De Lope F., Møller A.P. (1993). Female reproductive effort depends on the degree of ornamentation of their mates. Evolution 47(4): 1152-1160.

Deacon G. (1984). The Antarctic Circumpolar Ocean. Cambridge University Press, Cambridge, 180 pp.

Derome N., Chen W.J., Dettai A., Bonillo C., Lecointre G. (2002). Phylogeny of Antarctic dragonfishes (Bathydraconidae, Notothenioidei, Teleostei) and related families based on their anatomy and two mitochondrial genes. Molecular phylogenetics and evolution 24(1): 139-152.

Dettai A., Berkani M., Lautredou A-C., Couloux A., Lecointre G., Ozouf-Costaz C., Gallut C. (2012). Tracking the elusive monophyly of nototheniid fishes (Teleostei) with multiple mitochondrial and nuclear markers. Marine Genomics 8: 49-58.

DeVries A.L. (1988). The role of antifreeze glycopeptides and peptides in the freezing avoidance of Antarctic fishes. Comparative Biochemistry and Physiology 90B: 611-621.

DeVries A.L., Eastman J.T. (1981). Physiology and ecology of notothenioid fishes of the Ross Sea. Journal of the Royal Society of New Zealand 11: 329-340.

DeVries A.L., Steffensen J.F. (2009). The Arctic and Antarctic polar marine environments. In: Farrell A.P., Steffensen J.F. (eds) The physiology of polar fishes. Elsevier, 1-24.

Di Prisco G. (1998). Molecular adaptations of Antarctic fish hemoglobins. In: Di Prisco G., Pisano E., Clarke A. (eds) Fishes of Antarctica: a biological overview (2012). Springer Science & Business Media, 339-353.

Di Prisco G., Pisano E., Clarke A. (2012) Fishes of Antarctica: a biological overview. Springer Science & Business Media, 363 pp.

Duarte C.M., Alcaraz M. (1989). To produce many small or few large eggs: a size-independent reproductive tactic of fish. Oecologia 80: 401-404.

Eakin R.R., Riginella E., La Mesa M. (2015). A new species of *Artedidraco* (Pisces: Artedidraconidae) from the Weddell Sea, Antarctica. Polar Biology. doi: 10.1007/s00300-015-1721-0.

Eastman J.T. (1991). The fossil and modern fish faunas of Antarctica: evolution and diversity. In: Di Prisco G., Maresca B., Tota B. (eds) Biology of Antarctic fish (2012). Springer Science & Business Media, 116-130.

Eastman J.T. (1993). Antarctic fish biology: Evolution in a unique environment. Academic Press, San Diego, 322 pp.

Eastman J.T. (2000). Antarctic notothenioid fishes as subject for research in evolutionary biology. Antarctic Science 12(3): 276-287.

Eastman J.T. (2005). The nature of the diversity of Antarctic fishes. Polar Biology 28: 93-107.

Eastman J.T., Clarke A. (1998). A comparison of adaptive radiations of Antarctic fish with those of Non-Antarctic fish. In: Di Prisco G., Pisano E., Clarke A. (eds) Fishes of Antarctica: a biological overview (2012). Springer Science & Business Media, 3-28.

Eastman J.T., Eakin R.R. (2000). An updated species list for notothenioid fish (Perciformes; Notothenioidea), with comments on Antarctic species. Archive of Fishery and Marine Research 48(1): 11-20.

Eastman J.T., McCune A.R. (2000). Fishes on the Antarctic Continental Shelf: evolution of a marine species flock. Journal of Fish Biology 57: 84-102.

Eastman J.T., Eakin R.R. (2015). Notothenioid classification and list of species. http://www.oucom.ohiou.edu/dbms-eastman.

Ekau W. (1991). Morphological adaptations and mode of life in high Antarctic fish. In: Di Prisco G., Maresca B., Tota B. (eds) Biology of Antarctic fish (2012). Springer Science & Business Media, 23-39.

Everson I. (1994). Timescale of ovarian maturation in *Notothenia coriiceps*: evidence for a prolonged adolescent phase. Journal Fish Biology 44: 997-1004.

Everson I., Kock K.H., Parkes G. (1996). Ovarian development associated with first maturity in three Antarctic channichthyid species. Journal of Fish Biology 49(5): 1019-1026.

Falconer D.S., Mackay T.F.C. (1996). Introduction to quantitative genetics. 4th ed. Longman, Essex, U.K, 438 pp.

Felsenstein J. (1985). Phylogenies and the comparative method. The American Naturalist 125: 1-15.

Fisher J.A.D., Frank K.T., Leggett W.C. (2010). Global variation in marine fish body size and its role in biodiversity ecosystem functioning. Marine Ecology Progress Series 405: 1-13.

Foster T.D. (1984). The marine environment. In: Laws R.M. (eds) Antarctic Ecology Vol. 2, Academic Press, London, 345-371.

Frank K.T., Leggett W.C. (1994). Fisheries ecology in the context of ecological and evolutionary theory. Annual Review of Ecology and Systematics 25: 401-422.

Freckleton R.P., Harvey P.H., Pagel M. (2002). Phylogenetic analysis and comparative data: a test and review of evidence. The American Naturalist 160: 712-726.

Froese R., Pauly D. (2013). FishBase. World Wide Web electronic publication. www.fishbase.org, version (10/2013).

Gadgil M., Bossert W.H. (1970). Life historical consequences of natural selection. The American Naturalist 104(935): 1-24.

Garland T.J., Adolph S.C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. Physiological Zoology 67: 797-828.

Garland T.J., Bennett A.F., Rezende E.L. (2005). Phylogenetic approaches in comparative physiology. Journal of Experimental Biology 208: 3015-3035.

Gordon A.L. (1971). Recent physical oceanographic studies of Antarctic waters. In: Quam L.O. (eds) Research in the Antarctic, American Association for Advancement of Science, Washington, DC, 609-629.

Gordon A.L. (1988). Spatial and temporal variability within the Southern Ocean. In: Sahrhage D. (eds) Antarctic Ocean and Resources Variability, Springer-Verlag, Berlin and Heidelberg, 41-56.

Greenwood P.H. (1974). The cichlid fishes of Lake Victoria, East Africa: the biology and evolution of a species flock. Bulletin of the British Museum Natural History Zoology 6 (Monograph), London, 134 pp.

Harvey P.H., Pagel M.D. (1991). The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford, 239 pp.

Harvey P.H., Purvis A. (1991). Comparative methods for explaining adaptations. Nature 351: 619-624.

Helfman G., Collette B.B., Facey D.E., Bowen B.W. (1997). The diversity of fishes. Blackwell Science, Malden, Massachusetts, U.S.A. 528 pp.

Hislop J.R.G. (1984). A comparison of the reproductive tactics and strategies of cod, haddock, whiting and Norway pout in the North sea. In: Potts G.W., Wootton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, London, 311-329.

Hosken D.J., Jones K.E., Chipperfield K., Dixson A. (2001). Is the bat os penis sexually selected? Behavioural Ecology and Sociobiology 50: 450-460.

Hubold G. (1990). Seasonal patterns of ichthyoplankton distribution and abundance in the southern Weddell Sea. In: Kerry K.R., Hempel G. (eds) Antarctic Ecosystems. Springer Berlin Heidelberg, 149-158.

Huston M.A., Wolverton S. (2011). Regulation of animal size by eNPP, Bergmann's rule, and related phenomena. Ecological Monographs 81(3): 349-405.

Hutchings J.A. (1993). Behavioural implications of intraspecific life-history variation. Marine Behaviour and Physiology 23: 187-203.

Hutchings J.A. (1996). Adaptive phenotypic plasticity in brook trout, *Salvelinus fontinalis*, life histories. Ecoscience 3(1): 25-32.

Janko K., Marshall C., Musilová Z., Van Houdt J., Couloux A., Cruaud C., Lecointre G. (2011). Multilocus analyses of an Antarctic fish species flock (Teleostei, Notothenioidei, Trematominae): Phylogenetic approach and test of the early-radiation event. Molecular Phylogenetics and Evolution 60: 305-316.

Jennings S., Pinnegar J.K., Polunin N.V.C., Boon T. (2001). Weak cross-species relationships between body size and trophic level belie powerful size-based trophic structuring in fish communities. Journal of Animal Ecology 70: 934-944.

Johnston T.A. (1997). Within-population variability in egg characteristics of walleye (*Stizostedion vitreum*) and white sucker (*Catostomus commersoni*). Canadian Journal of Fisheries and Aquatic Sciences 54: 1006-1014.

Johnston T.A., Leggett W.C. (2002). Maternal and environmental gradients in the egg size of an iteroparous fish. Ecology 83: 1777-1791.

Julliard R., McCleery R.H., Clobert J., Perrins C.M. (1997). Phenotypic adjustment of clutch size due to nest predation in the great tit. Ecology 78: 394–404.

Kalleberg H. (1958). Observations in a streamtank of territoriality and competition in juvenile salmon and trout. Institute of Freshwater Research of Drottningholm Republic 39: 55-98.

Klingenberg C.P., Ekau W. (1996). A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). Biological Journal of the Linnean Society 59: 143-177.

Knox G.A. (1970). Antarctic marine ecosystems. In: Holdgate M.W. (eds) Antarctic Ecology Vol.1, Academic Press, London, 69-96.

Knox G.A. (2006). The biology of the Southern Ocean. Second Edition. CRC Press, Taylor & Francis Group, New York, 640 pp.

Knox G.A., Lowry J.K. (1977). A comparison between the benthos of the Southern Ocean and the North Polar Ocean with special reference to the Amphipoda and the Polychaeta. In: Dunbar M.J. (eds) Polar oceans. Arctic Institute of North America, Calgary, 423-462.

Knust R., Schröder M. (2014). The Expedition PS82 of the Research Vessel POLARSTERN to the southern Weddell Sea in 2013/2014 (ANT-XXIX/9). Berichte zur Polarforschung 680: 1-157.

Kock K.H. (2005). Antarctic icefishes (Channichthyidae): a unique family of fishes. A review, Part I. Polar Biology 28(11): 862-895.

Kock K.H. (1985). Krill consumption by Antarctic notothenioid fish. In: Siegfried W.R., Condy P.R., Laws R.M. (eds) Antarctic Nutrient Cycles and Food Webs (2013), Springer Science & Business Media 437-444.

Kock K.H. (1992). Antarctic Fish and Fisheries. Cambridge University Press, Cambridge, 359 pp.

Kock K.H., Duhamel G., Hureau J.C. (1985). Biology and status of exploited Antarctic fish stocks: a review. BIOMASS Scientific Series 6: 1-143.

Kock K.H., Kellermann A. (1991). Reproduction in Antarctic notothenioid fish. Antarctic Science 3: 125-150.

Kock K.H., Everson I. (1997). Biology and ecology of mackerel icefish, *Champsocephalus gunnari*: an Antarctic fish lacking haemoglobin. Comparative Biochemistry and Physiology 118(4): 1067-1077.

Kock K.H., Wilhelms S. (2003). The reproductive state of Antarctic fish in the Elephant Island – South Shetland Islands region in March-April 2003. Bundesforschungsanstalt fur Fischerei, Institut fur Seefischerei, 1-4.

Kolm N. (2001). Females produce larger eggs for large males in a paternal mouthbrooding fish. Proceedings of the Royal Society of London B 268: 2229-2234.

Kolm N., Ahnesjö N. (2005) Do egg size and parental care coevolve in fishes? Journal of Fish Biology 66: 1499-1515.

Konecki J.T., Targett T.E. (1989). Eggs and larvae of *Nototheniops larseni* from the spongocoel of a hexactinellid sponge near Hugo Island, Antarctic Peninsula. Polar Biology 10(3): 197-198.

Krist M. (2011). Egg size and offspring quality: a meta-analysis in birds. Biological Reviews 86(3): 692-716.

Kuhn K.L., Near T.J. (2009). Phylogeny of *Trematomus* (Notothenioidei: Nototheniidae) inferred from mitochondrial and nuclear gene sequences. Antarctic Science 21(6): 565-570.

Lack D. (1947). The significance of clutch size. Ibis 89: 302-351.

Laptikhovsky V. (2006). Latitudinal and bathymetric trends in egg size variation: a new look at Thorson's and Rass's rules. Marine Ecology 27: 7-14.

Law R. (1979). Ecological determinants in the evolution of life histories. In: Anderson R.M., Taylor B.D., Taylor L.R. (eds) Population dynamics. Blackwell Scientific, Oxford, UK.

Legg S., Briegleb B.P., Chang Y., Chassignet E.P., Danabasoglu G., Ezer T., Gordon A.L., Griffies S., Hallberg R., Jackson L., Large W.G., Özgökmen T.M., Peters H., Price J., Riemenschneider U., Wu W., Xu X., Yang J. (2009). Improving oceanic overflow representation in climate models: the gravity current entrainment climate process team. Bulletin of the American Meteorological Society 90(5): 657-670. doi: 10.1175/2008BAMS2667.1.

Levin L.A., Bridges T.S. (2001). Pattern and diversity in the reproduction and development. In: McEdward L. (eds) Ecology of Marine Invertebrate Larvae. CRC Press, London, 2-48.

Lindén M., Møller A.P. (1989). Cost of reproduction and covariation of live history traits in birds. Trends in Ecology & Evolution 4: 367–371.

Lynch M. (1991). Methods for the analysis of comparative data in evolutionary biology. Evolution 45: 1065-1080.

Lynch M., Walsh B. (1998). Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA, 980 pp.

Lucassen M. (2012). The expedition of the research vessel "Polarstern" to the Antarctic in 2012 (ANT-XXVIII/4). Berichte zur Polarforschung 652: 1-90.

Mappes T., Koskela E. (2004). Genetic basis of the trade-off between offspring number and quality in the bank vole. Evolution 58(3): 645-650.

Meiri S., Dayan T. (2003). On the validity of Bergmann's rule. Journal of Biogeography 30: 331-351. doi:10.1046/j.1365-2699.2003.00837.

Metcalf C.J.E., Koons D.N. (2007). Environmental uncertainty, autocorrelation and the evolution of survival. Proceedings of the Royal Society B, Biological Sciences 274(1622): 2153-2160.

Metz J.A.J., Nisbet R., Geritz S.A.H. (1992). How should we define 'fitness' for general ecological scenarios? Trends in Ecology & Evolution 7: 198-202.

Mileykovsky S.A. (1971). Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Marine Biology 10: 193–213.

Miller P.J. (1984). The tokology of gobioid fishes. In: Potts G.W., Wooton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, Oxford, 119-154

Moline M.A., Karnowski N.J., Brown Z., Divoki G.J., Frazer T.K., Jacoby C.A., Torres J.J., Fraser W.R. (2008). High latitude changes in ice dynamics and their impact on polar marine ecosystems. Annals of the New York Academy of Sciences 1134: 267-319.

Murphy G.I. (1968). Pattern in life history and the environment. The American Naturalist 102(927): 391-403.

Near T.J., Pesavento J.J., Cheng C.-H.C. (2004). Phylogenetic investigations of Antarctic notothenioid fishes (Perciformes: Notothenioidei) using complete gene sequences of the mitochondrial encoded 16S rRNA. Molecular Phylogenetics and Evolution 32: 881-891.

Near T.J., Parker S.K., Detrich H.W. (2006). A genomic fossil reveals key steps in hemoglobin loss by the Antarctic icefishes. Molecular Phylogenetics and Evolution 23: 2008-2016.

Near T.J., Cheng C.-H.C. (2008). Phylogenetics of notothenioid fishes (Teleostei: Acanthomorpha): Inferences from mitochondrial and nuclear gene sequenze. Molecular Phylogenetics and Evolution 47: 832-840.

Near T.J., Dornburg A., Kuhn K.L., Eastman J.T., Pennington J.N., Patarnello T., Zane L., Fernández D.A., Jones C.D. (2012). Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. Proceedings of the National Academy of Science 109(9): 3434-3439.

Near J.T., Dornburg A., Harrington R.C., Oliveira C., Pietsch T.W., Thacker C.E., Satoh T.P., Katayama E., Wainwright P.C., Eastman J.T., Beaulieu J.M. (2015). Identification of the notothenioid sister lineage illuminates the biogeographic history of an Antarctic adaptive radiation. BMC Evolutionary Biology 15: 109.

Nunn C.L. (2002). Spleen size, disease risk and sexual selection: a comparative study in primates. Evolutionary Ecology Research 4: 91-107.

Oksanen T.A., Koskela E., Mappes T. (2002). Hormonal manipulation of offspring number: maternal effort and reproductive costs. Evolution 56: 1530-1537.

Orzack S.H. (1985). Population dynamics in variable environments V. The genetics of homeostasis revisited. The American Naturalist 125(4): 550-572.

Orzack S.H., Tuljapurkar S. (1989). Population dynamics in variable environments VII. The demography and evolution of iteroparity. The American Naturalist 133(6): 901-923.

Orzack S.H., Tuljapurkar S. (2001). Reproductive effort in variable environments or environmental variation is for the birds. Ecology 82(9): 2659-2665.

Parker G.A., Begon M. (1986). Optimal egg size and clutch size: effects of environment and maternal phenotype. The American Naturalist 128: 573-592.

Pease C.M., Bull J.J. (1988). A critique of methods for measuring life history trade-offs. Journal of Evolutionary Biology 1: 293-303.

Petrie M., Williams A. (1993). Peahens lay more eggs for peacocks with larger trains. Proceedings of the Royal Society of London B 251(1331): 127-131.

Pettifor R.A., Perrins C.M., McCleery R.H. (1988). Individual optimization of clutch size in great tits. Nature 336: 160-162.

Place S.P., Hofmann G.E. (2005). Constitutive expression of a stress-inducible heat shock protein gene, hsp70, in phylogenetically distant Antarctic fish. Polar Biology 28: 261-267.

Potts G.W. (1984). Parental behaviour in temperate marine teleost with special reference to the development of nest structures. In: Potts G.W., Wootton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, London, 223-244.

Rass T.S. (1941). Geographic Parallelisms in Morphology and Development of Teleost Fish of Northern Seas. MOIP, Moscow (In Russian).

Rass T.S. (1986). Biogeographic rule of inverse relation between egg size and environmental temperature in poikilothermous animals. Trudy IOAN 116: 152-168. (In Russian).

Rees M., Childs D.Z., Metcalf J.C., Rose K.E., Sheppard A.W., Grubb P.J. (2006). Seed dormancy and delayed flowering in monocarpic plants: selective interactions in a stochastic environment. The American Naturalist 168(2): 53-71.

Reznick D.N. (1982). The impact of predation on life history evolution in Trinidadian guppies: genetic basis of observed life history patterns. Evolution 36(6): 1236-1250.

Reznick D.N., Butler M.J., Rodd F.H., Ross P. (1996). Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. Evolution 50(4): 1651-1660.

Ridley M. (2004). Evolution. 3rd ed. Blackwell Scientific Publishing, Malden, MA.

Rochet M.J., Cornillon P.A., Sabatier R., Pontier D. (2000). Comparative analysis of phylogenetic and fishing effects in life history patterns of teleost fishes. Oikos 91: 255-270.

Roff D.A. (1984). The evolution of life history parameters in teleosts. Canadian Journal of Fisheries and Aquatic Sciences 41: 984-1000.

Roff D.A. (1992). Evolution of life histories: theory and analysis. Chapman and Hall, New York, 548 pp.

Roff D.A. (1997). Evolutionary quantitative genetics. Chapman and Hall, New York.

Roff D.A. (2002). Life history evolution. Sinauer, Sunderland, 527 pp.

Rutschmann S., Matschiner M., Damerau M., Muschick M., Lehmann M.F., Hanel R., Salzburger W. (2011). Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Molecular Ecology 20: 4707-4721.

Ruud J.T. (1954). Vertebrates without erythrocytes and blood pigment. Nature 173: 848-850.

Sanchez S., Dettai A., Bonillo C., Ozouf-Costaz C., Detrich W., Lecointre G. (2007). Molecular and morphological phylogenies of the Antarctic teleostean family Nototheniidae, with emphasis on the Trematominae. Polar Biology 30: 155-166.

Sanford G.M., Lutterschmidt W.I., Hutchison V.H. (2002). The comparative method revisited. BioScience 52: 830-836.

Schaffer W.M. (1974). Optimal reproductive effort in fluctuating environments. The American Naturalist 108: 783-790.

Schmalhausen I.I. (1949). Factors of Evolution. Blakiston, Philadelphia, PA.

Schopka S.A., Hempel G. (1973). The spawning potential of populations of herring (*Clupea harengus* L.) and cod (*Gadus morhua* L.) in relation to the rate of exploitation. Rapports et Proce`s-Verbaux des Re´unions du Conseil International pour l'Exploration de la Mer 164: 178-185.

Shine R. (1978). Propagule size and parental care, the ''safe harbour'' hypothesis. Journal of Theoretical Biology 75: 417-424.

Sibly R.P., Calow P. (1983). An integrated approach to Iife-cycle evolution using selective landscapes. Journal of Theoretical Biology 102: 527-547.

Sinervo B. (2000). Adaptation, natural selection and optimal life-history allocation in the face of genetically based trade-offs. In: Mousseau T.A., Sinervo B., Endler J. (eds) Adaptive genetic variation in the wild. Oxford University Press, Oxford, UK, 41-64.

Smith C.C., Fretwell S.D. (1974). The optimal balance between size and number of offspring. The American Naturalist 108: 499-506.

Smith R.J., Cheverud J.M. (2002). Scaling of sexual size dimorphism in body mass: a phylogenetic analysis of Rensch's rule in primates. International Journal of Primatology 23: 1095-1135.

Smith S.L., Schnack-Schiel S.B. (1990). Polar zooplankton. In: Smith W.O. (eds) Polar Oceanography Vol. B. Academic Press, San Diego, 527-598.

Smith W.O., Sakshaug E. (1990). Polar phytoplankton. In: Smith W.O. (eds) Polar Oceanography Vol. B, Academic Press, San Diego, 477-525.

Snyder R.J. (1991). Quantitative genetic analyses of life histories in two freshwater populations of the threespined stickleback. Copeia 1991: 526-529.

Stearns S.C. (1976). Life-history tactics: a review of the ideas. Quarterly Review of Biology 51: 3-46.

Stearns S.C. (1989). Trade-offs in life history evolution. Functional Ecology 3: 259-268.

Stearns S.C. (1992). The Evolution of Life Histories. Oxford University Press, Oxford, 249 pp.

Stearns S.C. (2000). Life history evolution: successes, limitations, and prospects. Naturwissenschaften, 87: 476-486.

Stearns S.C., Koella J.C. (1986). The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40(5): 893-913.

Tuljapurkar S. (1990). Population dynamics in variable environments. Springer-Verlag, New York/Berlin, 154 pp.

Turner J., Bindschadler R., Convey P., Di Prisco G., Fahrbach E., Gutt J., Hodgson D., Mayewski P., Summerhayes C. (2009). SCAR's Antarctic Climate Change and the Environment (ACCE) Review Report, XXXII Antarctic Treaty Consultative Meeting, Baltimore, USA, 555 pp.

Via S., Lande R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39: 505-522.

Ware D.M. (1975). Relation between egg size, growth, and natural mortality of larval fish. Journal of Fish Research Board of Canada 32: 2503-2512.

Wedell N. (1996). Mate quality affects reproductive effort in a paternally investing species. The American Naturalist 148(6): 1075-1088.

Wilbur H.M., Rudolf V.H. (2006). Life-history evolution in uncertain environments: bet hedging in time. The American Naturalist 168(3): 398-411.

Winemiller K.O., Taphorn D.C. (1989). La evolucion de las estrategias de vida en los peces de los llanos occidentales de Venezuela. Biollania 6: 77-122.

Winemiller K.O., Rose A. (1992). Patterns of life-history diversification in North American fishes: implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences 49: 2196-2218.

Wootton R.J. (1979). Energy costs of egg production and environmental determinants of fecundity in Teleost fishes. In: Miller P.J. (eds) Fish Phenology: Anabolic Adaptiveness in Teleosts, Symposia of the Zoological Society of London 44, Academic Press, London 133-159.

PAPERS' EXTENDED ABSTRACTS

PAPER I

Reproductive resilience of ice-dependent Antarctic silverfish in a rapidly changing system along the Western Antarctic Peninsula

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The Western Antarctic Peninsula (wAP) is globally one of the systems most heavily impacted by climate change, notably steep declines in sea ice extent. In foraging species, reproductive resilience to change is particularly important because population fluctuations have rapid implications through the system due to trophic interactions. The reproductive traits of the ice-dependent foraging species Antarctic silverfish (*Pleuragramma antarctica*) from different areas along the wAP and at the tip of the Antarctic Peninsula were investigated through macroscopic and histological analyses of gonads, with the aim to assess its reproductive potential and to test for spatial differences in fecundity and spawning season. Fish samples were collected from late March to early May 2010, using a multiple opening/closing net and environmental sensing system (MOCNESS) on board the R/V *Nathaniel B. Palmer* in late summer off Charcot Island, in Marguerite Bay and off Joinville Island; no fish were caught in the central wAP.

Samples from Charcot Island and Marguerite Bay consisted of adults ranging 150-240 mm in size with gonads in developing stage, whereas those from Joinville consisted almost exclusively of juveniles (less than 160 mm in size), with the exception of a few adult specimens. Length at first maturity appeared therefore to be in the range of 150–160 mm, at approximately 65–70% of maximum size and at around 50% of maximum age, indicating a delay in energy expenditure for reproduction. Mean GSI was relatively low (2–3%) and similar in both sexes, as specimens were still far from being actively reproducing. Developing females exhibited group synchronous ovaries with two discrete, though partially overlapping, modes of oocytes of different size, the vitellogenic ones measuring 0.5-1.0 mm and the previtellogenic ones less than 0.4 mm in diameter. As already observed in other notothenioid species, the cohort of larger oocytes is likely to be spawned in the current breeding season, while the cohort of smaller oocytes is developing for the future reproductive season. Absolute and relative fecundity ranged between 3000 and 12000 eggs per female and between 80 and 190 eggs/g^{-1} (Tab. 1), with a strong relationship between absolute fecundity and body size (Fig. 1). Fecundity increased rapidly to reach 10000–12000 eggs per female after 200 mm length, highlighting the importance of larger, older fish in maintaining reproductive potential. There was no relationship between fish length and egg size. Comparing the two areas with larger sample size, Marguerite Bay and Charcot Island, females did not differ in fecundity, while GSI and oocyte size (mean egg size 0.52 and 0.59 mm, respectively) were significantly lower in fishes from Charcot Island, suggesting a slight temporal shift in female gonad maturation between the two areas. Mean absolute fecundity, of 10100 eggs per female off Joinville Island was consistent with the large size of the adult specimens sampled there.

Table 1. Absolute and relative fecundity in developing females of *Pleuragramma antarctica* collected along the Antarctic Peninsula in March-April.

Site	N	F (eggs/female) range	F (eggs/female) mean ± SE	F_{rel} (eggs/g) range	F_{rel} (eggs/g) mean ± SE
Charcot	12	2953-11613	6818 ± 1968	78-193	136 ± 39
Marguerite	12	3637-11492	6700 ± 1934	108-189	157 ± 45
Joinville	2	10043-10174	10109 ± 7148	119-171	145 ± 103

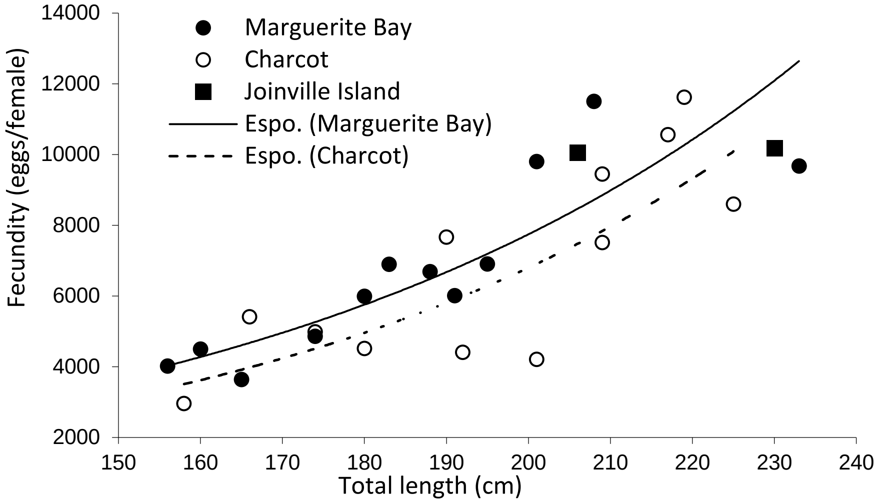

Figure 1. Relationships between absolute fecundity and fish size in developing females, showing experimental data and fitted exponential curves.

Overall these results were consistent with the occurrence of a single population at Charcot Island and Marguerite Bay. The reproductive investment of *P. antarctica* represents the second highest value of absolute fecundity recorded in high Antarctic notothenioids, lower only than the value of the related Antarctic toothfish (*Dissostichus mawsoni*), which spawns up to 1 million eggs and that is the largest sized species among Antarctic notothenioids. Considering relative fecundity, *P. antarctica* results to be the species with the highest investment in eggs. Due to its ice-dependence in spawning, egg deposition and as feeding ground for newly hatched larvae, Antarctic silverfish currently appears to be one of the species most vulnerable to climate change due to the effects of warming on sea-ice cover. Sampling from south to north of the Antarctic Peninsula a lack of catches of *P. antarctica* has been registered near Renaud and Anvers Islands, areas where this species was historically abundant. The disappearance of *P. antarctica* from these areas may have led to geographical fragmentation and reproductive and therefore a sharp loss in connectivity along the wAP and, as a consequence, the stabilizing effect of immigration as a subsidy to local self-recruitment.

The results of this study highlighted a considerable reproductive potential of Antarctic silverfish, that could therefore mitigate the effects of populations’ isolation, but cannot ultimately offset catastrophic loss of spawning habitats linked to sea-ice retreat.

EXTENDED ABSTRACT

PAPER II

Biological traits of a sub-Antarctic nototheniid, *Patagonotothen ramsayi*, from the Burdwood Bank

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The rockcod *Patagonotothen ramsayi* (Regan 1913) is the most abundant species of the genus *Patagonotothen*, which includes 14 species distributed in the south-western Atlantic Sea. It occurs along the Patagonian shelf from 35°S to the Burdwood Bank (55°S). Because of its abundance, it plays an important role in the demersal food web both as prey and predator, as well as by-catch in the local finfish and squid trawl fisheries. Adult specimens of *P. ramsayi* were collected during bottom trawling carried out in February 2011 on the eastern shelf of Burdwood Bank during the expedition ANT- XXVII/3 of the R/V *Polarstern*. They were successfully aged by otolith readings and their reproductive characteristics in terms of gonadosomatic index (GSI), gametogenesis and fecundity were assessed by macroscopical and histological analyses. Age, estimated on 106 specimens, was similar between sexes, ranging from 4 to 7 and from 4 to 8 years in males and females of comparable size respectively. Age data of both sex were pooled together and summarized in the age-length key (Tab. 1).

Table 1. Age-length key of the rock cod from the Burwood Bank. TL: total length.

Size TL (mm)	Age (years) 4	5	6	7	8
200	1				
210		1			
220	1	1			
230	1	11			
240	4	23	3		
250		30	3		
260		16	2		
270		1	4	1	
280			1		
290				1	1
n	7	83	13	2	1
Mean size at age (mm)	234	250	262	282	292

GSI was relatively low in females (<1.5%), as fish were sampled far from the spawning season reported for the species (i.e. June-August). In females, the gonad maturation process had started, with the most advanced oocytes being in the cortical alveoli stage or early vitellogenesis (Fig. 1a). Few large atretic oocytes (>1mm) were also found in two females (Fig. 1a). In males, all analysed testes had spermatocytes in the germinal epithelium, while the occurrence of spermatozoa in the lumen was observed only in two specimens (Fig 1b).

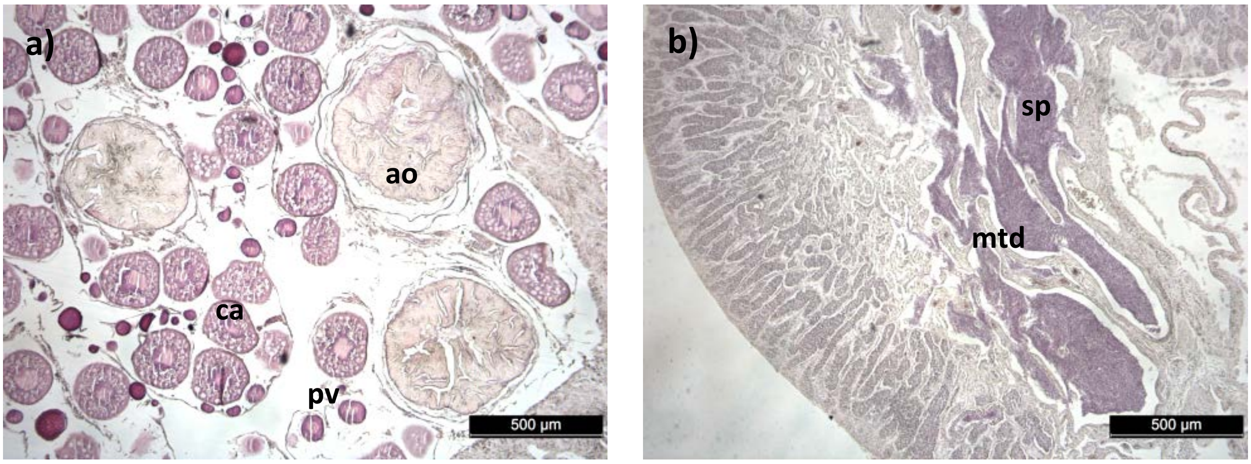

Figure 1. Transversal histological sections of gonads of *Patagonotothen ramsayi*. **a)** Female at early developing stage (TL 292 mm), showing previtellogenic, cortical alveoli stage and atretic oocytes; **ao** atretic oocytes, **ca** cortical alveoli oocyte, **pv** previtellogenic oocytes. **b)** Spent male (TL 249 mm) showing abundant sperm in the main testicular duct; **mtd** main testicular duct, **sp** sperm.

The oocytes size distribution was bimodal (i.e. group-synchronous ovaries), with a first mode consisting of oocytes from 0.08 to 0.16 mm and a second mode from 0.17 to 0.66 mm. Given that vitellogenesis lasts for 7-8 months in this species only the oocytes of the second mode are likely to be spawned in the next reproductive season and were considered for fecundity estimates. Mean absolute fecundity was 55445 ± 22351 (ranging from 30931 to 120789 oocytes). No relationship was found between female size and fecundity (R^2=0.03; $F_{1,18}$=0.48; p=0.49), probably due to the relatively narrow range of size of the analysed fishes. Youngest specimens attaining sexual maturity were 4 years old in both sexes.

Our results highlighted also the occurrence in the sampled period of spent males with considerable residual spermatozoa and females with atretic oocytes still in the resorption process. Oocytes and spermatozoa are likely from the previous spawning season, possibly indicating a later or extended spawning season in the study area than previously reported. Comparing the rock cod inhabiting the Burdwood Bank with the northern populations, some biological traits appear to differ to some extent, possibly driven by different environmental conditions. Firstly, differences among areas in the spawning season occur, since spawning takes place in austral autumn (April-May) on the northern Argentinean shelf, in winter (June-August) around the Falkland Islands and in spring-early summer (October-December, possibly also January as suggested by this study) on the Burdwood Bank.

The present study provided, for the first time, data on age and reproduction of the rock cod, *P. ramsayi*, from the Burdwood Bank, representing the southern boundaries of its distribution. According to literature data, both total and relative fecundities seem to be rather different between the populations inhabiting the shelf around the Falkland Islands and the Burdwood Bank. Taking into account the same fish size range (i.e. 20-30 cm TL) and fecundity estimates corrected for atretic losses, total and relative fecundities calculated from the southern population were higher than those from the northern one, where they were 24000-76000 eggs/female and 200–350 eggs/g, respectively. This pattern was unexpected, as Antarctic fish populations distributed at the higher latitudes usually show lower level of fecundity compared with those inhabiting lower latitudes. Differences in fecundity between populations of rock cod may be related to a different degree of atretic processes and therefore of oocyte resorption between sites, and/or to a difference in potential fecundity (i.e. the maximum number of oocytes that can mature). In both cases, more favourable environmental conditions (food availability or water temperature, among others) at Burdwood Bank might be responsible for such differences, though it deserves further investigations.

EXTENDED ABSTRACT

PAPER III

Life history strategies of the Scotia Sea icefish, *Chaenocephalus aceratus*, along the Southern Scotia Ridge

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Estimates of fecundity and reproductive biology are critical to assess reproductive potential over the life history, including length or age at first maturity relative to maximum age, and any relationships between size and absolute fecundity. Physical-biological interactions between circulation and life history processes can help to account for population distributions and abundance. Nevertheless, variability also depends on the reproductive capacity of each population in making larvae available for dispersal and self-recruitment. Additionally, spatial differences in vital rates and life history parameters can be used to corroborate isolation following settlement on shelf areas along the Antarctic Peninsula and southern Scotia Ridge.

We examined size and age distribution and reproductive traits in the Scotia Sea icefish, *Chaenocephalus aceratus*, an abundant benthic species with a long larval pelagic phase inhabiting continental shelves along the Southern Scotia Ridge. Biological traits of *C. aceratus* were investigated through macroscopic and histological analyses of gonads and otoliths reading. Life history strategies were compared between fish caught during surveys undertaken off the South Orkney Islands (SOI) and South Shetland Islands (SSI). Reproductive features of *C. aceratus* females obtained in the present study were compared to literature data (Tab. 1). Differences among areas emerged in fish size, weight and female fecundity. Overall, the mean size of fish caught off SOI was larger (SOI TL = 43.4 ± 10.6 cm; SSI: TL = 32.1 ± 14 cm) and the length frequencies differed sharply between regions, with relatively few smaller fish caught in SOI.

Compared to SSI, body weight increased faster with length and mean absolute fecundity was 46% lower in SOI population and increased more slowly with size (Fig. 1).

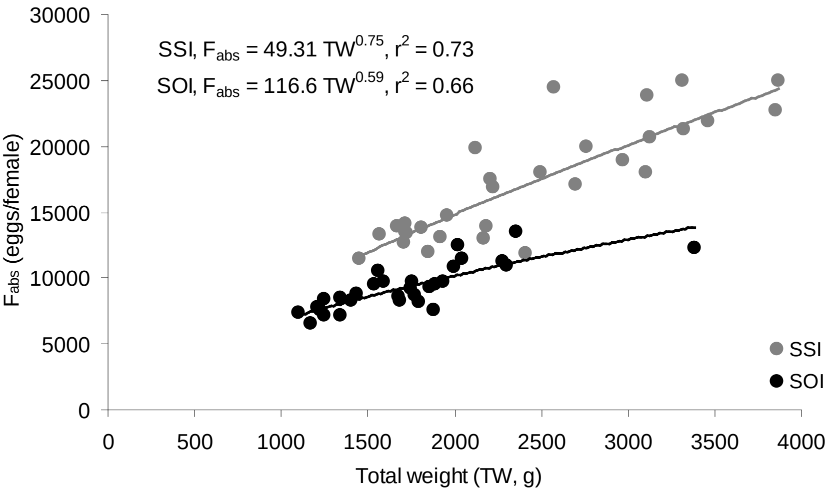

Figure 1. Relationships between fish total weight (TW) and absolute fecundity (F_{abs}) in *Chaenocephalus aceratus* from the South Orkney Islands (SOI) and South Shetland Islands (SSI).

The regional differences in relative and absolute fecundity were consistent with results of previous studies (Tab.1).

Table 1. Reproductive characteristics of *Chaenocephalus aceratus* females from different areas. F_{abs} = absolute fecundity; F_{rel} = relative fecundity; GSI = gonadosomatic index; -- = not available. Source: 1 = Kock and Kellermann 1991; 2 = Kock 2005; 3 = Kock 1989.

Area	Max egg size (mm)	Size at spawning (TL_{50}, cm)	F_{abs}	F_{rel}	Lenght range (cm)	Max GSI (female)	Source
South Georgia	4.4	47 ♂, 58 ♀	7358 - 22626	5 - 9	47.7 - 73	27	1 – 2
South Georgia	--	--	3082 - 22626	3.9 – 9.2	48 - 72	--	1 – 2
South Georgia	--	--	5898 - 18039	4 - 6.8	58 - 75	--	1
South Orkney Is.	4.9	--	4499 - 15174	3.9 - 6.8	52 - 67	28	1 – 2
South Orkney Is.	4.8	44 ♂, 57 ♀	6534 - 13529	4 - 7.4	52 - 66	15	Present study
Elephant Is.	4.7	46 ♂, 57 ♀	6711 - 19672	4.9 - 9.2	51 - 66	25	3
South Shetland Is.	--	--	7375 - 12450	4.6 - 8.2	53 - 62	--	3
South Shetland Is.	4.3	45 ♂, 58 ♀	11493 - 25012	5.2 - 12.2	56 - 68	28	Present study

Based on the von Bertalanffy models, age at first spawning in females was 14 years for SOI and 13 years for SSI. Considering the maximum ages for each area (15 years for SOI and 17 years for SSI), this results indicated the occurrence of only two female spawning cohorts at SOI and five at SSI. Finally there was evidence of significant differences in growth between regions, with L_∞ lower and k higher for SOI.

The differences between study areas are likely a phenotypic response to prevailing environmental and ecological conditions related to regional hydrography. In particular, the influence of cold water from the Weddell Sea is considerable over the South Orkney shelf and decreases northwards, and the seasonal effect of pack ice is longer and more intense with more and deeper benthic scouring by icebergs. Likely in relation to these hydrological conditions, krill abundance differs between areas, with greater biomass at SSI at least in some years. *C. aceratus* feeds mainly on krill during its juvenile phase and on fish as adults. Therefore the higher abundance of krill at SSI may support locally abundant juveniles of the Scotia Arc icefish, and indeed juveniles were caught mainly at SSI. In addition, SSI fish could take advantage of the warmer shelf waters and higher food availability of this area to invest more in reproduction.

Higher fecundity and a larger number of spawning cohorts combined with the much larger volume of eastward flow, suggest that the SSI have a much greater influence on the SOI than vice versa, being ultimately very important in influencing distributions, abundance and population connectivity of icefish along the southern Scotia Ridge.

EXTENDED ABSTRACT

PAPER IV

Life history strategies of *Notothenia rossii* and *N. coriiceps* along the Southern Scotia Arc

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The genus *Notothenia* includes 5 species, three are Sub-Antarctic (*Notothenia angustata, N. microlepidota* and *N. cyanobrancha*), whereas *N. rossii* and *N. coriiceps* present wide Antarctic distribution. With the exception of *N. rossii*, whose adults have bentho-pelagic habits, *Notothenia* species are typically benthic, inhabiting the continental-shelf. *N. rossii* and *N. coriiceps* produce large pelagic eggs and have a long larval pelagic phase, two reproductive features promoting the connections among populations inhabiting different areas. High female reproductive investment in gonads is common among notothenioids, including *N. rossii* and *N. coriiceps*, however these two species show high gonadal investment in males too, suggesting potential spawning aggregation and sperm competition. *Notothenia* genus represents a good study group to analyze the variation of life history traits at both inter- and intraspecific level, given their wide distribution in Antarctic waters, comprising different habitat conditions.

Starting from 1970s, *N. rossii* was one of the first Antarctic fish species overexploited by the commercial finfish fishery, showing an important decrease in the catches, even after the fishery ban established in 1991. *N. coriiceps* was fished too but was considered an accessory catch, therefore it did not show any marked decline. Both are among the reference species in monitoring programs of overexploited species of the Southern Ocean and a deepened knowledge of their biology is needed.

This study provides insights on some key aspects of the reproductive biology and age structure in *N. rossii* and *N. coriiceps* from South Shetland Islands (SS). Furthermore, the availability of *N. rossii* specimens sampled around South Georgia (SG), enabled to investigate the intraspecific variation of life history traits in populations inhabiting different areas. Samples were collected during two *R/V Polarstern* expeditions, carried out between February-April 2011 and 2012 (Tab. 1).

Table 1. Number of specimens in *Notothenia rossii* and *N. coriiceps* in the relative sampled area. Females and males abundances are reported; NA: not attributed

	Site	Sex	n	Tot n
N. rossii	South Shetland Is.	♀	50	
		♂	21	71
	South Georgia	♀	33	
		♂	26	
		NA	7	66
N. coriiceps	South Shetland Is.	♀	32	
		♂	12	44

The minimum size of a mature specimen was 44 cm of total length in *N. rossii* males (corresponding to an age of 5 years) and 50 mm in females (corresponding to 7 years old). In *N. coriiceps* females mature at 390 mm, males at 360 mm (corresponding to 7 years old in both sex). Absolute fecundity estimations ranged from 8300 to 52000 eggs/female in *N. coriiceps* and from 6700 to 68000 eggs/female in *N. rossii*, relating positively to size. No significant differences in fecundity, controlled for size, emerged between the populations of SS and SG of *N. rossii* (Fig.1). However, mean egg size in mature SG females were lower than in the SS population. The observed intraspecific differences may reflect a temporal shift in sampling, one month later in the season in SS, rather than a real difference in the egg size between the two populations.

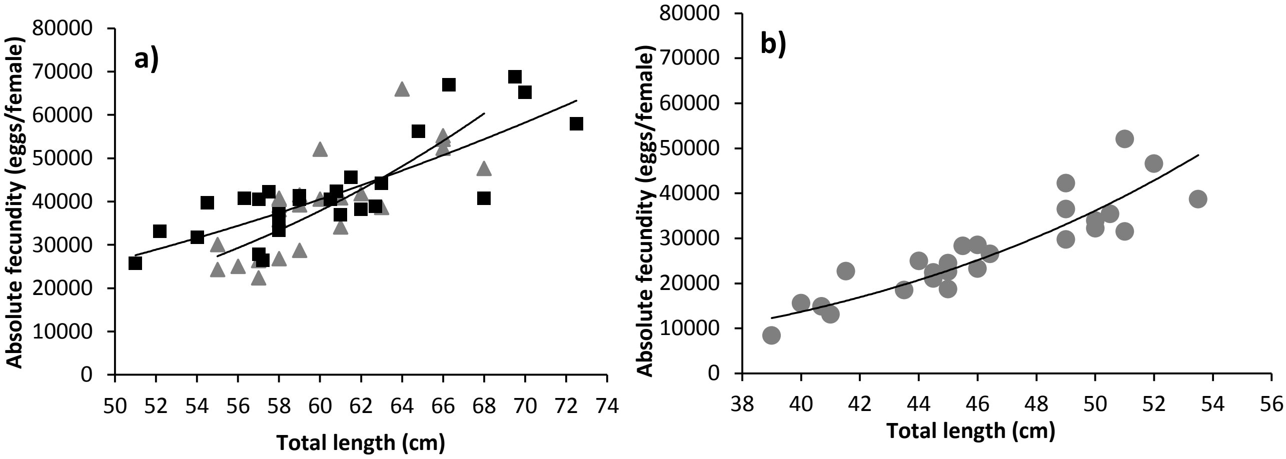

Figure 1. Relationships between total length and absolute fecundity in: **a)** *Notothenia rossii* from South Shetland Islands (grey triangles) and from South Georgia (black square) and **b)** *N. coriiceps* from South Shetland Islands.

Histological analyses confirmed the maturity stage macroscopically attributed in 35 out of 39 samples analysed and provide new descriptions of gonad morphology at microscopic level. Group synchronous ovaries with bimodal oocytes distribution and unrestricted spermatogonial testicular type were observed, indicating both species as total spawners. Both species release relatively large eggs (up to 5 mm) if compared with others pelagic spawners. Consistently with what emerged from intraspecific comparison of absolute fecundity, the relationship between fish size and age in *N. rossii* did not show any difference between specimens from SS and SG, strengthening the conclusion of no differentiation between populations.

N. coriiceps and *N. rossii* show high reproductive effort, including high gonadosomatic index (GSI), in particularly, males showed the highest gonads investment among notothenioids, reaching 23.8% and 51.4% of body weight, respectively. Such high values of male GSI, together with the abundance of sperm in testes, have been put in relation, in other teleost fish, to high levels of sperm competition occurring in spawning aggregations, therefore suggesting this type of mating system also in these species. Considering that the occurrence of spawning aggregations make the species particularly vulnerable to fishery exploitation, this aspect deserves further investigations.

EXTENDED ABSTRACT

PAPER V

Life strategies of two Antarctic plunderfishes (Artedidraconidae) from the Weddell Sea

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The plunderfishes belong to the Artedidraconidae, one of the five families of Notothenioidea endemic of the Southern Ocean. This family represents an important component of the benthic fauna inhabiting the continental shelf and upper slope of the High Antarctic Zone and accounts for 20-23% of the diversity of the Ross Sea and the Weddell Sea. Plunderfishes play an important role in the High Antarctic trophic webs, representing a main source of food for top predators, such as Emperor Penguins and Weddell Seals. Artedidraconids includes 4 genera (*Artedidraco*, *Dolloidraco*, *Histiodraco* and *Pogonophryne*) and 25 species, characterized by small to medium sizes, a sculpin-like appearance and the presence of a mental-barbel as distinctive trait. Overall scientific information on the biology of Artedidraconidae is still scattered and scarce, likely due to the difficult accessibility of specimens from the permanent pack-ice zone and their absence of commercial value. Few data are available on reproductive biology and age structures.

This study provides information on reproductive investment (i.e. gonadosomatic index, GSI), fecundity and size at first spawning in two species of plunderfishes from the Weddell Sea, *Artedidraco skottsbergi* and *Dolloidraco longedorsalis*. Moreover, histologic analyses of the gonads were performed to validate the maturity stage attribution and to describe, for the first time, gametogenesis process of ovaries and testes of *D. longedorsalis* and to enforce data on *A. skottsbergi*. Age-length relationships were assessed through otoliths reading, providing so far unknown information on the age of *A. skottsbergi*.

Overall 24 specimens of *A. skottsbergi* and 83 specimens of *D. longedorsalis* were collected in the Antarctic summer 2014, during the *RV Polarstern* expedition PS82 in the Weddell Sea.

From the reproductive point of view, *A. skottsbergi* and *D. longedorsalis* follow the same common features described for Notothenioids, a biennial process of gametogenesis and the production of few but very large eggs (Tab. 1), released during a single spawning event.

While *A. skottsbergi* and *D. longedorsalis* oocytes are similar in sizes to those of many other species of the Weddell Sea (2-4 mm), their absolute fecundities are instead 5 to 40 times lower than in other Bathydraconidae, Channithyidae and Nototheniidae species of the same area. This lower fecundity can be explained by the constraints related to their size, being *A. skottsbergi* and *D. longedorsalis* smaller than almost all species of the above mentioned families.

Table 1. Ranges of total length (TL), gonadosomatic index (GSI), absolute fecundity (F_{abs}) and relative fecundity (F_{rel}), mean, minimum and maximum diameter (Ø) of oocytes in advanced vitellogenesis, of females of *Artedidraco skottsbergi* (n=6) and *Dolloidraco longedorsalis* (n=11).

	TL (cm)	GSI (%)	F_{abs} (oocytes)	F_{rel} (oocytes/g)	Mean Ø. (mm)	Min Ø (mm)	Max Ø (mm)
Artedidraco skottsbergi	9.5-10.3	9.8-27.9	60-76	6.4-26	2.91±0.15	2.56	3.21
Dolloidraco longedorsalis	11-13.2	4.6-12.1	86-330	7-20.5	2.22±0.14	1.80	2.56

Late sexual maturity was confirmed in both species with females first spawning at 9.5 cm, and males at 8 cm in *A. Skottsbergi* (86% and 73% of reported maximum size respectively), while both sexes first spawn at 11 cm in *D longedorsalis* (80% of maximum size).

Otoliths reading allowed to estimate age-length key in *A. skottsbergi* (from 6 to 14 years) and in *D. longedorsalis* (from 3 to 14 years). Von Bertalanffy growth-curve for *D. longedorsalis* was set (Fig.1).

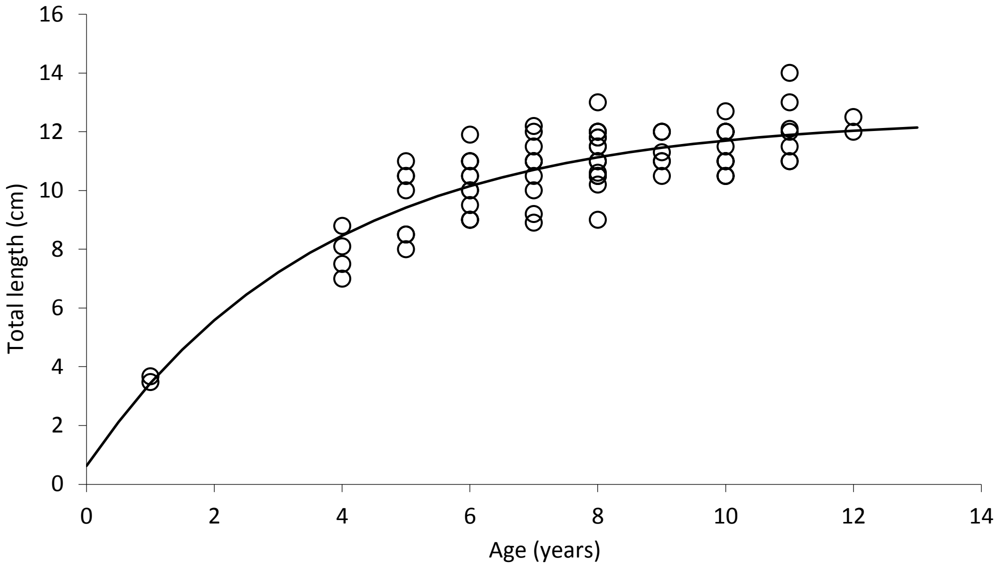

Figure 1. Von-Bertalanffy growth-curve fitted to the estimated age-length data of *Dolloidraco longedorsalis* from Weddell Sea. Data of two specimens from Voskoboinikova (2001) were also used to fill the gap in the first age classes.

The results of this study contributes to the understanding of the biology of *A. skottsbergi* and *D. longedorsalis*, with particular reference to the Weddell Sea populations. Furthermore the evaluation of the reproductive potential could help in assessing the capacity of resilience or potential of recovery of these populations in response to environmental disturbances. In fact, besides their lack of a commercial value, plunderfishes represent an important component of the diet of top Antarctic predators and thus a drop in their abundance could trigger a series of cascading effects in the very specialized Antarctic trophic webs.

EXTENDED ABSTRACT

PAPER VI

Evolution of life history traits in Notothenioid fishes

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Teleost fishes display a wide inter- and intraspecific variation in life history traits, reflecting the effects of evolutionary forces acting on them over time and across environmental conditions in their distribution. Growth rate, age at sexual maturity, hatching size, egg size, fecundity and reproductive investment are largely influenced by temperature and food availability. Therefore these two factors have been recognized as some of the major drivers of variation in life history traits.

Fish reveal a wide range of fecundity and egg size, two key features of reproductive strategies, generally with a trade-off between few large (quality) or many small eggs (quantity). In general, a trend of increasing egg size with the decrease of temperature towards high latitudes has been documented in several taxa, including fish. Notothenioid fishes inhabiting the Antarctic and sub-Antarctic regions represent an unique example of adaptive radiation in marine environment and an excellent model group for evolutionary studies. They dominate the waters surrounding the Antarctic continent shelf both in species number, with over 120 species (47%), and biomass (90-95%). Notothenioids display wide variability in life history traits at both inter- and intra-specific levels and a general trend of increasing egg size and decreasing relative fecundity towards higher latitudes was described in the Suborder.

To properly investigate the evolution of life histories in response to environmental conditions phylogenetic relationships have to be taken into account in the comparative approach, as species share characteristics through descendent from common ancestors. The evolution of some life history traits in notothenioids were investigated in relation to environmental variables: sea water temperature, primary production, salinity and sea ice cover, using the independent contrasts as comparative method. Gonadal investment, represented by gonadosomatic index, fecundity, egg size and maximum body size were the studied traits.

The frequency distribution of maximum fish size highly overlapped among species occurring in different zones (High, Low and Sub-Antarctic) (Fig. 1a). High Antarctic species in general exhibit large eggs, while Sub-Antarctic smaller ones (Fig. 1b).

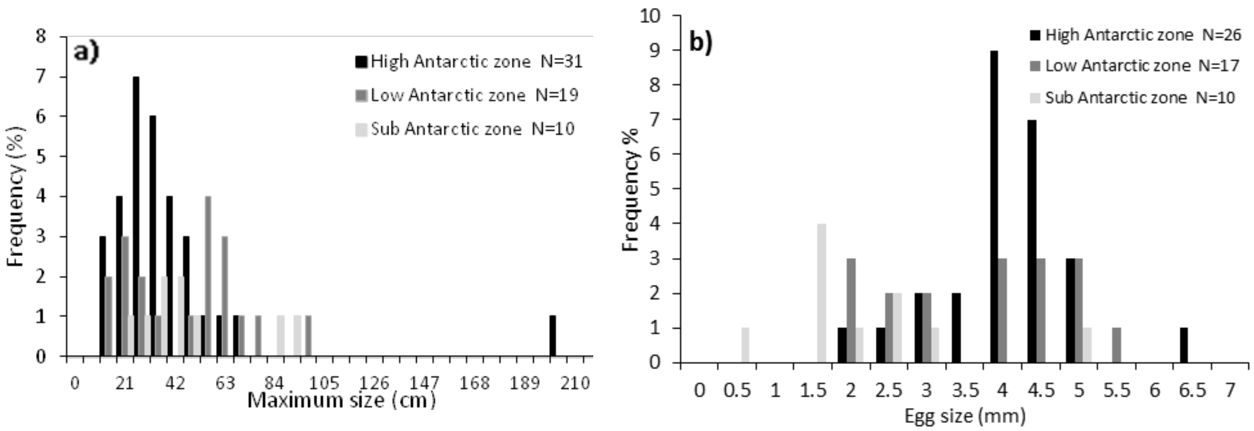

Figure 1. Frequency distribution in High, Low and Sub-Antarctic species of: **a)** maximum size; **b)** egg size.

Maximum size was not related to any environmental factor, suggesting that the Bergmann's rule predicting larger specimens found in colder environments does not persist at the high latitudes.

Egg size was related to the maximum parental body size, indicating a trend with larger fish laying larger eggs. In general, fish size appears to constrain, rather than to determine, the range of possible egg sizes. The wider spectrum of egg sizes, possible for large fish, implies that a greater range of tactics in the partition of reproductive effort between fecundity and egg size is available to them.

Egg size was negatively related to primary production (Fig 2a) and, in turn, negatively related to latitude. Both food availability and temperature have been claimed as factors driving these general trends of egg/offspring size with latitude. Primary production can be considered a proxy of food availability, therefore in notothenioids food availability appears to be the main factor influencing egg size.

Relative fecundity was not related to primary production, a result expected considering the trade-off between egg size and number, but was instead positively related to the water temperature (Fig. 2b). The influence of water temperature on relative fecundity remains an issue to be further investigated.

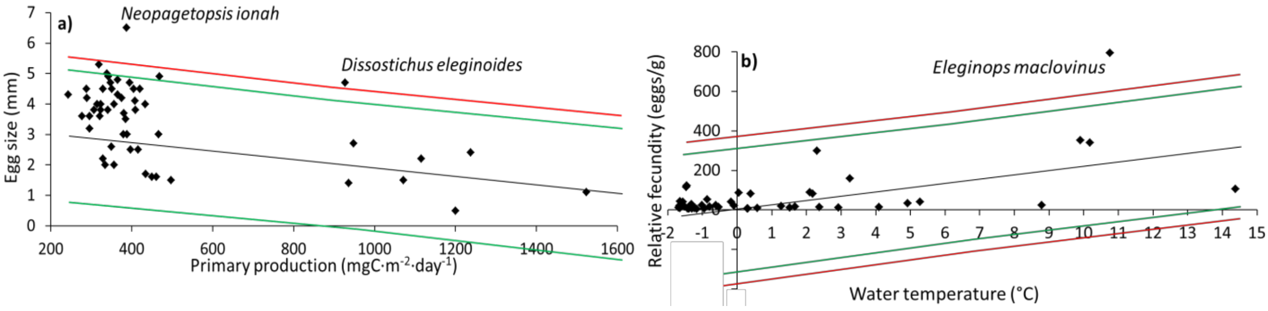

Figure 2. Linear regression of: **a)** egg size vs primary production; **b)** relative fecundity vs water temperature. Total least square regression (black line) and associated 90% (green lines) and 95% (red lines) confidence intervals have been corrected for phylogenetic relationships and mapped on the original data space. The names of the species presenting values of the life history traits outside the 95% confidence interval are reported.

The maximum female GSI, representing one component of the reproductive effort, showed a positive relationship with sea ice cover. Pack sea ice is a key factor for the primary production, the seasonal pack ice melting, trigging phytoplankton blooms, results in seasonally increased primary production. At higher latitudes the sea ice cover is almost permanent and the primary production is likely to remain low, being also influenced by the long and dark Antarctic winter. The relationship between the melting of pack ice and the seasonality of primary production may explain the observed relationship between GSI and mean sea ice cover. GSI estimates reported in this study are in agreement with the general patterns observed in Antarctic notothenioids, which attain higher values than in temperate species and an increasing trend towards higher latitudes. Higher GSI investment could be advantageous given the potentiality in increasing eggs/juveniles size and therefore survival rate.

To elucidate potential trend in strictly Antarctic notothenioids, the same trends were tested also excluding Sub-Antarctic species. The analyses produced the same results emerged from the total data set, with the exception of relative fecundity, positively related to maximum size. The consistency in the results strengthens the observed patterns, confirming the strong evolutionary pressure of environmental factors on life history traits.

In general, the observed trends in the different life history traits could be determined by other variables, different from those considered in this study but possibly related to them. In the case of a correlation between ecological and environmental factors, resulting trends could be a hint of a stronger relationship involving unmeasured ecological variables. Factors such as competition for resources or predation, that have been hypothesized to influence the evolution of life history traits, are often correlated with environmental variables, and their role in notothenioids remains to be investigated.

First rigorous evidences of the evolution of life history traits in notothenioids are provided in this study, shedding light on the major factors that could drive evolution in the Antarctic environment. In a more comprehensive framework, comprising other marine taxa, including invertebrates, and wider latitudinal range (implying a larger variation in environmental factors), more general statements on life history traits evolution could finally be traced.

The knowledge of life history traits of notothenioids is also crucial for conservation biology purposes, highlighting the high vulnerability of this taxon to potential impacts such as climate change and/or fishery. In fact the low fecundity and large egg size, the high reproductive effort in gonadal investment and in parental care, the low growth rate and the late sexual maturity in general, make this group of teleosts particularly sensitive to fishing. So notothenioids should be considered a very vulnerable taxon, especially at higher latitude where species vulnerability is likely to increase, and caution and conservation policies are recommended when operating in a climate change and potential fishery re-opening scenario.

GENERAL DISCUSSION

The present study shed light on the evolution of some life history traits in relation to environmental variables, using Notothenioidea Suborder as taxon model. The data used in the analyses include both original information and available literature ones. In particular, this study, applying the rigorous comparative method, highlights some of the factors possibly driving the evolutionary processes in the Southern Ocean (Paper VI). Notothenioids have proved to be an excellent model group for evolutionary studies (see also Eastman 1993, 2005). In the last 20-30 years the research on Southern Ocean fishes has made considerable progresses gaining new knowledge on the mechanisms underlying the life history strategies achieved by notothenioids to successfully cope with the cold and the high seasonality of their environment. However, the reproductive biology of many species is still poorly known and, very often, the available data are referred to few specimens, potentially biasing the information on life history traits. In the present research, I investigated the reproductive biology of 17 species (Papers I, II, III, IV, V, VI), including specimens of the same species sampled in different areas, allowing intraspecific comparisons (Papers I, III and IV). Unpublished data of species life history traits, belonging the eight notothenioid families, were used in the interspecific comparison (Paper VI), starting from sampled species collected during 2 cruises carried out onboard the German *R/V Polarstern* (ANT-XXVIII/4, 2012 and ANT-XXIX/9, 2014) off the Antarctic Peninsula and in the Weddell Sea (Lucassen 2012; Knust and Schröder 2014). Further samples, belonging to 4 notothenioid species, were provided by partner scientists during past Antarctic expeditions (2009, 2010 and 2011 during the austral summer) in different areas (i.e. South Orkney, South Georgia, Burdwood Bank and Western Antarctic Peninsula) (Jones and Kock 2009; Knust *et al*. 2012).

The comparisons at intraspecific level in three species highlighted differences that may reflect the influence of environmental factor variability at local levels, on reproductive traits and on particular life history aspects (Papers I, III and IV). For instance, the spatial differences of absolute fecundity estimated for *Chaenocephalus aceratus*, a benthic icefish with demersal eggs and pelagic larvae, are likely linked to local differences in environmental conditions (Paper III). In particular, the colder water masses and lower food availability characterizing the northern site, South Orkney, may explain the lower values of absolute fecundity recorded there. Thus the initial unexpected latitudinal trend (lower fecundity at lower latitudes), has turned in an expected result when detailed local environmental conditions (lower fecundities at lower temperature/lower food availability). This result is in line with those emerged in the comparison at interspecific level (see further discussion). Moreover, other aspects of the life history of this species, such as sedentary habits and long parental care, might contribute to a partial reproductive isolation between neighbouring populations (~260 nautical miles - NM - in this case). By contrast, geographic distance (~ 700 NM) does not seem to lead to reproductive isolation in *Notothenia rossii*, one of the few notothenioids with pelagic eggs and a relatively long larval period, both promoting long-distance dispersal (Paper IV). In the Antarctic silverfish *Pleuragramma antarctica*, the comparison between Marguerite Bay and Charcot Island populations (~260 NM apart) showed no differences in fecundity, while gonadosomatic index (GSI) and oocyte size were significantly lower in fishes from Charcot Island, suggesting a slight temporal shift in female gonad maturation between the two areas. In general, the intraspecific variation in life history traits observed in several species showed a temporal shift in reproductive period, with variability in gametogenesis processes, suggesting a potential, at least partial, reproductive isolation. The intraspecific results highlight the role of local environmental conditions and of some life history traits in influencing the connectivity among populations inhabiting different areas.

The interspecific comparative analyses highlighted some correlation between environmental factors and life history traits, revealing potential evolutionary forces. Notothenioids' maximum size was the only biological trait not correlated to any environmental variable, also when only Antarctic notothenioids (i.e. excluding Sub-Antarctic species) were considered. The patterns, causes, and consequences of geographic variation in body size are fundamental themes in the literature on life history evolution (Roff 2002). The so called Bergmann's rule, stating that larger specimens are found at higher latitudes, was firstly postulated for warm-blooded animals, as the internal heat conservation was suggested to be the underlying mechanism (Bergmann, 1847). Because latitude provides a reasonable proxy of temperature (Blackburn *et al*. 1999), Bergmann's rule has been commonly discussed as a relationship between body size and both temperature and latitude. Several studies on endotherms and ectotherms, including fish (Fisher *et al.* 2010), have questioned the generality of both the pattern and mechanisms of Bergmann's rule (reviewed by Blackburn *et al.* 1999; Meiri and Thomas 2007; Olson *et al*. 2009), pointing out other possible drivers to body-size variation such as food availability (Huston and Wolverton 2011). Despite several researches, both the geographic patterns of body-size variation and their underlying mechanisms remain controversial (McNab 1971; Geist 1987; Cousins 1989; Blackburn *et al*. 1999; Meiri and Thomas 2007) and, to my knowledge, investigations in Antarctic area were still lacking, due to the paucity and unreliability of existing data, at least on fish (Mora *et al*. 2008; Fisher *et al*. 2010). However it was proposed an opposite trend (Huston and Wolverton 2011). This study suggests that general rules on body-size variation do not persist in the Antarctic area, when rigorously controlling for phylogeny. However using raw data, body size correlated to temperature variation (Δt), showed a tendency with water temperature, and was negatively related to sea ice cover. Despite these results emerged exclusively considering raw data (i.e. not controlling for phylogeny), a general trend in size decreasing toward the Pole seems to be suggested, with small sized species abundant at high latitudes and very large sized species, such as *Dissostichus eleginoides*, occurring in Sub-Antarctic areas. The loss of significance if these relationships when considering the phylogenetic relationships might be related to the occurrence of several small-sized species occurring in the High Antarctic that are phylogenetically closely related.

Notothenioids' egg size was negatively related to primary production and, therefore, positively to latitude, as described from theoretical models and relevant studies (Mileykovsky 1971; Alekseev 1981; Levin and Bridges 2001; Laptikhovsky 2006). Egg size was also related to the maximum parental body size, however as already stated, large fishes are not more likely to have large eggs than small fish (Miller 1984; Hislop 1984; Duarte and Alcaraz 1989), and in notothenioids several small-size species present higher values than predicted.

Temperature influences the yolk composition and accumulation pattern, changing the amount of nutritional sources available for embryonic development (Laptikhovsky 2006) and, in many notothenioids, for early larval growth (Kellermann 1990). The increasing in egg size toward latitude was put in negative relationship to temperature (Rass 1941, 1986) and larval mortality, with a gradual shift from complex food webs with high predator diversity in tropical seas to simpler food webs in seasonal seas and in deep waters (Alekseev 1981). However in this study no relationship between egg size and temperature emerged.

Taking into account the trade-off between egg size and number, a positive relationship between relative fecundity and primary production was expected. However this relationship was not found and relative fecundity results to increase with water temperature. The influence of water temperature on relative fecundity therefore remains an aspect to be further investigated. To corroborate the poleward increasing in reproductive effort, as expected, maximum female GSI, representing one component of the reproductive effort, showed a positive relationship to sea ice cover and in turn negative to latitude. Obviously sea-ice cover is higher at higher latitude, and the influence of pack ice on primary production is an aspect to take in consideration.

The analyses on a narrower latitudinal range considering only Antarctic notothenioids (i.e. excluding Sub-Antarctic species distributed also along South America and South-East Australia), gave the same results emerged from the total data set, with the exception of relative fecundity, positively related to maximum size, underlining the influence of fish-size in eggs production. Therefore, the consistency in the results, strengthens the observed patterns, confirming the strong evolutionary pressure of environmental factors on life history traits.

This study represents, to my knowledge, the first attempts to examine the largescale interspecific variation in life history traits of fish species from Southern Ocean and Sub-Antarctic areas, in relation to environmental variables and controlling for phylogeny. Overall, the results of this study 1) extend the current knowledge of notothenioids' life history traits, providing original information and enhancing the reliability of the existing ones, and 2) allow the deciphering of some latitudinal and geographical trends linked to the environmental variables (at local and broader scale) both within and among species. The unveiling of such relationships is an important issue in evolutionary biology in order to hypothesize the drivers of evolution acting on organisms. The observed patterns in life history traits deserve further investigations in polar regions, accounting for more species and increasing the life history traits investigated to include, for instance, size and age at sexual maturity, growth rate and the influence of parental care occurrence, and taking into account local and seasonal environmental factors. Moreover other marine taxa could be added, including invertebrates. In a more comprehensive framework, comprising also a wider latitudinal range (implying a larger variation in environmental factors), more general statements on life history traits evolution could finally be traced. Nonetheless, even if focused on notothenioids, the wide breath of the present study is also due to the wide latitudinal range covered (mean species distribution, 38.5°÷73° S; min-max 33°÷79° S), making the observed trends likely applicable, or at least expected, in other areas or organisms of the same area.

Antarctica is a unique environment in its climatic and hydrographical conditions and the knowledge of organisms' adaptations in such extreme context is still an interesting topic in an evolutionary context. The present results are also crucial for conservation biology purposes. Low fecundity, large egg size, high reproductive effort in gonadal investment and, in some cases, parental care, low growth rate and late sexual maturity in general, depict notothenioids as a highly vulnerable taxon in a climate change and fishery re-opening scenario. Notothenioids should be considered a very vulnerable taxon, especially at higher latitude where vulnerability seems to increase, so caution and conservation policies are strongly recommended. Furthermore the knowledge of the relationship between life histories and environmental variables, may permit to better understand organisms’ vulnerability and even to predict it, starting from the knowledge of abiotic factors.

REFERENCES

Alekseev F.E. (1981). Rass-Thorson - Marshall rule and biological structure of marine communities. In: G.G. Vinberg. 4th Congress of All-Union Hydrobiological Society. Theses of reports. Part I. Naukova Dumka. Kiev 4-6.

Bergmann C. (1847). Über die Verhältnisse der Wärmeö-konomie der Thiere zu ihrer Grösse. Gottinger Studien 3: 595-708.

Blackburn T.M., Gaston K.J., Loder N. (1999). Geographic gradients in body size: a clarification of Bergmann's rule. Diversity and Distributions 5: 165-174.

Calì F., Riginella E., La Mesa M., Mazzoldi C. Life history strategies of *Notothenia rossii* and *N. coriiceps* along the Southern Scotia Arc. Paper.

Colombo M., Damerau M., Hanel R., Salzburger W., Matschiner M. (2015). Diversity and disparity through time in the adaptive radiation of Antarctic notothenioid fishes. Journal of Evolutionary Biology 28: 376-394.

Duarte C.M., Alcaraz M. (1989). To produce many small or few large eggs: a size-independent reproductive tactic of fish. Oecologia 80: 401-404.

Eastman J.T. (2005). The nature of the diversity of Antarctic fishes. Polar Biology 28: 93-107.

Eastman J.T. (1993). Antarctic fish biology: Evolution in a unique environment. Academic Press, San Diego, 322 pp.

Fisher J.A.D., Frank K.T., Leggett W.C. (2010). Global variation in marine fish body size and its role in biodiversity ecosystem functioning. Marine Ecology Progress Series 405: 1-13.

Geist V. (1987). Bergmann's rule is invalid. Canadian Journal of Zoology 65(4): 1035-1038.

Hislop J.R.G. (1984). A comparison of the reproductive tactics and strategies of cod, haddock, whiting and Norway pout in the North sea. In: Potts G.W., Wootton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, London 311-329 pp.

Huston M.A., Wolverton S. (2011). Regulation of animal size by eNPP, Bergmann's rule, and related phenomena. Ecological Monographs 81(3): 349-405.

Jones C.D., Kock K.H. (2009). Standing stock, spatial distribution, and biological features of demersal finfish from the 2009 US AMLR bottom trawl survey of the South Orkney Islands (Subarea 48.2). Document WG-FSA-09/19, CCAMLR, Hobart, Australia.

Kellermann A. (1990). Identification key and catalogue of larval Antarctic fishes. Ber. Polarforsch (eds). 67: 1-136.

Knust R., Gerdes D., Mintenbeck K. (2012). The expedition of the research vessel "Polarstern" to the Antarctic in 2011 (ANT-XXVII/3) (CAMBIO). Berichte zur Polar und Meeresforschung. 644: 1-202 pp.

Knust R., Schröder M. (2014). The Expedition PS82 of the Research Vessel POLARSTERN to the southern Weddell Sea in 2013/2014 (ANT-XXIX/9). Berichte zur Polar und Meeresforschung 680: 1-157 pp.

La Mesa M., Riginella E., Mazzoldi C., Ashford J. (2015). Reproductive resilience of ice-dependent Antarctic silverfish in a rapidly changing system along the western Antarctic Peninsula. Marine Ecology 36: 235-245.

La Mesa M., Riginella E., Melli V., Bartolini F., Mazzoldi C. (2015). Biological traits of a sub-Antarctic nototheniid, *Patagonotothen ramsayi*, from the Burdwood Bank. Polar Biology 1-9. doi 10.1007/s00300-015-1663-6.

Laptikhovsky V. (2006). Latitudinal and bathymetric trends in egg size variation: a new look at Thorson's and Rass's rules. Marine Ecology 27: 7-14.

Levin L.A., Bridges T.S. (2001). Pattern and diversity in the reproduction and development. In: McEdward L. Ecology of Marine Invertebrate Larvae. CRC Press. London 2-48.

Lucassen M. (2012). The expedition of the research vessel "Polarstern" to the Antarctic in 2012 (ANT-XXVIII/4). Berichte zur Polar und Meeresforschung. 652:1-90 pp.

McNab B.K. (1971). On the ecological significance of Bergmann's rule. Ecology 52: 845-854.

Meneghesso C., Riginella E., La Mesa M., Donato F., Mazzoldi C. Unveiling the biology of two Antarctic Artedidraconidae species: reproduction and age-length relationship of *Artedidraco skottsbergi* and *Dolloidraco longedorsalis* from the Weddell Sea. Paper.

Meiri S., Thomas G.H. (2007). The geography of body size - challenges of the interspecific approach. Global Ecology and Biogeography 16(6): 689-693.

Miller P.J. (1984) The tokology of gobioid fishes. In: Potts G.W., Wooton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, Oxford 119-154.

Mileykovsky S.A. (1971). Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Marine Biology 10: 193-213.

Mora C., Tittensor D.P., Myers R.A. (2008). The completeness of taxonomic inventories for describing the global diversity and distribution of marine fishes. Prociding of the Royal Society of London B 275: 149-155.

Olson V.A., Davies R.G., Orme C.D.L., Thomas G.H., Meiri S., Blackburn T.M., Gaston K.J, Owens I.P.F, Bennett P.M. (2009). Global biogeography and ecology of body size in birds. Ecology Letters 12(3): 249-259.

Rass T.S. (1941). Geographic Parallelisms in Morphology and Development of Teleost Fish of Northern Seas. MOIP, Moscow (In Russian).

Rass T.S. (1986). Biogeographic rule of inverse relation between egg size and environmental temperature in poikilothermous animals. Trudy IOAN, 116:152-168. (In Russian).

Riginella E., La Mesa M., Mazzoldi C. Evolution of life history traits in Notothenioid fishes. Paper.

Riginella E., Mazzoldi C., Ashford J,. Jones C.D., Morgan C., La Mesa M. Life history strategies of the Scotia Sea icefish, *Chaenocephalus aceratus*, along the Southern Scotia Ridge. Paper.

Roff D.A. (2002). Life history evolution. Sinauer, Sunderland.

Rogers A.D., Johnston N.M., Murphy E.J., Clarke A. (2012). Antarctic ecosystems: an extreme environment in a changing world. John Wiley and Sons (eds.).

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VI. **Evolution of life history traits in Notothenioid fishes**
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PAPER I

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ORIGINAL ARTICLE

Reproductive resilience of ice-dependent Antarctic silverfish in a rapidly changing system along the Western Antarctic Peninsula

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Abstract

The Western Antarctic Peninsula (wAP) is globally one of the systems most heavily impacted by climate change, notably steep declines in sea ice extent. In forage species, reproductive resilience to change is particularly important because population fluctuations are rapidly communicated through the system via trophic interactions. The reproductive traits of the ice-dependent forage species Antarctic silverfish (*Pleuragramma antarctica*) from different areas along the wAP and at the tip of the Antarctic Peninsula were investigated through macroscopic and histological analyses of gonads, with the aim to assess its reproductive potential and to test for spatial differences in fecundity and spawning season. Fish samples were collected in late summer off Charcot Island, in Marguerite Bay and off Joinville Island; no fish were caught in the central wAP. Samples from Charcot Island and Marguerite Bay consisted of adults in developing gonad stage, whereas those from Joinville consisted almost exclusively of juveniles. Mean GSI was relatively low (2–3%) and similar in both sexes, as specimens were still far from being actively reproducing. Developing females exhibited two discrete, though partially overlapping modes of oocytes of different size, with vitellogenic oocytes measuring 0.5–1.0 mm. Absolute and relative fecundity ranged between 3000 and 12,000 eggs per female and between 80 and 190 eggs$\cdot$g^{-1}, with a strong relationship between absolute fecundity and body size. These results were consistent with a single population at Charcot Island and Marguerite Bay and indicated substantial reproductive potential, which may mitigate population isolation and reductions in habitat availability but cannot ultimately offset catastrophic loss of spawning habitat linked to sea-ice retreat.

Introduction

Studies examining climate-induced effects on the vital rates of individual populations are rare, yet recent evidence of strong interactions between life history connectivity and circulation (*e.g.* Hanchet *et al.* 2008; Ashford *et al.* 2010, 2012) suggest that variation in population processes is closely linked to ocean structure. As a result, geographic distributions of fish species experiencing rapid change in the marine environment are likely to reflect population boundaries and connectivity. However, abundances will also depend on the reproductive resilience of individual populations and their capacity to offset increases in mortality. Estimating fecundity, and understanding the reproductive potential over the life history, is therefore a critical part of assessing how individual populations will respond to systemic change.

In the case of forage species, reproductive resilience is particularly important because variations in abundance and distribution are rapidly communicated upward through the system via trophic interactions. The Antarctic silverfish (*Pleuragramma antarctica*) is a widely distributed, high latitude species found between the continental margin and the shelf break around the Antarctic (Dewitt *et al.* 1990). It is overwhelmingly the most abundant pelagic fish on the continental shelves of the Ross Sea and Weddell Sea, representing more than 90% the total ichthyoplankton (Hubold 1985; Guglielmo *et al.* 1998). Considered a high-Antarctic species (*sensu* Kock 1992), it is also found in the seasonal pack-ice zone, such as the shelf waters of the Western Antarctic Peninsula (wAP) (Lancraft *et al.* 2004; Donnelly & Torres 2008), and plays a fundamental role in the Antarctic food web as an important food resource for high-level predators such as seals, penguins and whales, as well as for other notothenioid fishes. It is characterized by a considerable feeding plasticity, taking advantage of biomass peaks of either small (copepods) or large (krill) zooplankton throughout its life span (Hubold & Ekau 1990).

Along the wAP, the continental shelf hosts a biologically rich community, in which silverfish and Antarctic krill (*Euphausia superba*) sustain a number of higher trophic levels in the local food web (Lancraft *et al.* 2004; Friedlander *et al.* 2011). The wAP shelf region has been the subject of an extensive field programmes (such as the Palmer Station Long-Term Ecological Research and the Southern Ocean Global Ecosystem Dynamics), aimed at understanding the dominant physical and biological processes (Smith *et al.* 1995; Hofmann *et al.* 2004 and references therein). Globally, the wAP is currently one of the most heavily impacted regions by climate change, with the fastest rate of warming water and declining sea ice around Antarctica (Clarke *et al.* 2007). Changes in the maximum extent of sea ice in winter and timing of its advance and retreat potentially influence fish populations living in both coastal and oceanic systems; moreover, the most critical changes are expected to be those that affect the most vulnerable periods in the life history, such as the early life stages (Moline *et al.* 2008).

Silverfish need sea ice for spawning, egg deposition and feeding grounds for newly hatched larvae (Vacchi *et al.* 2004). The occurrence of dense spawning aggregations has been suggested by the observation of thousands of individuals under land-fast sea ice along the Antarctic Peninsula between June and October (Daniels & Lipps 1982), and eggs of *P. antarctica* were consistently found floating in the platelet ice underneath thick sea ice in Terra Nova Bay in the Ross Sea until larval hatching in mid-November (Vacchi *et al.* 2004).

Mitochondrial DNA sequencing indicated a weak population structure around the Antarctic Continent, characterized by a small but highly significant differentiation between samples along with a lack of association between clades and geographical locations (Zane *et al.* 2006; La Mesa & Eastman 2012). More recent data based on stable isotope and trace element analyses performed on otoliths revealed strong heterogeneity between the northern tip of the Antarctic Peninsula (AP) near Joinville Island and the southern wAP in Marguerite Bay and near Charcot Island, suggesting that *P. antarctica* are distributed in independent, discrete populations (Ferguson *et al.* 2011; Ferguson 2012). This is consistent with the different water masses characterizing the northern tip of the AP and the southern wAP, respectively from the Weddell Sea and Bellingshausen Sea origin (Loeb *et al.* 1993). Nevertheless, the otolith chemistry, when coupled with particle simulations, suggested a single population in the southern wAP, with transport by the Antarctic Peninsula Coastal Current (APCC) (Moffat *et al.* 2008) from Marguerite Bay to Charcot Island during the first 2 years of life (Ferguson *et al.* 2011). As a result, spatial segregation and the southward reduction in sea ice extension along the wAP might increase the risk of extinction of local populations through mortality of ice-dependent early life stages (Torres *et al.* 2010).

However, potential future impacts of climate change on silverfish biology also depend on their resilience to the risk of extinction in the case of climate-linked increases in mortality and changes in habitat availability. Key information on fecundity and reproductive biology are critical to assess the reproductive potential of individual populations over their life history. In particular, the estimate of length or age at first sexual maturity with respect to the maximum size or life span and the relationship between absolute fecundity and fish size give more insights on the actual reproductive potential of the species and, hence, on its capacity of resilience as the potential for recovery from environmental disturbance.

Despite this, available data on the reproductive biology of *Pleuragramma antarctica* are rather scarce and fragmentary, geographically restricted (East Antarctica and Weddell Sea), and often based on the macroscopic appearance of gonads (La Mesa & Eastman 2012). Therefore, in the present study we examined the reproductive traits of *P. antarctica* collected in different locations along the wAP and at the tip of the AP through histological and macroscopic analyses of gonads, aiming (i) to geographically extend the knowledge of the reproductive biology of this species based on a more accurate methodological approach, (ii) to test whether populations from different areas are characterized by differences in fecundity or spawning cycle and (iii) to assess the reproductive potential of the species in the face of a rapidly changing system along the wAP.

Material and Methods

A multiple opening/closing net and environmental sensing system (MOCNESS) was used on board the R/V *Nathaniel B. Palmer* to sample areas along the wAP off Charcot Island, in Marguerite Bay, south of Renaud Island, off Anvers Island and in Croker Passage, as well as in Antarctic Sound between Joinville Island and the tip of Antarctic Peninsula from late March to early May 2010 (Fig. 1). The sampling system consisted of six 10-m^2 nets (MOC-10) with a mesh size of 3 mm, each of them deployed at a specific depth interval (0–500, 500–300, 300–200, 200–100, 100–50, 50–0 m). Each trawl sequence lasted approximately 2 h. Overall, 48 tows were undertaken, of which 18 were off Charcot Island, 11 in Marguerite Bay and four in each of the other areas (only one in the Croker Passage).

Fish were measured to the nearest mm (total length, TL and standard length, SL) and sexed through direct examination of gonads. Gonad stage of maturity was macroscopically assessed according to a five-point scale for notothenioids (Kock & Kellermann 1991). Individual fish weight (TW) was estimated by applying the length–weight relationship for the species reported elsewhere (Kock *et al.* 1985). Whenever possible, gonads were excised, weighed to the nearest 0.01 g (GW) and the gonadosomatic index (GSI) calculated as percentage of gonad weight to total weight of fish.

Generally, the number of oocytes and size frequency distribution do not differ between left and right ovaries (Hunter *et al.* 1985); however, to ensure no bias, fecundity was estimated from a single randomly selected ovary from each female. Using the gravimetric method (Murua *et al.* 2003), the number of most advanced oocytes in a weighed subsample was counted and related to the weight of the entire ovary. A subsample of each ovary, representing 3–20% of the total gonad weight, was weighed and immersed in commercial sodium hypochlorite (10% in filtered seawater, Choy 1985) for 3 min to facilitate the disintegration of ovarian lamellae, and then immersed in filtered seawater in a Petri dish. Oocytes were spaced and photographed by a Leica DFC 420 videocamera to count and to measure the size of oocytes using IMAQ VISION BUILDER 6 software (National Instrument Corporation, Austin, TX, USA).

The number of oocytes taken from different ovary portions (anterior, median and posterior) was compared in six specimens to check any variation across the ovary. As no statistically significant differences among portions were observed (Friedman test for dependent data, $\chi^2 = 1.33$, $P = 0.513$, $df = 2$), fecundity was estimated from a randomly selected ovary portion from all other females.

On a subsample of 26 females, the size frequency distribution of all oocytes (previtellogenic and vitellogenic) in the gonad subsample was assessed by measuring the

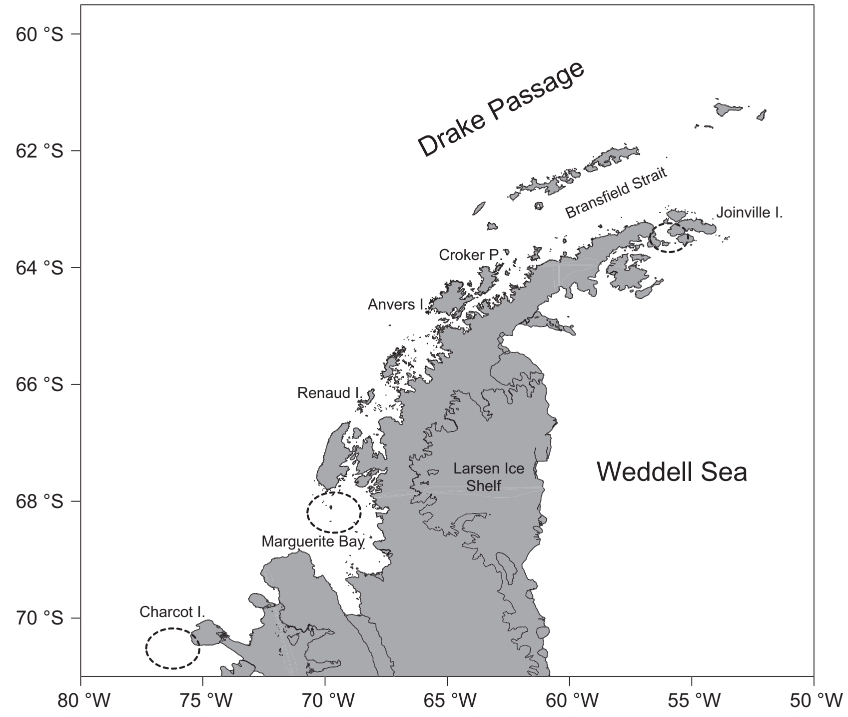

Fig. 1. Map of the Antarctic Peninsula, showing the sampling areas of *Pleuragramma antarctica* located off Charcot Island, in Marguerite Bay and south of Joinville Island.

average diameter. Based on the size frequency distribution and the size of vitellogenic oocytes (both endogenous and exogenous), a threshold size of 400 μm was set to estimate fecundity in order to exclude previtellogenic oocytes. Absolute fecundity (F_{ij}), the number of vitellogenic oocytes per female j in area i, was then estimated as:

$$F_{ij} = n_{ij} \times GW_{ij}/w_{ij},$$

where n is the number of vitellogenic oocytes in the subsample, w is the weight of the subsample and GW is the gonad weight. Relative fecundity (F_{rel}) was calculated as the number of vitellogenic oocytes per gram of fish body weight (TW).

The relationship between fish size (TL, mm) and absolute fecundity (F) was investigated using linear regression analysis. Data for both fish size and absolute fecundity were $\log_e$-transformed to fulfil assumptions. Normality and homogeneity of variances were tested using the Shapiro–Wilk test and the F-ratio test, respectively. No relationship was found between F_{rel} or size of vitellogenic oocytes and fish size. Because few adult specimens were available from Joinville Island, comparisons were made exclusively between Charcot Island and Marguerite Bay. As a result, differences in absolute fecundity between geographic areas were tested using the analysis of covariance (ANCOVA, test for homogeneity of regression slopes), with female size as covariate and areas as fixed factors. All statistical analyses were performed using STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA) software.

Histological analyses were carried out on both sexes to evaluate the stage of gonad development and to validate the macroscopical stage attribution. Particular care was taken to record the presence of postovulatory follicles (POF, indicating the occurrence of recent spawning event) and atretic oocytes. Gonad samples were dehydrated through increasing concentrations of ethanol solution, embedded in paraplast, cut in a series of transverse sections (7 μm) and mounted on slides. Sections were stained with Harrys' Haematoxylin and Eosin (Pearse 1985) and examined with a Leica DM LB light microscope at 100–630× magnification. Based on histological appearance and cell structure (West 1990), ovarian follicles were classified in the following developmental stages: I – chromatin nucleolar (immature); II – perinucleolar (immature); III – yolk vesicle or cortical alveoli formation (early maturation, endogenous vitellogenesis); IV – vitellogenic (late maturation, exogenous vitellogenesis); V – ripe (mature); VI – postovulatory follicles (post-reproductive). Each individual was staged considering the most advanced histological stage observed in ovarian sections, as notothenioids typically exhibit group synchronous ovaries (*sensu* Wallace & Selman 1990). Care was taken in comparing the size of oocytes at different stages of maturity between macroscopic and histological procedures, owing to the considerable shrinkage of oocytes observed after dehydration and embedding in paraplast. Based on the presence of different types of gametocytes in the seminiferous lobules (Billard 1986), male testes were classified in the following developmental stages: I – spermatogonia and spermatogonial mitoses (immature); II – first meiotic division (early maturation), spermatocytes I; III – second meiotic division (advanced maturation), spermatocytes II and spermatids; IV – spermatozoa cysts (mature); V – residual spermatozoa in collapsed lobules (post-reproductive).

Results

Fish samples

Pleuragramma antarctica were only caught off Charcot Island, in Marguerite Bay and south of Joinville Island. Off Charcot Island, gonad samples consisted of 15 males of 163–196 mm TL and 41 females of 152–225 mm TL (Fig. 2). All specimens were developing (stage 3 of macroscopic scale of gonad maturity). In Marguerite Bay, gonad samples consisted only of 48 developing females ranging from 124 to 233 mm TL (Fig. 2). Off Joinville Island, only four adult fish were collected, and these were all females ranging from 148 to 230 mm TL; two were maturing virgin (stage 2) and two were developing (stage 3). All other fishes (n = 7) were unsexed juveniles ranging from 103 to 144 mm TL, at stage 1 of gonad maturity (immature) (Fig. 2).

Macroscopic analysis

Gonadosomatic index values calculated for each sex and macroscopic stage of gonad maturity are summarized in Table 1. Mean GSI was significantly different between Charcot and Marguerite Bay (two-sample *t*-test, $t = 3.45$, df = 85, $P = 0.0008$). Mean GSI of developing males collected off Charcot was slightly higher than that of females (Table 1). The macroscopic analysis of ovaries in developing females revealed the presence of two discrete although partially overlapping modes of oocytes of different size (Fig. 3), confirming the group synchronous oocyte development in this species. Based on histological analysis (see below), the first mode consisted of smaller previtellogenic oocytes and the second of relatively larger vitellogenic oocytes at yolk vesicle or yolk granule stages. The two modes were separated by a threshold size of approximately 400 μm (Fig. 3). Consistent with GSI, mean and maximum size of vitellogenic oocytes were both significantly different between the two sampling

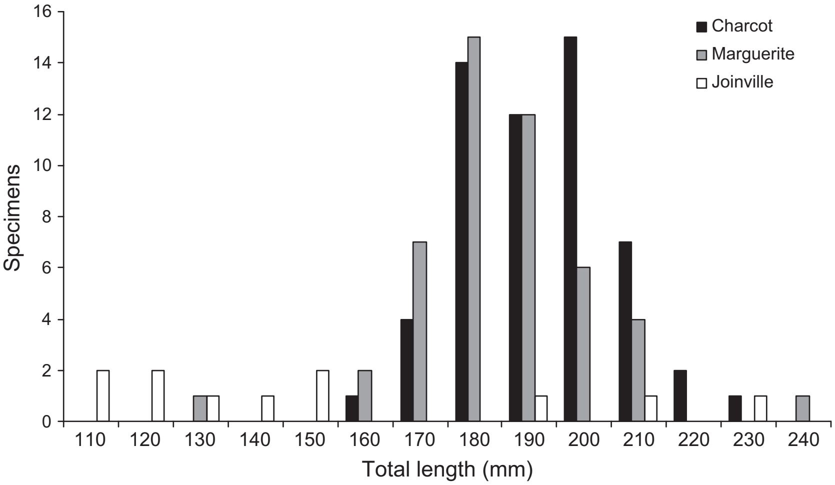

Fig. 2. Length-frequency distributions of *Pleuragramma antarctica* collected in each sampling area.

Table 1. Gonadosomatic index (GSI) of *Pleuragramma antarctica* collected along the Antarctic Peninsula in March–April.

Site	Sex	n	Maturity stage	GSI (%) range	GSI (%) mean ± SE
Charcot	♂	15	3	1.09–4.00	2.87 ± 0.20
Charcot	♀	41	3	1.48–3.46	2.25 ± 0.06
Marguerite	♀	46	3	1.54–4.35	2.62 ± 0.08
Joinville	♀	2	3	1.29–5.25	3.27 ± 1.98
Joinville	♀	1	2	0.05	–

areas (two-sample *t*-test = 4.10, df = 22, P = 0.0004; t = 4.16, df = 22, P = 0.0004, respectively), with samples from Marguerite Bay possessing slightly larger oocytes than samples from Charcot (Table 2). The two developing females from Joinville exhibited oocytes within the size range of samples from the other areas (Table 2).

Absolute and relative fecundity of developing females were estimated from the number of vitellogenic oocytes (Table 3). No significant differences in relative fecundity were found between females sampled off Charcot and those in Marguerite Bay (two-sample *t*-test; t = 0.11, df = 22, P = 0.91). Females sampled off Joinville showed absolute and relative fecundity within the values observed in the other areas (Table 3, Fig. 4). The relationships between absolute fecundity (F) and fish size (TL, mm) from Charcot and Marguerite Bay followed the exponential equations

Charcot $F = 0.000915\ TL^{2.99}$ $(n = 12, r^2 = 0.61)$
Marguerite Bay $F = 0.002181\ TL^{2.85}$ $(n = 12, r^2 = 0.80)$

Comparing the regression slopes of the above equations with female size as covariate and areas as fixed factors, absolute fecundity was positively related to fish size in both areas, with no significant difference between them (ANCOVA, Table 4).

Histological analysis

From a histological point of view, the different stages of development were very similar in the samples from the

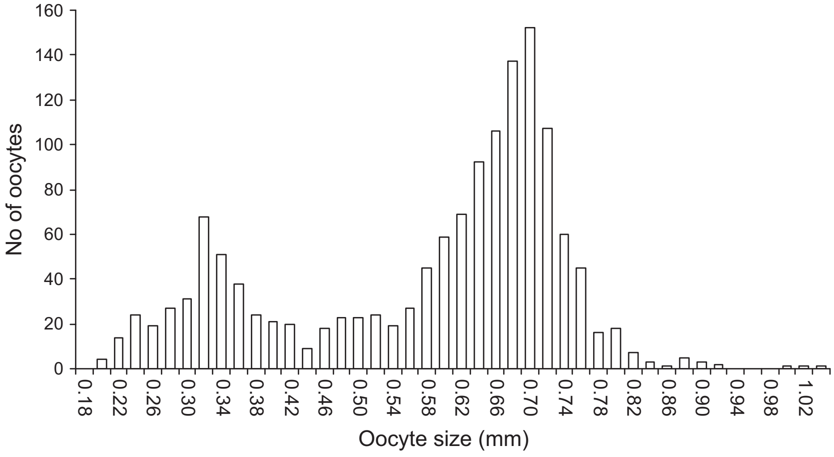

Fig. 3. Size frequency distribution of oocytes in ovaries of *Pleuragramma antarctica* developing female sampled along the wAP in March–April.

Table 2. Mean and maximum size of vitellogenic oocytes in developing females of *Pleuragramma antarctica* collected along the Antarctic Peninsula in March–April.

Site	n	Mean size (mm) range	Mean size (mm) ± SE	Max. size (mm) range	Max. size (mm) ± SE
Charcot	12	0.46–0.56	0.52 ± 0.01	0.61–0.81	0.68 ± 0.01
Marguerite	12	0.54–0.70	0.59 ± 0.02	0.68–1.00	0.81 ± 0.02
Joinville	2	0.48–0.69	0.58 ± 0.10	0.57–0.99	0.78 ± 0.21

Table 3. Absolute and relative fecundity in developing females of *Pleuragramma antarctica* collected along the Antarctic Peninsula in March–April.

Site	N	F (eggs per female) range	F (eggs per female) mean ± SE	F_{rel} (eggs per g) range	F_{rel} (eggs per g) mean ± SE
Charcot	12	2953–11,613	6818 ± 1968	78–193	136 ± 39
Marguerite	12	3637–11,492	6700 ± 1934	108–189	157 ± 45
Joinville	2	10,043–10,174	10,109 ± 7148	119–171	145 ± 103

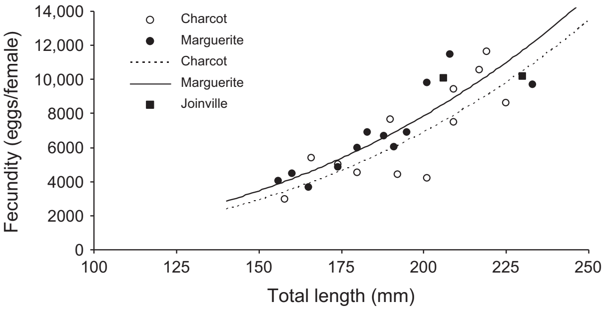

Fig. 4. Relationships between absolute fecundity and fish size in *Pleuragramma antarctica* developing females, showing experimental data and fitted exponential curves.

Table 4. ANCOVA table results (test for homogeneity of regression slopes), testing the relationships between absolute fecundity and fish size (TL) in different sites.

Parameter	Degree of freedom	Wald statistic	P
Site	1	0.098	0.753
$\log_e$ TL	1	59.73*	0.00000
Site * $\log_e$ TL	1	0.078	0.780

*indicates statistically significant value.

different areas, so that they are described regardless of the geographical origin. The analyses of ovaries in females (n = 18) revealed the presence of oocytes at different development stages, such as previtellogenic, cortical alveoli and vitellogenic. A single small maturing female showed small ovaries filled exclusively with previtellogenic oocytes of two different sizes (62 ± 14 and 142 ± 15 µm; histological stage II). The developing females (17) had relatively larger ovaries. Most of them (13) exhibited mainly oocytes at late cortical alveoli stage (367 ± 73 µm, histological stage III, Fig. 5a), whereas ovaries of all other females (n = 4) consisted of oocytes in exogenous vitellogenesis, completely filled with yolk granules (418 ± 32 µm, histological stage IV; Fig. 5c) and a few oocytes at an early cortical alveoli stage (143 ± 38 µm). Only two developing females showed oocytes under resorption through atretic processes (430 ± 51 µm, from histological sections; Fig. 5b). No post-ovulatory follicles were recorded.

Males were in two different stages of maturity. Four immature males (staged macroscopically unsexed juveniles) presented very small and compact testes, with only cysts of spermatogonia in the lobule epithelium (histological stage I). Lobule lumina were not distinguishable (Fig. 6a). Developing males (n = 5) showed testes with spermatocytes I in the lobule epithelium, and enlarged and empty lobule lumina (histological stage II), possible signs of a previous reproduction (Fig. 6b).

Discussion

Reproductive biology of Antarctic silverfish

Examining the gonads of Antarctic silverfish collected along the wAP and at the tip of the AP, we found two discrete modes of oocytes of different size in the ovaries of developing females (Fig. 3), confirming that oocyte development is group synchronous in this species. The first mode consisted of previtellogenic oocytes and the second of vitellogenic oocytes at yolk vesicle or yolk granule stages. Mean absolute fecundity was estimated at 6800 eggs per female sampled off Charcot Island and 6700 eggs per female in Marguerite Bay. However, there was a strong relationship with fish size: those larger than

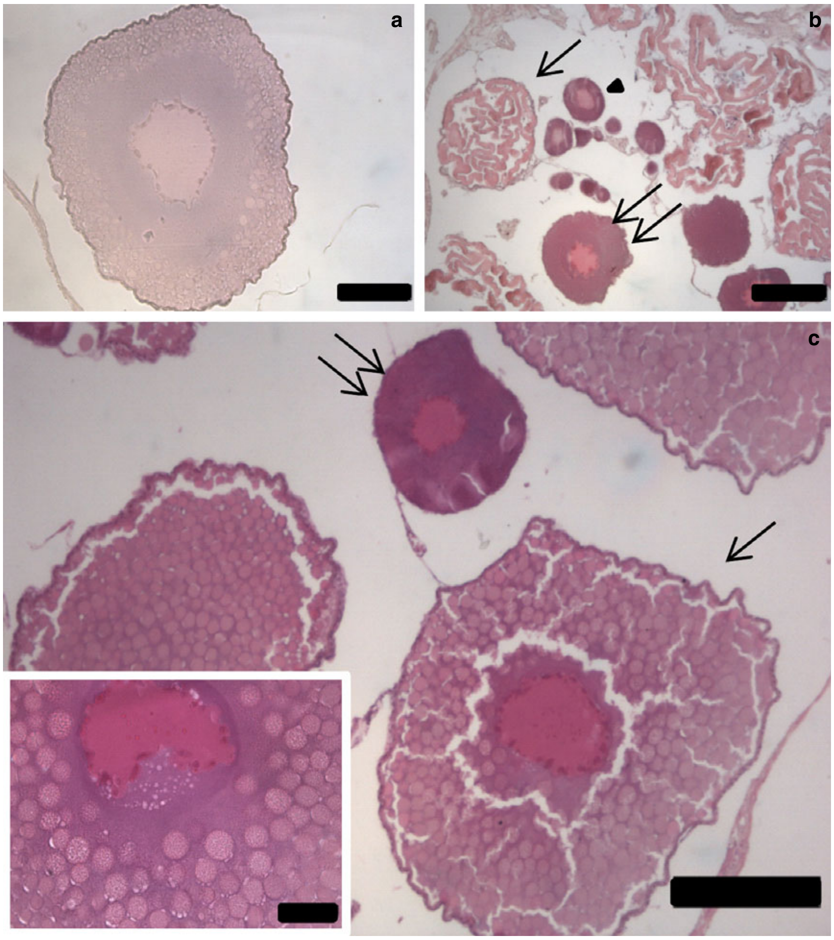

Fig. 5. Transverse histological sections of ovary of *Pleuragramma antarctica*. (a) Developing female, oocyte at late cortical alveoli stage, with unstained yolk vesicles in the peripheral cytoplasm. Female TL 158 mm, scale bar 100 μm. (b) Developing female showing mainly atretic oocytes (arrow) presenting convoluted chorion, oocytes at early cortical alveoli stage (double arrow) and at pre-vitellogenic stage (arrowhead). Female TL 201 mm, scale bar 200 μm. (c) Developing female with oocytes in the vitellogenic yolk stage, completely filled with yolk granules (arrow), and at early cortical alveoli stage (double arrow). Female TL 230 mm, scale bar 200 μm. Inset: enlargement of a vitellogenic oocyte with yolk granules. Scale bar 50 μm.

200 mm reached absolute fecundities larger than 10,000 eggs per female, compared with only 2000–6000 eggs per female in those smaller than 175 mm. In contrast, there was no relationship between fish length and size of eggs, estimated at 0.52 mm in fish sampled off Charcot Island and 0.59 mm in fish in Marguerite Bay. Mean absolute fecundity of 10,100 eggs per female off Joinville Island was consistent with the large size of the adult fish sampled there.

The knowledge of the Antarctic silverfish reproductive traits is still largely based on a few scattered data, geographically restricted to the Weddell Sea and Indian Ocean sector of the Southern Ocean (La Mesa & Eastman 2012). In this study, we were able to sample during late summer into early winter prior to ice formation, and extend the geographic limits to the wAP. Sampling from south to north of the Peninsula, we found no silverfish in historically abundant areas south of Renaud Island and off Anvers Island, and only one in Croker Passage, suggesting population fragmentation as a result of rapid change in the shelf system (Ferguson 2012). Nevertheless, we were able to capture fish from the southern wAP, in Marguerite Bay and off Charcot Island, and at the tip of the AP in Antarctic Sound that covered the range of life stages from juvenile to large adult.

Comparing the reproductive traits of *Plueragramma antarctica* from the wAP with other regions, the timing of gonad development and the reproductive effort represented by the gonadosomatic index (GSI) were consistent with a similar annual gonad development cycle around the Antarctic. Thus, in the Weddell Sea and in the Cosmonaut, Cooperation and Mawson Seas of Eastern Antarctica, most specimens collected in January–February were in their resting stage, with a mean GSI comprising approximately 1–2% in both sexes (Faleeva & Gerasimchuk 1990; Hubold 1992; Duhamel *et al.* 1993). At this stage of gonad maturity, two well defined groups of oocytes of different size were already distinguishable in females, with a threshold size of about 0.4 mm. The larger group consisted of oocytes starting the vitellogenic process (Faleeva & Gerasimchuk 1990), with a maximum size of 0.6 mm (Hubold 1992). In late summer (March–April) GSI started to increase rapidly in both sexes, attaining a mean value of 2.5–3.5% in populations from

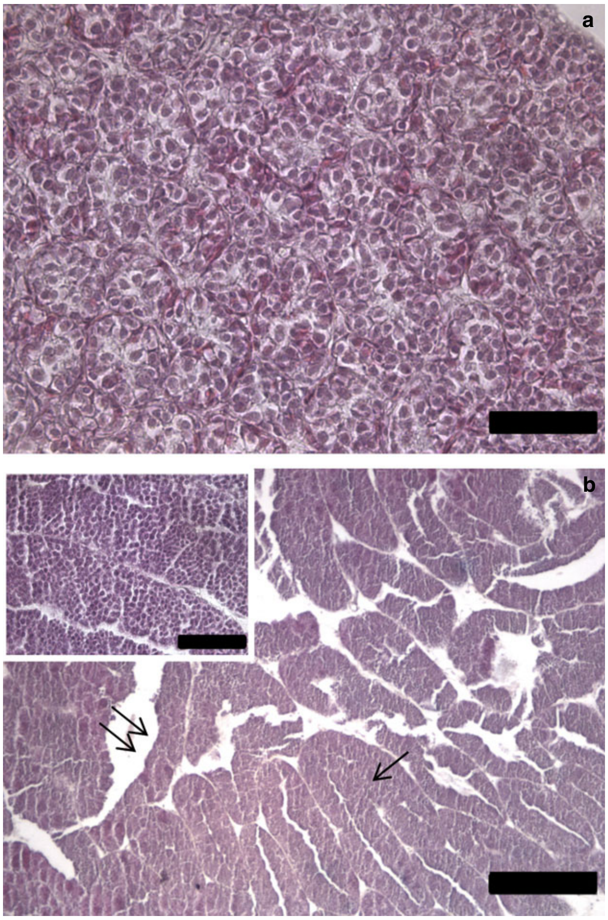

Fig. 6. Transverse histological sections of testis of *Pleuragramma antarctica*. (a) Immature male showing cysts of spermatogonia. Male TL 129 mm, scale bar 50 μm. (b) Developing testis, showing spermatocytes I in the lobule epithelium (arrow), and enlarged and empty lobule lumina (double arrow). Male TL 171 mm, scale bar 100 μm. Inset: enlargement of the developing testis with spermatocytes I. Scale bar 50 μm.

Eastern Antarctica and the Western Antarctic Peninsula. At the same time, vitellogenic oocytes showed a steady increase in size (up to about 1 mm), leading to a clear separation between vitellogenic and previtellogenic oocytes, which formed the batch to be spawned in the current season and the reserve stock, respectively (Faleeva & Gerasimchuk 1990; present data). GSI of males and females were also comparable at this stage of gonad development, and this has been tentatively considered an adaptation to pelagic spawning and egg release (Faleeva & Gerasimchuk 1990), as has been observed in the marbled rockcod, *Notothenia rossii* (Camus & Duhamel 1985; Kock & Kellermann 1991).

Histological analyses also showed similarities geographically, with those from the wAP closely resembling those reported from Eastern Antarctica (Faleeva & Gerasimchuk 1990). In late summer (March–April), adult specimens stopped the resting stage and showed an active gametogenesis, vitellogenic oocytes and primary spermatocytes being the most advanced gametocytes in females and males, respectively. According to Wallace & Selman (1990), at least two cohorts of oocytes can be distinguished in maturing ovaries, corresponding to a fairly synchronous population of larger oocytes to be spawned during the current breeding season, and a more heterogeneous population of smaller oocytes to be spawned in future breeding seasons. Two maturing females showed individual large oocytes at different stages of resorption, perhaps making up the fraction of unspawned eggs of the previous spawning season, as observed in the Eastern Antarctica (Faleeva & Gerasimchuk 1990; present data). Assuming that this species is a winter spawner (Kock & Kellermann 1991), ovarian follicle atresia is therefore a very slow, long-lasting process.

Population structure and reproductive potential

In contrast, fecundity estimates showed evidence of differentiation geographically. Populations from the Weddell Sea showed maximum values of 12,000–13,000 eggs per female and 190–200 eggs·g^{-1} (Hubold 1992), similar to our data, whereas absolute and relative fecundity estimates in the Mawson Sea were approximately 4000–18000 eggs per female and 70–160 egg·g^{-1}, respectively (Gerasimchuk 1987). Nevertheless, reproductive traits observed in fishes collected from Marguerite Bay and Charcot showed little evidence of differentiation: in particular, absolute and relative fecundity and its positive relationship with fish size were similar between the two areas, indicating similar parental investment in reproduction. Although significantly lower gonadosomatic index and egg size in fishes from Charcot Island suggested a slight temporal shift in female gonad maturation between the two areas, the difference was very small. Consequently, the effect was unlikely to have been biologically meaningful, especially as fish were still far from being in reproductive condition. As a result, we were unable to refute the hypothesis of a single southern wAP population (Ferguson 2012) based on the reproductive data.

Length–frequency distributions were also consistent with a single population (Ashford *et al.* 2010; Ferguson 2012). Thus, specimens caught in Marguerite Bay and near Charcot Island showed almost the same size range, consisting solely of adult fish 150–240 mm in size. In contrast, those sampled near Joinville Island contained only a few adult specimens, and consisted almost exclusively of juveniles smaller than 160 mm. Length at first maturity appeared therefore to be in the range of 150–160 mm, at approximately 65–70% of maximum size.

Converting to age using the von Bertalanffy curve published by Hubold & Tomo (1989), age-at-first maturity occurred at approximately 50% of maximum age, suggesting a significant delay in energy expenditure for reproduction. Moreover, although egg size remained constant, fecundity rose rapidly to reach 10,000–12,000 eggs per female after 200 mm length, highlighting the importance of larger, older fish in maintaining reproductive potential.

The demographic differences indicated that very different population constraints operate spatially. Thus, in the southern wAP, a large proportion (>30%) of fish were 200 mm or larger, consistent with substantial reproductive potential, but the lack of smaller fish suggested a failure in recent recruitment, potentially as a result of continuing sea-ice retreat. Conversely, at the tip of the AP, the large numbers of juveniles demonstrated recent recruitment, which could be partially explained by a longer persistence of sea ice in the Antarctic Sound than in the southern wAP. However, the low relative number of adults found in our sampling suggested that the mortality rate may be higher and reproductive potential consequently lower than in the southern wAP. Alternatively, connectivity may be important, with juveniles transported from spawning areas in the Western Weddell Sea.

Comparing reproductive investment with other high-Antarctic notothenioids, *Pleuragramma antarctica* exhibited the highest absolute fecundity except for the related Antarctic toothfish (*Dissostichus mawsoni*), which spawns up to 1 million eggs (Kock & Kellermann 1991). Even so, taking into account relative fecundity, *P. antarctica* is overwhelmingly the species with the highest maternal contribution, as a result of the trade-off between small body size and reproductive effort in terms of potential fecundity and egg size. Yet, Antarctic silverfish also currently appear to be one of the species most vulnerable to climate change (La Mesa & Eastman 2012). Several effects may be implicated. Geographical fragmentation and reproductive isolation implied by the lack of catches recorded from historically abundant areas near Renaud and Anvers Islands may have led to a sharp loss in connectivity along the wAP and, with it, the stabilizing effect of immigration as a subsidy to local self-recruitment (Pulliam 1988).

Inshore, the northward dispersal or transport of juvenile *Pleuragramma* from the Charcot Island/Marguerite Bay population is prevented for most of the year by the southward flow of the APCC, which is triggered by the melt water input in spring and persists until June, before the onset of the sea-ice formation. The flow is virtually absent in winter, rising again in early November at the sea-ice retreat, concomitantly with the hatching peak of *Pleuragramma* (Moffat *et al.* 2008; La Mesa & Eastman 2012). Offshore, Lagrangian particle simulations indicated a potential transport by the Antarctic Circumpolar Current (ACC) from Marguerite Bay north as far as Anvers Island, with trajectories further north passing along the outer shelf of South Shetlands away from the Northern Peninsula, consistent with the lack of mixing between the tip of the AP and southern wAP (Ferguson *et al.* 2011).

Sea ice melting and increases in seawater temperature may also reduce the duration of larval stages of *P. antarctica* (Vacchi *et al.* 2004; O'Connor *et al.* 2007), in turn reducing larval dispersal and connectivity. Most important, however, is their dependence on sea ice throughout their life history (La Mesa & Eastman 2012), and in particular the loss of critical spawning habitat due to sea-ice retreat. The substantial reproductive potential of silverfish may mitigate the effect of isolation and moderate reductions in habitat availability but it cannot ultimately offset catastrophic loss of spawning habitat in a rapidly changing system.

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References

Ashford J.R., La Mesa M., Fach B.A., Jones C., Everson I. (2010) Testing early life connectivity using otolith chemistry and particle-tracking simulations. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1303–1315.

Ashford J.R., Dinniman M., Brooks C., Andrews A.H., Hofmann E., Cailliet G., Jones C.D., Ramanna N. (2012) Does large-scale ocean circulation structure life history connectivity in Antarctic toothfish (*Dissostichus mawsoni*)? *Canadian Journal of Fisheries and Aquatic Sciences*, **69**, 1903–1919.

Billard R. (1986) Spermatogenesis and spermatology of some teleost fish species. *Reproduction, Nutrition, Development*, **26**, 877–920.

Camus P., Duhamel G. (1985) Ponte et développement embryonnaire de *Notothenia rossii rossii* (Richardson 1844). Nototheniidae des Iles Kerguelen. *Cybium*, **9**, 283–293.

Choy S.C. (1985) A rapid method for removing and counting eggs from fresh and preserved decapod crustaceans. *Aquaculture*, **48**, 369–372.

Clarke A., Murphy E.J., Meredith M.P., King J.C., Peck L.S., Barnes D.K.A., Smith R.C. (2007) Climate change and the marine ecosystem of the Western Antarctic Peninsula. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, **362**, 149–166.

Daniels R.A., Lipps J.H. (1982) Distribution and ecology of fishes of the Antarctic Peninsula. *Journal of Biogeography*, **9**, 1–9.

Dewitt H.H., Heemstra P.C., Gon O. (1990) Nototheniidae. In: Heemstra P.C. (Ed), *Gon O*. Fishes of the Southern Ocean. JLB Smith Institute of Ichthyology, Grahamstown: 279–331.

Donnelly J., Torres J.J. (2008) Pelagic fishes in the Marguerite Bay region of the West Antarctic Peninsula continental shelf. *Deep-Sea Research II*, **55**, 523–539.

Duhamel G., Kock K.H., Balguerias E., Hureau J.C. (1993) Reproduction in fish of the Weddell Sea. *Polar Biology*, **13**, 193–200.

Faleeva T.I., Gerasimchuk V.V. (1990) Features of reproduction in the Antarctic sidestripe, *Pleuragramma antarcticum* (Nototheniidae). *Journal of Ichthyology*, **30**, 67–79.

Ferguson J.W. (2012) Population structure and connectivity of an important pelagic forage fish in the antarctic ecosystem, *Pleuragramma antarcticum*, in relation to large scale circulation. CCAMLR, WG-FSA-12/23.

Ferguson J.W., Ashford J., Piñones A., Torres J., Fraser W., Jones C., Pinkerton M. (2011) Connectivity and population structure in *Pleuragramma antarcticum*. CCAMLR, WG-FSA-11/19.

Friedlander A.S., Johnston D.W., Fraser W.R., Burns J., Halpin P.N., Costa D.P. (2011) Ecological niche modeling of sympatric krill predators around Marguerite Bay, Western Antarctic Peninsula. *Deep-Sea Research II*, **58**, 1729–1740.

Gerasimchuk V.V. (1987) On the fecundity of the Antarctic silverfish *Pleuragramma antarcticum*. *Journal of Ichthyology*, **28**, 98–100.

Guglielmo L., Granata A., Greco S. (1998) Distribution and abundance of postlarval and juvenile *Pleuragramma antarcticum* (Pisces, Nototheniidae) off Terra Nova Bay (Ross Sea, Antarctica). *Polar Biology*, **19**, 37–51.

Hanchet S.M., Rickard G.J., Fenaughty J.M., Dunn A., Williams M.J. (2008) A hypothetical life cycle for Antarctic toothfish *Dissostichus mawsoni* in Antarctic waters of CCAMLR Statistical Area 88. *CCAMLR Science*, **15**, 35–54.

Hofmann E.E., Wiebe P.H., Costa D.P., Torres J.J. (2004) An overview of the southern ocean global ocean ecosystems dynamics program. *Deep-Sea Research II*, **51**, 1921–1924.

Hubold G. (1985) The early life history of the high-Antarctic silverfish, *Pleuragramma antarcticum*. In: Siegfried W.R., Condy P.R., LAWS R.M. (Eds). *Antarctic Nutrient Cycles and Food Webs*. Springer, Berlin: 445-451.

Hubold G. (1992) Ecology of Weddell sea fishes. *Berichte zur Polarforschung*, **103**, 1–157.

Hubold G., Ekau W. (1990) Feeding patterns of post-larval and juvenile Notothenioids in the southern Weddell Sea (Antarctica). *Polar Biology*, **10**, 255–260.

Hubold G., Tomo A.P. (1989) Age and growth of Antarctic silverfish *Pleuragramma antarcticum* Boulenger, 1902, from the southern Weddell Sea and Antarctic Peninsula. *Polar Biology*, **9**, 205–212.

Hunter J.R., Lo N.C.H., Leong R.J.H. (1985) Batch fecundity in multiple spawning fishes. *NOAA Technical Report NMFS*, **36**, 67–77.

Kock K.H. (1992) *Antarctic Fish and Fisheries*. Cambridge University Press, Cambridge: 359.

Kock K.H., Kellermann A. (1991) Reproduction in Antarctic notothenioid fish – a review. *Antarctic Science*, **3**, 125–150.

Kock K.H., Duhamel G., Hureau J.C. (1985) Biology and status of exploited Antarctic fish stocks: a review. *BIOMASS Scientific Series*, **6**, 1–143.

La Mesa M., Eastman J.T. (2012) Antarctic silverfish: life strategies of a key species in the high-Antarctic ecosystem. *Fish and Fisheries*, **13**, 241–266.

Lancraft T.M., Reisenbichler K.R., Robison B.H., Hopkins T.L., Torres J.J. (2004) A krill-dominatedmicronekton and macrozooplankton community in Croker Passage, Antarctica with an estimate of fish predation. *Deep-Sea Research II*, **51**, 2247–2260.

Loeb V., Kellermann A., Koubbi P., North A.W., White M.G. (1993) Antarctic larval fish assemblages: a review. *Bulletin of Marine Science*, **53**, 416–449.

Moffat C., Beardsley R.C., Owens B., Van Lipzigc N. (2008) A first description of the Antarctic Peninsula Coastal Current. *Deep-Sea Research II*, **55**, 277–293.

Moline M.A., Karnowski N.J., Brown Z., Divoki G.J., Frazer T.K., Jacoby C.A., Torres J.J., Fraser W.R. (2008) High latitude changes in ice dynamics and their impact on polar marine ecosystems. *Annals of the New York Academy of Sciences*, **1134**, 267–319.

Murua H., Kraus G., Saborido-Rey F., Witthames P.R., Thorsen A., Junquera S. (2003) Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. *Journal of Northwest Atlantic Fisheries Science*, **33**, 33–54.

O'Connor M.I., Bruno J.F., Gaines S.D., Halpern B.S., Lester S.E., Kinlan B.P., Weiss J.M. (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 1266–1271.

Pearse A.G.E. (1985) *Histochemistry, Theoretical and Applied Analytical Technology*. Churchill Livingstone, Edinburgh: 624.

Pulliam H.R. (1988) Sources, sinks and population dynamics. *The American Naturalist*, **132**, 652–661.

Smith R.C., Baker K.S., Fraser W.R., Hofmann E.E., Karl D.M., Klinck J.M., Quetin L.B., Prezelin B.B., Ross R.M., Trivelpiece W.Z., Vernet M. (1995) The Palmer LTER: a long-term ecological research program at Palmer Station, Antarctica. *Oceanography*, **8**, 77–86.

Torres J., Piñones A., Ashford J., Fraser W., Ferguson J.W. (2010) Connectivity and population structure in *Pleuragramma antarcticum* along the west Antarctic Peninsula. CCAMLR, WG-FSA-10/16.

Vacchi M., La Mesa M., Dalù M., Macdonald J. (2004) Early life stages in the life cycle of Antarctic silverfish, *Pleuragramma antarcticum* in Terra Nova Bay, Ross Sea. *Antarctic Science*, **16**, 299–305.

Wallace R.A., Selman K. (1990) Ultrastructural aspects of oogenesis and oocyte growth in fish and amphibians. *Journal of Electron Microscopy Techniques*, **16**, 175–201.

West G. (1990) Methods of assessing ovarian development in fishes: a review. *Australian Journal of Marine and Freshwater Research*, **41**, 199–222.

Zane L., Marcato S., Bargelloni L., Bortolotto E., Papetti C., Simonato M., Varotto V., Patarnello T. (2006) Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum*. *Molecular Ecology*, **15**, 4499–4511.

PAPER II

Polar Biol
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Biological traits of a sub-Antarctic nototheniid, *Patagonotothen ramsayi*, from the Burdwood Bank

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Abstract The rock cod *Patagonotothen ramsayi* (Regan 1913) is the most abundant species of the genus *Patagonotothen*, occurring along the Patagonian shelf. It plays an important role in the demersal food web both as prey and predator, showing an increasing importance for the local finfish and squid trawl fisheries. Age structure and the reproductive traits were investigated from the population inhabiting the eastern shelf of Burdwood Bank, which represents the southernmost area of its geographical distribution. Adult specimens of *P. ramsayi* were collected during bottom trawling carried out in the austral summer. The specimens were aged by otolith readings, and their reproductive characteristics were assessed by macroscopical and histological analyses. Age was similar between sexes, ranging from 4 to 7 and from 4 to 8 years in males and females of comparable size, respectively. GSI was relatively low in females (<1.5 %), as fish were sampled far from the reported spawning season (June–August). Females were all in the early developing stage (III) on the macroscopic maturity scale, with the most advanced oocytes being in early vitellogenesis. The oocyte size distribution was bi-modal, with two partially overlapping modes consisting of oocytes of a maximum size of 0.66 mm. A few large atretic oocytes (diameter > 1 mm) were found in three females. Absolute fecundity ranged from approximately 30 to 120 thousands of eggs. No relationship was found between female size and fecundity, probably due to the relatively narrow range of the investigated fish sizes. Males were in the spent (VII) or resting (II) stages.

Keywords Age structure · Reproduction · *Patagonotothen* · Subantarctic · Southern Ocean

Introduction

The genus *Patagonotothen* includes 14 species distributed almost exclusively in the Patagonian Region of South America (De Witt et al. 1990; Eastman and Eakin 2000). The rock cod, *Patagonotothen ramsayi* (Regan, 1913), is the most abundant species of the genus, inhabiting the outer Patagonian shelf and upper slope from 35°S to 55°S (Burdwood Bank) at depths between 50 and 960 m. Compared with other nototheniids, it is a medium-sized species, attaining 41 cm total length, with a benthopelagic mode of life.

Because of its abundance, *P. ramsayi* plays an important role in the demersal food web of the south-western Atlantic shelf, both as predator and prey. The rock cod is a near-bottom feeder, relying on a variety of prey which differ seasonally and with fish size (Laptikhovsky and Arkhipkin 2003). Diet includes mostly gelatinous and crustacean plankton, such as jellies or salps and copepods or hyperiid amphipods, but also benthic prey, such as ophiuroids, polychaetes and lobster krill. Occasionally, it also scavenges on fishing discards, especially squid (Ekau 1982;

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Laptikhovsky and Arkhipkin 2003; Laptikhovsky 2004; Padovani et al. 2012; Arkhipkin and Laptikhovsky 2013). In turn, the rock cod is the common prey of several large fishes, including hakes, toothfish, kingclip, redcod and rajids (Garcia de la Rosa et al. 1997; Koen Alonso et al. 2001; Arkhipkin et al. 2003; Brickle et al. 2003; Nyegaard et al. 2004), as well as of gentoo penguin (Clausen and Pütz 2003).

Since the 1980s, the rock cod has represented a significant portion of the bycatch of commercial fishing in the south-western Atlantic area conducted by Polish vessels, which targeted mainly southern blue whiting and squids (Sosiński and Janusz 2003). After the collapse of southern blue whiting in 2004–2007, the abundance of the rock cod increased considerably, with 20- to 30-fold increases in catches and CPUEs (Laptikhovsky et al. 2013). The rock cod is currently an important target species in the local finfish and squid trawl fisheries, yielding an annual catch of approximately 55–75 thousand tonnes around the Falkland Islands (Falkland Islands Government 2013).

The increasing importance of rock cod as fishing resource for vessels operating on the shelf around the Falkland Islands in the last decade has stimulated studies on several aspects of its biology, such as age and growth (Brickle et al. 2006a), reproduction (Brickle et al. 2006b; Arkhipkin et al. 2013), feeding and predation (Laptikhovsky and Arkhipkin 2003; Brickle et al. 2005; Arkhipkin and Laptikhovsky 2013; Laptikhovsky et al. 2013), as well as stock assessment (Winter et al. 2010). Nevertheless, the biological traits of this species have been studied mostly in the Patagonian shelf around the Falkland Islands, whereas few data are available from the population inhabiting the Burdwood Bank, which represents the southernmost area of its geographical distribution (Ekau 1982; Sosiński and Janusz 2003).

As part of the North Scotia Ridge, the Burdwood Bank is an undersea plateau delimited by the 200 m isobath and located at about 200 km south of the Falkland Islands. It is characterized by two main different cold water masses of subantarctic origin, the subantarctic surface water (SASW) and the subantarctic mode water (SAMW) (Smith et al. 2010), and by the northward flow of the subantarctic front (SAF) crossing the North Scotia Ridge through a 2000-m-deep gap located to the east of the Burdwood Bank (Arhan et al. 2002). The local fish community is dominated by typical low latitude species, such as *Micromesistius australis* (Gadidae) and the nototheniids *Patagonotothen guntheri*, *P. ramsayi* and *Dissostichus eleginoides* (Knust et al. 2012). Within the genus *Patagonotothen*, *P. tessellata* is the third more common species generally found on fishery grounds of the southern shelf of the Falkland Islands including the Burdwood Bank, while other species are found there occasionally (Laptikhovsky 2004).

Adult specimens of rock cod were collected during bottom trawling carried out in the austral summer (February 2011) on the eastern slope of Burdwood Bank, giving us the opportunity to provide more insights on some aspects of the biology of the local population. The present study provides data on the age structure, through the seasonal growth patterns in sagittal otoliths, as well as gonad stage of maturity and fecundity estimated through macroscopic and histological analyses.

Materials and methods

Rock cod samples were collected during the expedition ANT-XXVII/3 of the R/V *Polarstern* conducted in the Atlantic sector of the Southern Ocean between February and April 2011 (Knust et al. 2012). Fish were captured on 11 February 2011 in two close stations located on the eastern slope of Burdwood Bank (Fig. 1), at approximately 280 m depth. Sampling was made using a 130-ft bottom trawl with a codend mesh size of 10 mm, hauled for 25 min. Fish were sorted from the catches, identified to species level and measured to the nearest lower mm (total length, TL, and standard length, SL). Sex was determined through direct examination of gonads, and gonad stage of maturity was macroscopically assessed according to the FIFD eight-stage maturity scale (Brickle et al. 2006b). Individual fish weight (TW, g) was estimated using the length–weight relationship reported by the Falkland Islands Government Fisheries Department for 2011 (Falkland Islands Government 2012). Gonads were weighed to the nearest mg (GW), and the gonadosomatic index (GSI) was calculated as percentage of gonad weight to total weight of fish. To estimate fecundity and for histological analysis, gonad samples were fixed in 10 % seawater formaldehyde or Dietrich solutions, respectively.

To ensure no bias, the right or left ovary was randomly selected. Using the gravimetric method, fecundity was estimated as the product of gonad weight and oocyte density, which represented the number of oocytes per gram of ovarian tissue evaluated by counting the number of oocytes in a weighed subsample (Murua et al. 2003). Once separated by the ovarian lamellae, oocytes were placed in a Petri dish and photographed with a LEICA DFC 420 video camera. Pictures were then analysed using the IMAQ Vision Builder 6 software to recording number and size of oocytes. Three different portions of ovary (anterior, median and posterior) were sampled from six individuals, aiming to test for differences in oocyte density across the ovary. As differences were statistically significant (Friedman's test, $\chi^2 = 12$, $p = 0.002$, $df = 2$), with a decreasing trend from anterior to posterior portions, we used the median portion of the ovaries from the other females, taking tissue subsamples ranging from 1 to 2 % of total gonad weight.

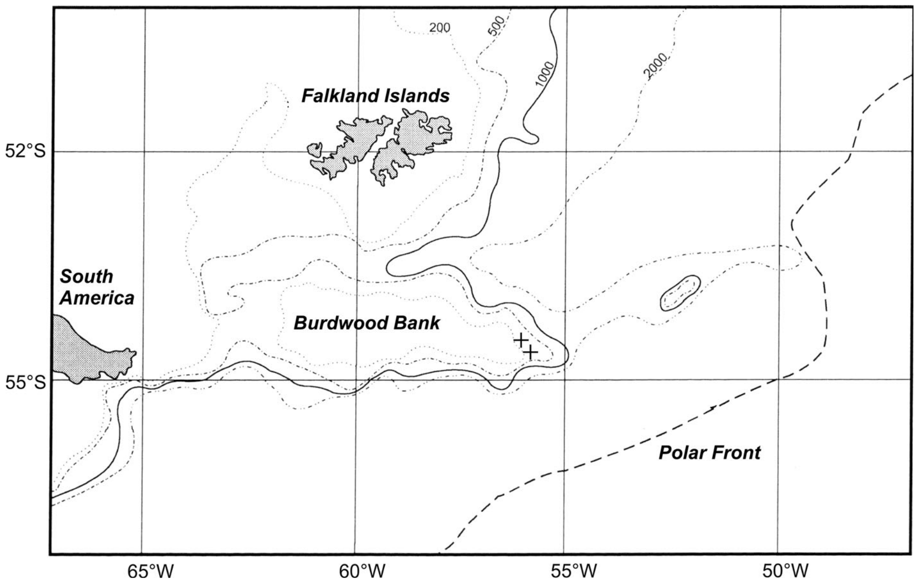

Fig. 1 Map of the southern Patagonian shelf, showing the Burdwood Bank and sampling sites of *Patagonotothen ramsayi* (*crosses*)

Based on the size frequency distribution of oocytes observed in the gonad subsamples and according to previous studies (Brickle et al. 2005, 2006b), oocytes larger than 150 µm were counted to estimate fecundity, assuming that they represented vitellogenic oocytes. Total fecundity (F_{tot}), defined as the standing stock of vitellogenic oocytes (Hunter et al. 1992), was estimated applying the following relationship:

$$F_{\mathrm{tot}} = (n/w)\ \mathrm{GW}$$

where n is the number of vitellogenic oocytes in the subsample, w is the weight of the subsample and GW is the gonad weight. Relative fecundity (F_{rel}) was then calculated as the number of vitellogenic oocytes per gram of body weight (TW). Based on previous study (Brickle et al. 2006b), we paid particular attention to evaluate the presence and amount of atretic oocytes in all females investigated, in order to assess as much as possible the realized fecundity corrected for atretic losses. In the present study, all females were all in the early developing stage (III), which previously represented the stage with higher percentage of atretic oocytes (up to 10 %) (Brickle et al. 2006b). However, the atretic process decreased with maturation (Brickle et al. 2006b), so that it is rather unlikely that we have greatly overestimated the total fecundity. In any case, such a limitation should be considered when comparing present and previous data. The relationship between fish size and total fecundity was assessed applying the least-square regression analysis to ln-transformed data. Normality and homogeneity of variances were tested by the Shapiro–Wilk and F-ratio tests.

The histological analyses were carried out on gonads from both sexes, in order to assess more accurately the stage of development complementing the macroscopic stage attribution. Gonadal tissues were dehydrated through a series of increasing alcohol concentrations, embedded in paraplast, sectioned at 7 µm and stained with Mayer's haematoxylin and eosin (Pearse 1985). The histological sections were examined under a light microscope (LEICA DM LB) at 100–630× magnifications. The ovaries and testes were assigned to five stages of development, based on histological appearance and cell structure (Brickle et al. 2005).

For each individual, sagittal otoliths were removed, rinsed in water and stored dry in vials. To reveal the growth ring pattern, otoliths were soaked in ethanol to enhance the contrast and examined whole on dark background with reflected light using a stereomicroscope at 25–40× magnification. Under reflected light, the nucleus and the opaque zones appeared as light rings and the translucent zones as dark rings (Fig. 2). The combination of each opaque and subsequent translucent zone was considered to form an annulus, based on the annual periodicity of growth ring deposition validated in the rock cod in a previous study (Brickle et al. 2006a). The count path was generally from the core towards the posterior margin of the otolith (Fig. 2). The whole set of otoliths was read twice, without any auxiliary information (blind reading). The ageing precision between readings was assessed by calculating the percentage agreement, the index of average percentage error (APE) (Beamish and Fournier 1981) and the mean coefficient of variation ($\mathrm{CV}_{\mathrm{mean}}$) (Chang 1982). Age difference and bias plot were computed to measure systematic difference between readings (Campana et al. 1995).

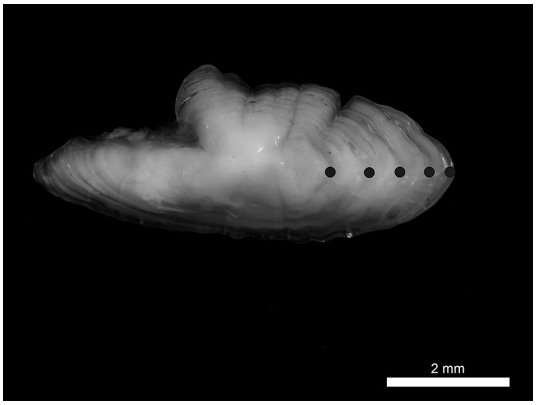

Fig. 2 Whole sagittal otolith of a 5-year-old *Patagonotothen ramsayi*, indicating the counting path and the translucent zones (*black dots*)

Results

Fish sample

The rock cod sample consisted of 109 adult fish ranging between 205 and 292 mm TL. The sex ratio of males and females was 1:0.87, not significantly different from 1:1 ($\chi^2 = 0.4$; $df = 1$; $p > 0.5$). The Kolmogorov–Smirnov test did not detect any significant difference ($p > 0.05$) in length frequency distribution (LFD) between sexes (Fig. 3). The estimated total body weight ranged from 86 to 272 g and from 97 to 278 g for males and females, respectively.

Age structure

The annulation pattern was generally evident in whole otoliths though with individual variability, allowing age determination in 106 individuals (97 % of the total sample). Edge analysis showed that most otoliths were laying down the opaque rings. Ageing data estimated for each sex were pooled together and summarized in the age–length key (Table 1). Age was similar between sexes, ranging from 4 to 7 and from 4 to 8 years in males and females of comparable size, respectively. Nevertheless, most of sampled population consisted of 5- to 6-year-old fishes (approximately 90 %). Concerning ageing precision, the index of average percentage error (APE) and the mean coefficient of variation (CV_{mean}) were both relatively low (1.3 and 1.9 %, respectively), indicating a good consistency between readings. The percentage agreement was approximately 87 %, with 13 % of age estimates differing by 1 year. The bias plot showed no systematic difference or bias between readings across the age classes (Fig. 4).

Maturity and reproduction

Based on the FIFD scale of gonad maturity, almost all females were in the early developing stage (stage III). A single small female was immature (stage I). GSI of developing females ranged between 0.33 and 1.59 % (mean ± SE, 0.72 ± 0.04 %). The macroscopic analysis of ovaries in developing females (30 individuals) showed the presence of two discrete, though partially overlapping, modes of oocytes of different sizes roughly separated by a threshold size of approximately 150 µm (Fig. 5), indicating the group synchronous type of oocyte development (sensu Wallace and Selman 1981) in this species. Based on histological analysis (see below), the first mode consisted of smaller previtellogenic oocytes and the second one of relatively larger oocytes at late cortical alveoli stage (endogenous vitellogenesis). The mean and maximum size of oocytes composing the second mode ranged between 217

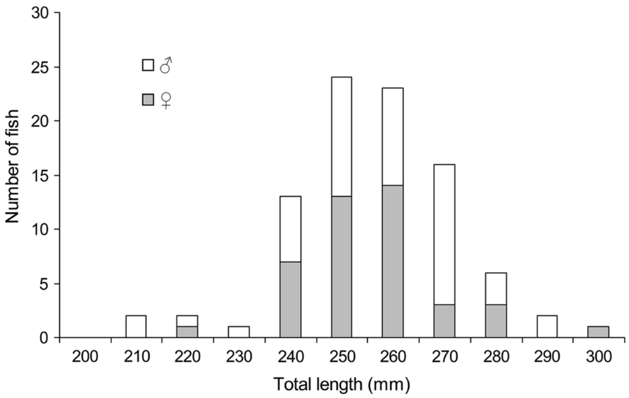

Fig. 3 Cumulated length frequency distribution of both sexes of *Patagonotothen ramsayi* sampled on the Burwood Bank in February 2011

Table 1 Age–length key of the rock cod from the Burwood Bank

Size TL (mm)	Age (years)				
	4	5	6	7	8
200	1				
210		1			
220	1	1			
230	1	11			
240	4	23	3		
250		30	3		
260		16	2		
270		1	4	1	
280			1		
290				1	1
n	7	83	13	2	1
Mean size at age (mm)	234	250	262	282	292

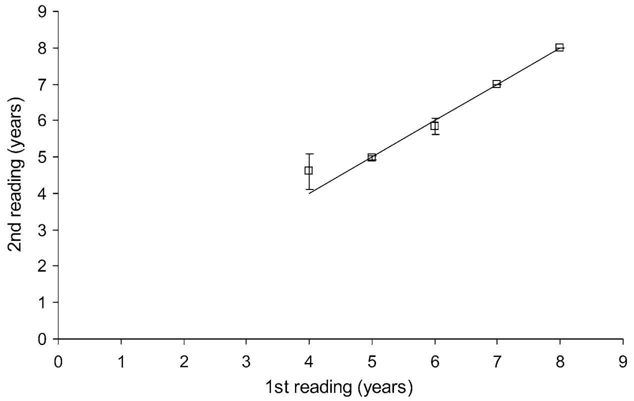

Fig. 4 Bias plot between readings, showing the mean age and 95 % confidence interval of second readings (*error bars*) for each age class assigned in the first reading

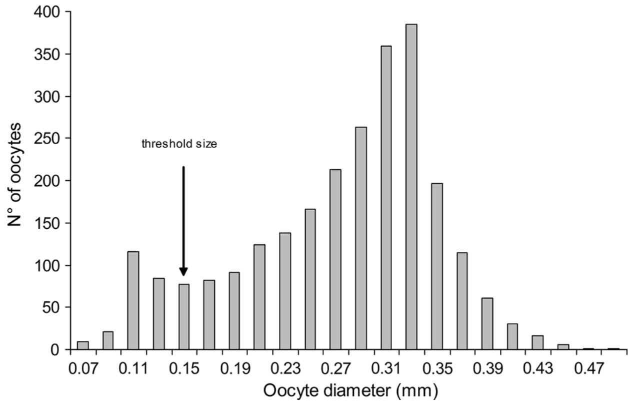

Fig. 5 Size frequency distribution of oocytes in ovaries of early developing female (stage III) of *Patagonotothen ramsayi*

and 390 μm (mean ± SE, 282 ± 7 μm) and between 320 and 680 μm (mean ± SE, 416 ± 17 μm), respectively. A few relatively large atretic oocytes measuring between 1020 and 1440 μm (mean ± SE, 1150 ± 140 μm) were found in three females. In these individuals, fecundity was calculated excluding the atretic oocytes.

Total fecundity (F_{tot}) and relative fecundity (F_{rel}), estimated by counting vitellogenic oocytes from the second mode, ranged between 30,874 and 113,493 eggs/female (mean ± SE, 51,909 ± 3332 eggs/female) and between 138 and 604 eggs/g (mean ± SE, 297 ± 18 eggs/g), respectively. No relationship was found between total fecundity and fish size (Fig. 6; $R^2 = 0.02$, $F_{1,28} = 1.58$, $p = 0.22$).

The histological analysis of gonads of females in the early developing stage (stage III) revealed the presence of previtellogenic oocytes measuring between 42 and 137 μm (mean ± SE, 86 ± 1 μm) and oocytes at a late cortical alveoli stage measuring between 124 and 363 μm (mean ± SE, 240 ± 4 μm) (Fig. 7a, b). The discrepancy of oocyte sizes assessed by macroscopic and histological analyses was due to their shrinkage after dehydration and embedding in paraplast. Atretic oocytes were observed in three females (Fig. 7c). Ovarian follicles of the single immature female consisted almost of previtellogenic oocytes, with a very few oocytes starting endogenous vitellogenesis interspersed between them (Fig. 7d).

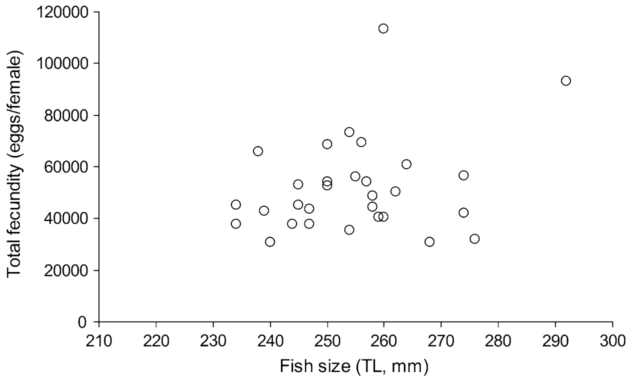

Fig. 6 Relationship between total fecundity and fish size in females of *Patagonotothen ramsayi*

Based on the terminology of Grier (1981), the testes in the rock cod were of the "unrestricted spermatogonial" type, characterized by a random distribution of spermatogonia along the entire length of the lobules. Males were at two different stages of gonad development, namely resting (stage II) or spent (stage VII).

Resting males showed compact testicular lobules, with spermatogonia and abundant cysts of spermatocytes; lobule lumina were small and no sperm were found in the main testicular and spermatic ducts (Fig. 8a). In spent individuals, lobule walls consisted of spermatogonia and abundant cysts of spermatocytes, as in resting males, with lobule lumina rather small (Fig. 8b, c). Residual spermatozoa were still abundant in the main testicular (Fig. 8c) and spermatic ducts (Fig. 8d), and in some lobule lumina. Main testicular and sperm ducts were large and convoluted (Fig. 8c, d).

Discussion

The results of this study provided new data on age and reproduction of the rock cod, *P. ramsayi*, in the Burdwood Bank, representing the southern boundaries of its distribution. Indeed, compared with the northernmost populations, the rock cod inhabiting the cold waters of the Burdwood Bank have received little attention. Available data on biological aspects of this species from this area come from two relatively old studies carried out between 1978 and 1984 (Ekau 1982; Sosiński and Janusz 2003).

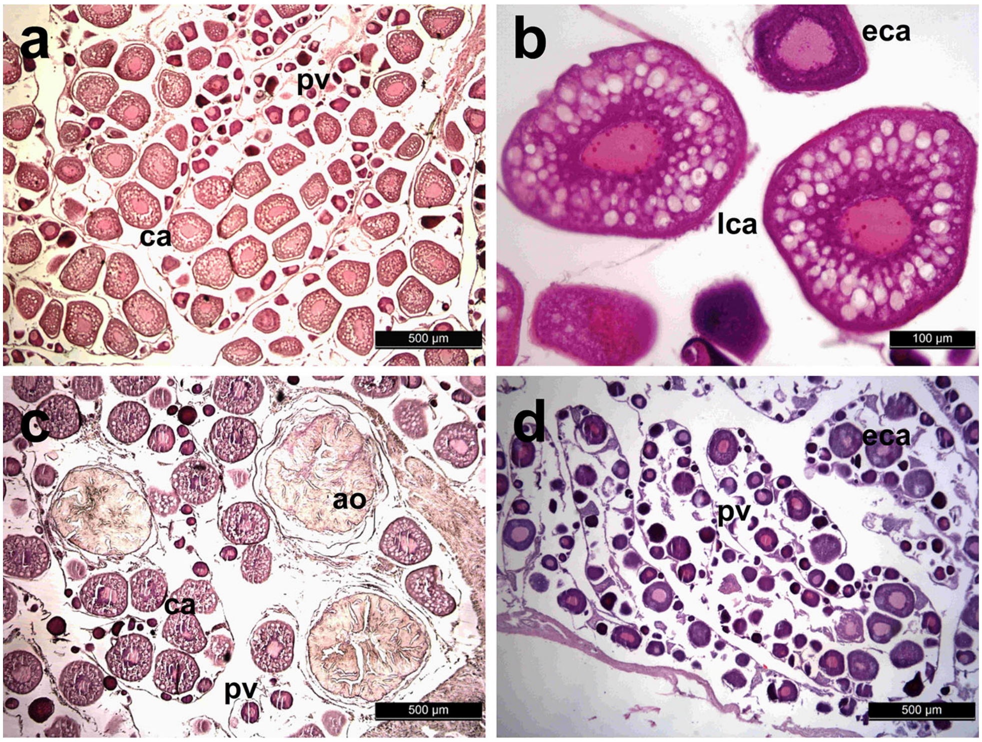

Fig. 7 Transversal sections of ovaries of *Patagonotothen ramsayi*, haematoxylin and eosin staining. **a** Female at early developing stage (TL 250 mm) showing oocytes at two different stages of development; **b** late and early cortical alveoli stages in a female at early developing stage (TL 245 mm); **c** female at early developing stage (TL 292 mm) with atretic oocytes; **d** immature female (TL 253 mm) showing previtellogenic oocytes and a few cortical alveoli stage oocytes. *ao* atretic oocytes, ca cortical alveoli oocyte, *eca* early cortical alveoli oocyte, *lca* late cortical alveoli oocyte, *pv* previtellogenic oocytes

From an ecological perspective, the rock cod is of particular interest for two main reasons. The first one is that recently their abundance has greatly increased in Falkland waters, attaining a standing biomass of at least 300,000 tons, thus becoming a key component of the local food web and one of the main target species of local fishing fleet (Arkhipkin et al. 2012). The other reason is that, compared with the other nototheniid species, it is distributed over a wide latitudinal range along the Patagonian shelf, inhabiting mixed subantarctic and temperate shelf waters. Across their geographical range of distribution, the rock cod populations experience different environmental conditions in terms of sea water temperature and salinity, ranging from 7.5 to 12.5 °C and 33.1–33.8 ‰ in the northern Patagonian shelf to 5.4–6.4 °C and 34.1 ‰ in the Burdwood Bank (Sosiński and Janusz 2003).

The rock cod population we sampled in the easternmost part of the Burdwood Bank consisted exclusively of medium-sized adult fish between 4 and 8 years of age. The mean lengths at age estimated in our samples were consistent with those previously reported for the same age classes from the Burdwood Bank (Sosiński and Janusz 2003), strengthening the reliability of the ageing method through otolith reading in this species. Similarly, the bulk of catches consisted of 5- to 6-year-old fish in both studies, corresponding to approximately 70–90 % of sampled population. Unfortunately, we did not catch fish larger than 30 cm TL; therefore, our sample lacks the older classes from 9 to 14 years previously reported from the same area (Sosiński and Janusz 2003).

Most of the sampled individuals exhibited gonads in resting (males) or in early developing stage (females). Previous studies, based on the macroscopic stage of gonad maturity, reported that most individuals caught in winter (i.e. from mid July to mid September) were late developing or ripe, suggesting that spawning would take place in spring (Ekau 1982). At these stages of maturity, the anal and ventral fins and the throat of males turned deep black (Ekau 1982), evidence of a sexual dimorphism recently described in the rock cod maintained in captivity just before spawning (Laptikhovsky et al. 2013). Later on, between November and December, the rock cod population sampled on the Burdwood Bank consisted almost exclusively of resting individuals (Sosiński and Janusz 2003). The stages of gonad maturity we observed in fish caught in February are consistent with the aforementioned data.

The most advanced oocytes observed in females were still in endogenous vitellogenesis (cortical alveoli stage),

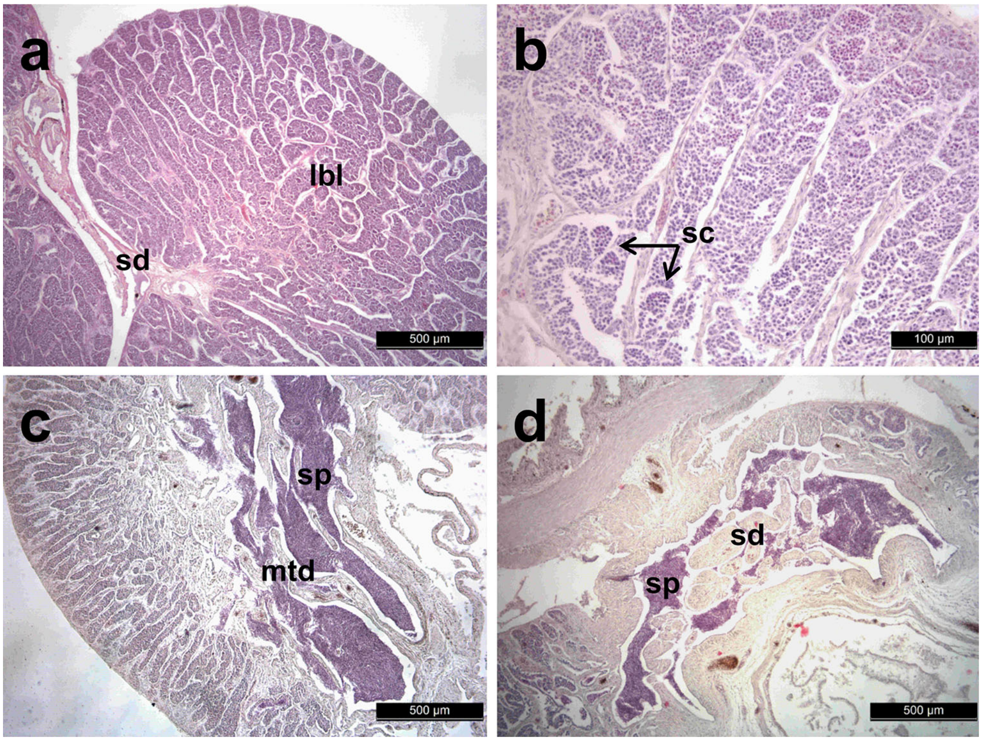

Fig. 8 Transversal sections of testes of *Patagonotothen ramsayi*, haematoxylin and eosin staining. **a** Resting male (Tl 262 mm) showing compact testicular lobules and no sperm in the spermatic ducts; **b** spent male (TL 249 mm) with abundant cysts of spermatocytes; **c** spent male (TL 249 mm) showing abundant sperm in the main testicular duct; **d** spent male (TL 249 mm) with sperm in the posterior part of the spermatic duct. *lbl* lobule lumina, *mtd* main testicular duct, *sc* spermatocytes, *sd* spermatic duct, *sp* sperm

suggesting that yolk accumulation or exogenous vitellogenesis in this species is a rather slow process lasting for about 7–8 months. Our sample was noteworthy in the presence of spent males with considerable residual spermatozoa still not being phagocyted and females with atretic oocytes still in the resorption process likely from the previous spawning season, indicating perhaps a later spawning season than previously reported.

Comparing the rock cod inhabiting the Burdwood Bank with the northern populations, some biological traits appear to differ to some extent, possibly driven by different environmental conditions across their wide geographical distribution. According to previous studies (Ekau 1982; Sosiński and Janusz 2003; Brickle et al. 2006a), maximum size, growth rate and longevity show an evident latitudinal cline, with the Burdwood Bank rock cod population attaining larger size, lower growth rate and higher longevity than the northern populations. Similarly, the spawning occurs in austral autumn (April–May) on the northern Argentinean shelf (Ekau 1982; Sosiński and Janusz 2003), in winter (June–August) around the Falkland Islands (Brickle et al. 2006b) and in spring–early summer (October–December or possibly later) on the Burdwood Bank (Ekau 1982; Sosiński and Janusz 2003; present study).

The present study provided also for the first time data on fecundity of the rock cod from the Burdwood Bank. Both total and relative fecundities seem to be rather different between the populations inhabiting the shelf around the Falkland Islands (Brickle et al. 2006b) and the Burdwood Bank. Taking into account the same fish size range (i.e. 20–30 cm TL) and fecundity estimates corrected for atretic losses, total and relative fecundities calculated from the southern population were higher than those from the northern one, ranging approximately between 30,000 and 113,000 eggs/female and 130–600 eggs/g and 24,000–76,000 eggs/female and 200–350 eggs/g, respectively. This pattern was unexpected, as Antarctic fish populations distributed at the higher latitudes usually show lower level of fecundity compared with those inhabiting lower latitudes (Kock and Kellermann 1991). Differences in fecundity between populations of rock cod may be related to a different degree of atretic processes and therefore of oocyte resorption between sites, and/or to a difference in potential fecundity (i.e. the maximum number of oocytes that can mature). In both cases, more favourable environmental conditions (food availability or water temperature, among others) at Burdwood Bank might be responsible for such differences, though it deserves further investigations.

On the Burdwood Bank, we did not find any relationship between female size and fecundity, possibly due to the small size range of fish sampled. Within the subantarctic genus *Patagonotothen*, fish size seems to largely determine both total and relative fecundities. A size-related trend is evident when comparing the rock cod with the smaller species of *Patagonotothen* with similar geographical distribution. *P. guntheri*, for example, attains a maximum size of 23 cm TL and spawn about 8000–28,000 eggs/female and 200–280 eggs/g off South Georgia (Lisovenko 1987). *P. tessellata*, a medium-sized species, attains a maximum size of 25 cm TL and spawn about 7600–62,000 eggs/female and 200–450 eggs/g in the Beagle Channel (Rae and Calvo 1995, 1996). Hence, fecundity or maternal output may show a high degree of intra- and inter-specific variation also among closely related species of nototheniids, modulated by biological constraints such as fish size and local environmental conditions.

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References

Arhan M, Naveira Garabato AC, Heywood KJ, Stevens DP (2002) The Antarctic Circumpolar Current between the Falkland Islands and South Georgia. J Phys Oceanogr 32:1914–1931

Arkhipkin A, Laptikhovsky V (2013) From gelatinous to muscle food chain: rock cod *Patagonotothen ramsayi* recycles coelenterate and tunicate resources on the Patagonian Shelf. J Fish Biol 83:1210–1220

Arkhipkin A, Brickle P, Laptikhovsky V (2003) Variation in the diet of the Patagonian toothfish, *Dissostichus eleginoides* (Perciformes: Nototheniidae), with size, depth and season around the Falkland Islands (Southwest Atlantic). J Fish Biol 63:428–441

Arkhipkin A, Brickle P, Laptikhovsky VV, Winter A (2012) Dining hall at sea: feeding migrations of nektonic predators to the eastern Patagonian Shelf. J Fish Biol 81:882–902

Arkhipkin A, Jurgens E, Howes PN (2013) Spawning, egg development and early ontogenesis in rock cod *Patagonotothen ramsayi* (Regan, 1913) caught on the Patagonian Shelf and maintained in captivity. Polar Biol 36:1195–1204

Beamish RJ, Fournier DA (1981) A method of comparing the precision of a set of age determinations. Can J Fish Aquat Sci 38:982–983

Brickle P, Laptikhovsky V, Pompert J, Bishop A (2003) Ontogenetic changes in the feeding habits and dietary overlap between three abundant rajid species on the Falkland Islands' shelf. J Mar Biol Assoc UK 83:1119–1125

Brickle P, Shcherbich Z, Laptikhovsky V, Arkhipkin A (2005) Scientific report. Aspects of the biology of the Falkland's rock cod *Patagonotothen ramsayi* (Regan, 1913) on the southern Patagonian Shelf. Falkland Islands Government

Brickle P, Arkhipkin A, Shcherbich Z (2006a) Age and growth of a sub-Antarctic notothenioid, *Patagonotothen ramsayi* (Regan 1913), from the Falkland Islands. Polar Biol 29:633–639

Brickle P, Laptikhovsky V, Arkhipkin A, Portela J (2006b) Reproductive biology of *Patagonotothen ramsayi* (Regan, 1913) (Pisces: Nototheniidae) around the Falkland Islands. Polar Biol 29:570–580

Campana SE, Annand MC, McMillan JI (1995) Graphical and statistical methods for determining the consistency of age determinations. Trans Am Fish Soc 124:131–138

Chang WYB (1982) A statistical method for evaluating the reproducibility of age determination. Can J Fish Aquat Sci 39:1208–1210

Clausen A, Pütz K (2003) Winter diet and foraging range of gentoo penguins (*Pygoscelis papua*) from Kidney Cove, Falkland Islands. Polar Biol 26:32–40

De Witt HH, Heemstra PC, Gon O (1990) Nototheniidae. In: Gon O, Heemstra PC (eds) Fishes of the Southern Ocean. JLB Smith Institute of Ichthyology, Grahamstown, pp 279–331

Eastman JT, Eakin RR (2000) An updated species list for notothenioid fish (Perciformes; Notothenioidei), with comments on Antarctic species. Arch Fish Mar Res 48:11–20

Ekau W (1982) Biological investigations on *Notothenia ramsayi* Regan 1913 (Pisces, Notothenioidei, Nototheniidae). Arch Fisch Wiss 33:43–68

Falkland Islands Government (2012) Fisheries statistics, vol 16. Fisheries Department, Stanley

Falkland Islands Government (2013) Fisheries Statistics, vol 17. Fisheries Department, Stanley

Garcia de la Rosa SB, Sanchez F, Figueroa D (1997) Comparative feeding ecology of Patagonian toothfish (*Dissostichus eleginoides*) in the southwestern Atlantic. CCAMLR Sci 4:105–124

Grier HJ (1981) Cellular organization of the testis and spermatogenesis in fishes. Am Zool 21:345–357

Hunter JR, Macewicz BJ, Lo NCH, Kimbrell CA (1992) Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fish Bull 90:101–128

Knust R, Gerdes D, Mintenbeck K (2012) The expedition of the research vessel "Polarstern" to the Antarctic in 2011 (ANT-XXVII/3) (CAMBIO). Rep Polar Mar Res 644:1–202

Kock KH, Kellermann A (1991) Reproduction in Antarctic notothenioid fish. Antarct Sci 3:125–150

Koen Alonso M, Crespo EA, Garcia NA, Pedraza SN, Mariotti PA, Beron Vera B, Mora NJ (2001) Food habits of *Dipturus chilensis* (Pisces: Rajidae) off Patagonia, Argentina. ICES J Mar Sci 58:288–297

Laptikhovsky V (2004) A comparative study of diet in three sympatric populations of *Patagonotothen* species (Pisces: Nototheniidae). Polar Biol 27:202–205

Laptikhovsky V, Arkhipkin A (2003) An impact of seasonal squid migrations and fishery on the feeding spectra of notothenioids *Patagonotothen ramsayi* and *Cottoperca gobio* around the Falkland Islands. J Appl Ichthyol 19:35–39

Laptikhovsky V, Arkhipkin A, Brickle P (2013) From small bycatch to main commercial species: explosion of stocks of rock cod *Patagonotothen ramsayi* (Regan) in the Southwest Atlantic. Fish Res 147:399–403

Lisovenko LA (1987) Reproductive biology of Antarctic icefish in relation to their environment. In: Skarlato OA, Alekseev AP, Liubimova TG (eds) Biological resources of the Arctic and Antarctic. Nauka, Moscow, pp 337–357 (in Russian)

Murua H, Kraus G, Saborido-Rey F, Witthames PR, Thorsen A, Junquera S (2003) Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. J Northwest Atl Fish Sci 33:33–54

Nyegaard M, Arkhipkin A, Brickle P (2004) An alternating discard scavenger: variation in the diet of kingclip, *Genypterus blacodes* (Ophidiidae) around the Falkland Islands. J Fish Biol 65:666–682

Padovani LN, Viñas MD, Sanchez F, Mianzan H (2012) Amphipod-supported food web: *Themisto gaudichaudii*, a key food resource for fishes in the southern Patagonian Shelf. J Sea Res 67:85–90

Pearse AGE (1985) Histochemistry, theoretical and applied analytical technology. Churchill Livingstone, Edinburgh

Rae GA, Calvo J (1995) Fecundity and reproductive habits in *Patagonotothen tessellata* (Richardson, 1845) from the Beagle Channel, Argentina. Antarct Sci 7:235–240

Rae GA, Calvo J (1996) Histological analysis of gonadal development in *Patagonotothen tessellata* (Richardson 1845) (Nototheniidae: Pisces) from the Beagle Channel, Argentina. J App Ichthyol 12:31–38

Smith IJ, Stevens DP, Heywood KJ, Meredith MP (2010) The flow of the Antarctic Circumpolar Current over the North Scotia Ridge. Deep Sea Res I 57:14–28

Sosiński J, Janusz J (2003) The distribution and biology of *Patagonotothen ramsayi* (Regan, 1913): results of polish studies on the Patagonian Shelf, 1979-1993. Sea Fisheries Research Institute, Gdynia

Wallace RA, Selman K (1981) Cellular and dynamic aspects of oocyte growth in teleosts. Am Zool 21:325–343

Winter A, Laptikhovsky V, Brickle P, Arkhipkin A (2010) Rock cod *Patagonotothen ramsayi* (Regan, 1913) stock assessment in the Falkland Islands. Falkland Islands Government Fisheries Department, Stanley

PAPER III

Life history strategies of the Scotia Sea icefish, *Chaenocephalus aceratus*, along the Southern Scotia Ridge

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Abstract

Reproductive capacity can influence distribution and abundance over large spatial scales through larval dispersal, even when adult stages remain isolated following settlement. We examined size distribution, reproductive traits and age structure in Scotia Sea icefish, *Chaenocephalus aceratus*, an abundant benthic species with a long larval pelagic phase found on continental shelves along the Southern Scotia Ridge. In particular, we compared life history strategies between fish caught during surveys undertaken off the South Orkney Islands (SOI) and South Shetland Islands (SSI). Results corroborated regional separation after settlement, and suggested distinct life history strategies, in which fish from SOI invested much less in reproduction, and somewhat more in somatic growth earlier in their life history. Compared to SSI, body weight increased faster with length and absolute fecundity was 46% lower and increased more slowly with size for SOI population. In addition, the proportion of spawning cohorts and L_∞ was lower and k higher for SOI. The differences appeared to be a phenotypic response to environmental conditions related to regional hydrography. Lower reproductive capacity around the SOI, and strong eastward flow in the large-scale circulation, suggest that the SSI may be more important in influencing distributions and abundance of icefish along the southern Scotia Ridge.

Introduction

For many marine species, adult stages are restricted to shelf areas that are often geographically distant. Nevertheless, dispersal during early life can overcome isolation (e.g. Cowen and Spanaugle 2009) and generate complex populations structured by ocean circulation through the advection of young stages (e.g. Pulliam 1988; Sinclair 1988). Transport can be predicted using particle simulations in a circulation model, and potential connectivity estimated as the per capita probability of larvae reaching one or more juvenile habitats (Watson *et al*. 2012). However, another critical measure that can be overlooked is the number of individuals that are made available for dispersal. As well as transport pathways connecting shelf areas, the number of sexually mature fish at source, their fecundity and growth can have wide-ranging consequences on distribution and abundance, even when adult stages remain isolated.

Understanding the reproductive capacities of connected populations is particularly important when fish are exposed to diverse environments that modify vital rates and life history traits. In the Southern Ocean, warm, nutrient-rich Circumpolar Deep Water (CDW) is brought close to the surface near the southern boundary of the eastward-flowing Antarctic Circumpolar Current (ACC) (e.g. Orsi *et al*. 1995). Along the western Antarctic Peninsula (AP), the boundary is located close to the continental slope, flooding the shelf with CDW. Above the CDW, a layer of cold Winter Water (WW) persists into summer. However, the WW layer is eroded by mixing with warmer Antarctic

Surface Water above and deep water below, creating modified CDW that occupies depths below 200m (Smith *et al*. 1999). Along the eastern AP by contrast, water in the Weddell Sea flows northwards and into the Bransfield Strait along the shelf and slope, via the Antarctic Coastal Current (CC) and Antarctic Slope Front (ASF) (Thompson *et al*. 2009) (Fig. 1). In the Bransfield Strait, it is entrained in a large standing eddy, or returned to the South Scotia Ridge by a strong western boundary current off the South Shetland Islands. Water transported northwards along the eastern AP, but further offshore in the Weddell Front, flows around the Powell Basin to reach the South Orkney Islands (Fig. 1). Cold and fresh water from the ASF, and higher salinity water from the CC, contrast with the modified CDW on the western shelf of the AP and Warm Deep Water (WDW) from the Weddell Sea on the South Orkney shelf (Gordon *et al*. 2001; Heywood *et al*. 2004).

Icefish (family Channichthyidae) include 16 endemic species (Iwami and Kock 1990; La Mesa *et al*. 2002) that are distributed in the Southern Ocean and characterized by polar adaptations that include a lack of haemoglobin (Eastman 1993). Typical life history features include a high reproductive effort with production of large eggs (Kock 2005), a long pelagic larval and juvenile phase, slow somatic growth and late sexual maturity (Kock and Kellermann 1991; Kock 2005). The Scotia Sea icefish (*Chaenocephalus aceratus)* is a previously exploited species that has been protected by a fishing moratorium since 1990. Found on continental shelves from the South Shetland Islands and northern tip of the Antarctic Peninsula eastward along the southern Scotia Ridge, it is among the most abundant fish species occupying depths down to 770 m (Kock and Stransky 2000). Its life history is marked by sharp ontogenetic shifts in the spatial scales of movement. Demersal eggs are spawned between April and June (Kock and Kellermann 1991; Kock and Jones 2005); spawning fish are found between 130-320 m and males guard the eggs in nests in benthic depressions (Detrich *et al*. 2005). Hatching lasts from July-August to December (Kock and Kellermann 1991) peaking in November (La Mesa and Ashford 2008). Based on counts of otolith micro-increments from young juveniles after settlement, the pelagic phase has been estimated at an upper bound of between 400 and 508 days duration (La Mesa and Ashford 2008). Like most notothenioid species, the adults are negatively buoyant (Eastman and Sidell 2002), assuming more sedentary behavior distributed in benthic habitats along the shelf. Recovery from earlier exploitation has been slow, and fisheries surveys have found considerable fluctuations in abundance between years that may be related to variation in the strength of recruiting year classes (Kock and Jones 2005).

Complex population structuring, with connectivity during the pelagic phase and isolation over the rest of the life history, may help account for the variability. Aggregations of spawning fish occur on the shelves of the northern Antarctic Peninsula, notably north and west of Elephant Island and King George Island in the South Shetland Islands, off the South Orkney Islands, and further downstream in the ACC off South Georgia and Bouvet Island. In *C. aceratus*, growth rates (Gubsch 1980; Kompowski 1990; La Mesa *et al*. 2004), morphometric and meristic measurements (Kock 1981), and larval nematode infestations (Siegel 1980) showed differentiation between the AP, South Orkney Islands and South Georgia. Often strongly influenced by environmental differences, these techniques used to evaluate biological traits of the species usefully indicate population structuring over the life history that fish movement and mixing would otherwise homogenize (e.g. Ihssen *et al*. 1981). However, they are unreliable in detecting situations in which larvae from a single population disperse across disparate environments and heterogeneity develops as a result of subsequent isolation. Microsatellite and mitochondrial markers have been examined as well, and showed genetic structuring between the South Shetland Islands and the South Orkney Islands. However, differentiation was weak, with high gene flow especially in an eastward direction, periodic restrictions in effective population size, and considerable temporal variation, congruent with connectivity through larval dispersal (Papetti *et al*. 2007, 2009; Damerau *et al*. 2012).

Accumulating evidence of connectivity has been found for several Antarctic species. Krill distributions (Hofmann *et al*. 1998) and particle simulations using circulation models (Fach and Klinck 2006) indicated advection in the ACC from the western AP to South Georgia. Transport pathways connect widely separated regions of the Southern Ocean (Thorpe *et al*. 2007), and empirical studies suggest population structuring in the toothfish *Dissostichus eleginoides* that is related to the position of fronts and the direction of water flow (e.g. Shaw *et al*. 2004; Ashford *et al*. 2006, 2008, 2012). The life history of *D. mawsoni* has also been linked to transport in the Ross Gyre (Hanchet *et al*. 2008; Ashford *et al*. 2012), and distributions of *Pleuragramma antarctica* off the northern AP appear related to dispersal along the CC (Kellermann 1986; La Mesa *et al*. 2015). In *C. aceratus*, La Mesa and Ashford (2008) hypothesized that the long pelagic phase might promote connectivity from the western AP along the transport pathways used by krill; larvae reaching suitable habitats off South Georgia and the South Orkney Islands then mix with locally spawned fish. Ashford *et al*. (2010) subsequently tested the hypothesis using a combination of otolith chemistry and simulations using a circulation model. Contrary to expectation, they found evidence suggesting a population boundary between the Peninsula and South Georgia. However, the circulation model showed that the separation could be explained by simulated eastward trajectories that reached the South Orkney Islands but passed too far south to arrive at South Georgia. The study indicated that nesting behavior in icefish fundamentally influenced the direction of connectivity: releases from the shelf benthos off Elephant Island and the South Shetland Islands in late winter/spring resulted in dispersal along the southern Scotia Ridge and further east, whereas trajectories towards South Georgia depended on summer releases from the surface layer over the outer continental slope (Ashford *et al*. 2010).

Thus, physical-biological interactions between circulation and life history processes can help account for population distributions and abundance. Nevertheless, variability also depends on the reproductive capacity of each population in making larvae available for dispersal and self-recruitment. Estimates of fecundity and reproductive biology are critical to assess reproductive potential over the life history, including length or age at first maturity relative to maximum age, and any relationships between size and absolute fecundity (e.g. La Mesa *et al*. 2014). Additionally, spatial differences in vital rates and life history parameters can be used (e.g. Begg 2005) to corroborate isolation following settlement on shelf areas along the AP and southern Scotia Ridge. Consequently, in this study, we investigated the size and age distributions, reproductive characteristics, growth and mortality of *C. aceratus* off the South Shetland Islands and South Orkney Islands, aiming to a) provide estimates of the gonadic and somatic investment of populations exposed to different environments, b) test for population structuring expected following settlement, and c) assess spatial differences in life history strategies and reproductive capacity.

Materials and methods

Sampling and preliminary analyses

Samples of *C. aceratus* were collected around the South Orkney Islands (SOI) by the RV *Yuzhmorgeologya* and compared to a sample set taken off the South Shetland Islands (SSI) by the RV *Polarstern* (Fig. 1). The two cruises were part of a series of fisheries surveys of the two regions, and the vessels deployed equipment used on previous expeditions; sampling activities were undertaken using a commercial benthic trawl with a codend mesh size of 40 mm, following a random stratified survey design. A total of 75 hauls, each lasting 30 minutes, were carried out over the continental shelf and slope around the SOI from 9 February to 9 March 2009, within three main depth strata of 50 - 150 m, 150 - 250 m and 250 - 500 m (Jones and Kock 2009). Similarly, a total of 71 hauls of 30 minutes were conducted around Elephant Island, off the SSI and the tip of the Antarctic Peninsula from 17 March to 5 April 2012, within five depth strata set at 50 – 100 m, 101 - 200 m, 201 - 300 m, 301 - 400 m, and 401 - 500 m (Lucassen 2012).

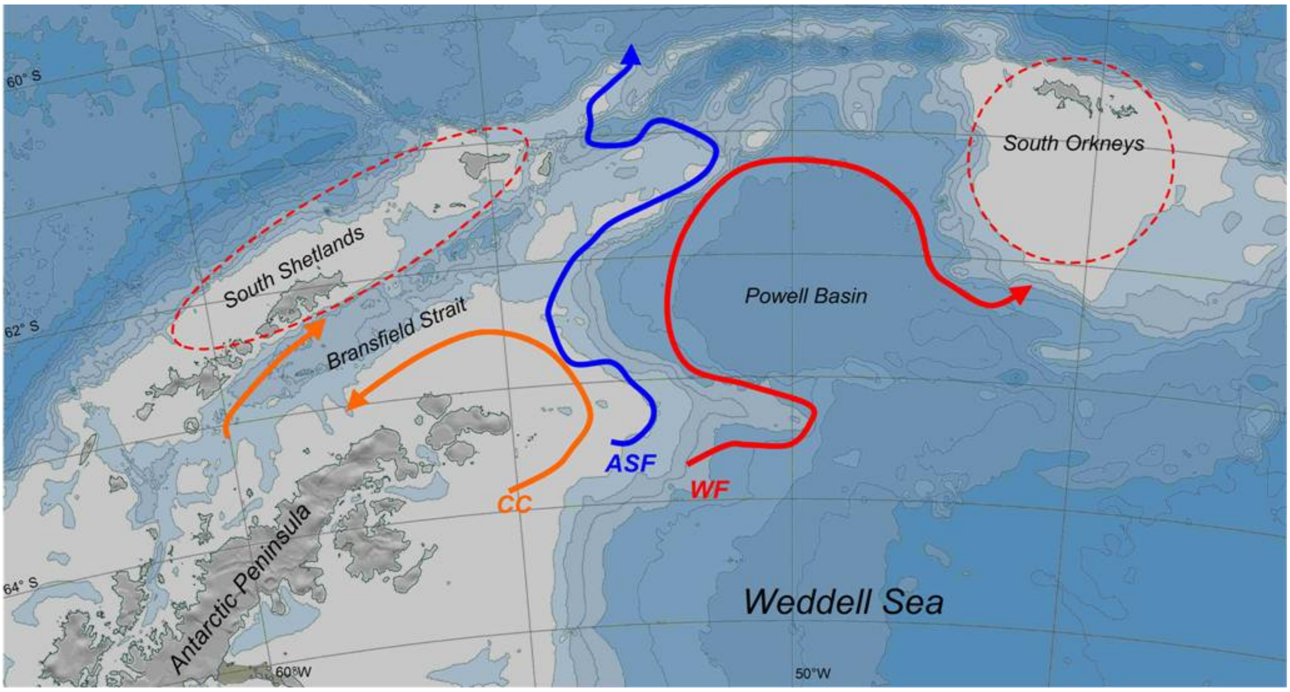

Figure 1. Map of the Southern Scotia Ridge, indicating the study area off the South Orkney Islands and South Shetland Islands (red dashed circles). Schematic representation of surface circulation at the tip of the Antarctic Peninsula from the north-western Weddell Sea according to Thompson *et al*. (2009) is also shown. Abbreviations: ASF, Antarctic Slope Front (blue arrow); CC, Antarctic Coastal Current (orange arrow); WF, Weddell Front (red arrow). Depth contours set at 500 m depth interval.

A set of standard measurements were recorded for each specimen, including total length (TL, cm), total weight (TW, g), and gonad weight (GW, g). Sex and stage of sexual maturity were assigned according to a standardized five-point scale (Kock and Kellermann 1991). Gonadosomatic index (GSI) was calculated as the percentage of gonad weight to total somatic weight of fish: GSI = 100*GW/(TW - GW). Gonad samples were preserved respectively in Dietrich’s solution or in 7% formaldehyde in sea water for histological analyses and fecundity estimates. Otoliths were removed and stored dry for ageing.

We tested for differences between sites and sexes by applying a mixed model with log-transformed TL as response variable and sex (fixed) and area (random) as orthogonal factors, using the Permutational Analysis of Variance starting from an Euclidean matrix of distances. For all the analyses, the number of permutations was set equal to 9999 (Anderson *et al*. 2008). Individuals for which no sex was recorded (n = 3 for SOI; n = 79 for SSI) were not included in the analyses; results on size differences between sites did not change if they were included. Length-weight relationships were calculated by region as well, and by sex within each region. The equation $TW = a\ TL^b$, where TW is total weight (g), TL is total length (cm) of fish and a and b are the regression parameters, was fitted to the data. All relationships fulfilled assumptions of normality and homogeneity of variances and the residuals showed no trends. An F-test was applied to test for differences in b between SOI and SSI and between sexes within region (Sokal and Rohlf 1995). Furthermore, a t-test was applied to determine whether b values obtained from the linear regression differed significantly from isometric growth (b = 3) using the equation: $t = (b - 3)\ sb^{-1}$, where sb is the standard error (SE) of b.

Analysis of Covariance was used to examine differences between areas in the gonad weight of developing females (macroscopic stage 3), with gonad weight as the dependent variable, area as the factor with two levels, and total weight as the covariate. After log-transformation of gonad weight and total weight, residuals fulfilled the assumptions of normality and equality of variances. A similar analysis was undertaken for males.

Gonad morphology and maturity

Histological analyses were performed on a sub-sample of 49 specimens to analyse gonad morphology and to validate the macroscopic stage of maturity. Histological analyses on developing and ripe ovaries were aimed at evaluating the presence of post-ovulatory follicles (POF) that indicate the occurrence of a recent spawning event, as well as of atretic oocytes for correctly evaluating absolute fecundity. Gonad samples, preserved in the fixative for at least 30 days, were dehydrated, embedded in paraplast, cut in transverse serial sections (7 μm) and mounted on slides. Slides were then stained with Mayer's haematoxylin and eosin (Pearse 1985).

Ovaries were classified according to six stages of development (West 1990): I – chromatin nucleolar (immature); II – perinucleolar (immature); III – yolk vesicle (cortical alveoli) formation (early maturation, endogenous vitellogenesis); IV – vitellogenic (late maturation, exogenous vitellogenesis); V – ripe (mature); VI – post-ovulatory follicles (post-reproduction). The stage of maturity of males was assigned according to five stages of gametogenesis (Billard 1986): I – spermatogonia (immature); II – spermatocytes I (early maturation); III – spermatocytes II and spermatids (advanced maturation); IV spermatozoa (mature), –; V – residual spermatozoa in collapsed lobules (post-reproduction). Maturity stage was attributed considering the most advanced histological stage observed in ovarian or testicular sections.

Estimating fecundity

Oocyte number and size frequency distribution generally do not differ between left and right ovaries (Hunter *et al.* 1985), and the ovary used for fecundity estimate was randomly chosen.

Fecundity was estimated in developing females (macroscopic stage 3, histological stage IV) using the gravimetric method (Murua *et al*. 2003), in which oocytes are counted in a subsample of the ovary that has been weighed. The gonad subsamples represented between 1.5% and 7% of the gonad total weight. The oocytes were manually separated in a petri dish, photographed under a stereomicroscope (magnification 80 X) using a Leica DFC 420 photo camera and counted manually. Diameters were measured using the IMAQ VISION BUILDER 6 software (National Instrument Corporation, Austin, TX, USA).

Since notothenioids generally present a group synchronous ovary (Wallace and Selman 1981) with a bimodal oocyte size distribution, the larger mode represents the oocytes corresponding to the current reproductive event (Kock and Kellermann 1991), and hence the potential fecundity (Nagahama 1983). In order to identify the two modes, the size frequency distribution of the oocytes was described by measuring and counting all the oocytes present in the ovaries of five females from each region. The two modes completely separated from each other, and the minimum size of the oocytes of the largest mode (1.5 mm) was used as a lower limit for the estimate of potential fecundity (Fig. 2). In the remaining females, the total number of oocytes above the limit was manually counted and a subsample of 100 oocytes was measured. Since no significant differences were found in the number of oocytes from anterior, middle and posterior ovary sections from six fish (Friedman test for dependent data, $\chi^2 = 4.33$, df = 2, $p > 0.05$), all subsamples were randomly chosen.

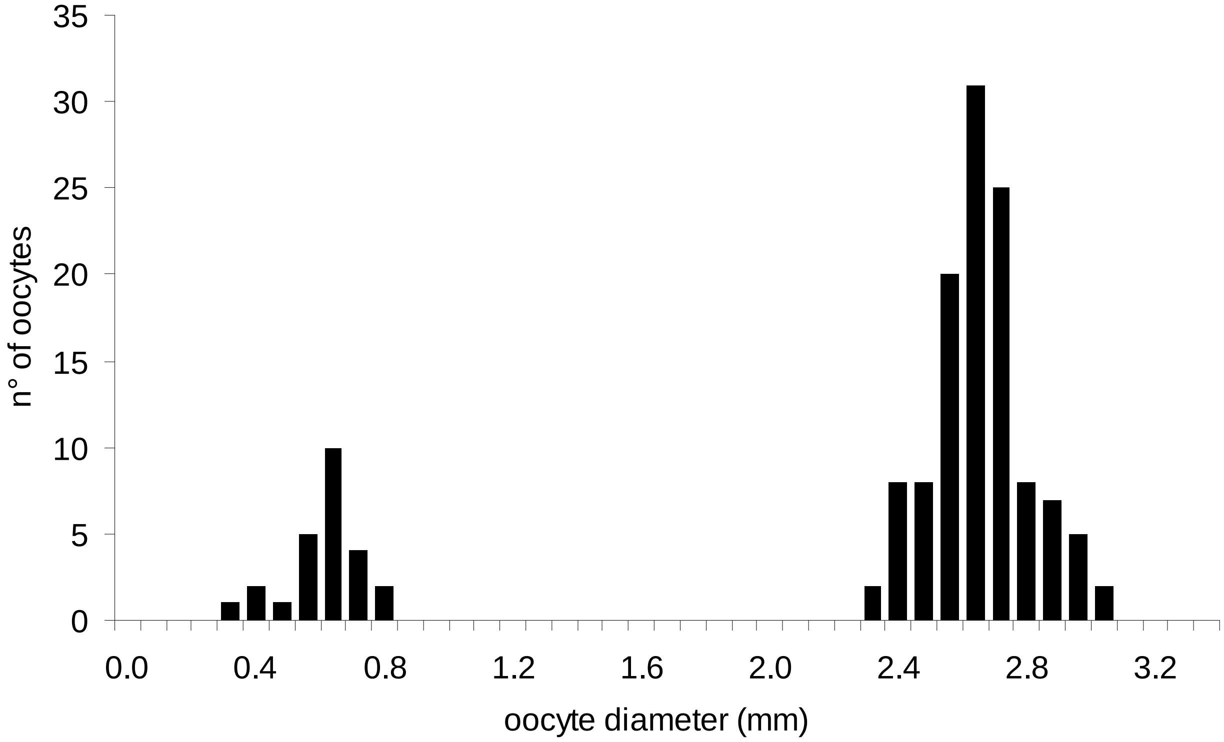

Figure 2. Frequency distributions of oocytes diameter of *Chaenocephalus aceratus* developing female (macroscopic stage 3), with the two modes representing pre-vitellogenic and vitellogenic oocytes completely separated from each other.

We used t-tests for independent data to compare the mean size of vitellogenic oocytes between the two regions. Both absolute (F_{abs}) and relative fecundities (F_{rel}) were estimated for each female with developing gonads. Absolute fecundity was calculated as: $F_{abs} = (n_s/w)$ GW, where n_s is the number of vitellogenic oocytes present in the subsample, w is the weight of the subsample and GW is the total weight of the gonads. Relative fecundity was calculated as the number of vitellogenic oocytes per gram of total body weight (TW) ($F_{rel} = F_{abs}/TW$). The relationship between the total body weight and absolute fecundity was examined using linear regression: after log-transformation, data fulfilled assumptions of normality and homogeneity of variances, and the residuals showed no trends. Analysis of covariance (ANCOVA) was used to compare absolute fecundity between regions, with total body weight as the covariate. A t test was applied to compare the relative fecundity between regions. For both analyses, assumptions were fulfilled after log-transformation.

Age, growth and mortality

In both regions, individuals were randomly selected from the sample taken. Sex could not be determined for young juveniles and these fish were excluded from the analysis, almost all from SSI. No differences in weight were found between left and right otoliths (t-test for paired comparison, $t = 0.14$, $df = 220$, $p > 0.05$ for SOI; $t = 0.48$, $df = 208$, $p > 0.05$ for SSI), and age estimates were performed on a single otolith randomly selected from each pair. Given the opacity and the presence of a dense calcareous matrix in the sagittal otoliths of Antarctic fish (Everson 1980; White 1991), the following procedure was used to prepare sagittal sections. For each specimen, otoliths were baked in an oven at 350° C, embedded in resin (Crystalbond 509 Amber, Aremo products, inc.), grinded using an abrasive paper and polished on a lapping film (with 0.05 µm alumina powder) to reveal the pattern of growth increments. As reported in other notothenioids (Everson 1980; North 1988), an annulus consisted of an opaque zone and an adjacent translucent zone, which appeared respectively as light and dark rings when observed under reflected light. To enhance the contrast, otolith sections were immersed in water and observed using a stereomicroscope at a magnification of 25-50 X. The count path began at the nucleus and followed the posterior-dorsal axis to the otolith margin (Fig. 3). Otoliths were read blind twice by a single reader, and when age estimates differed from each other by more than one year, a third reading was undertaken. Average percent error (APE) (Beamish and Fournier 1981) and mean coefficient of variation (CV) (Chang 1982) were used to measure the consistency of age estimates (Campana 2001).

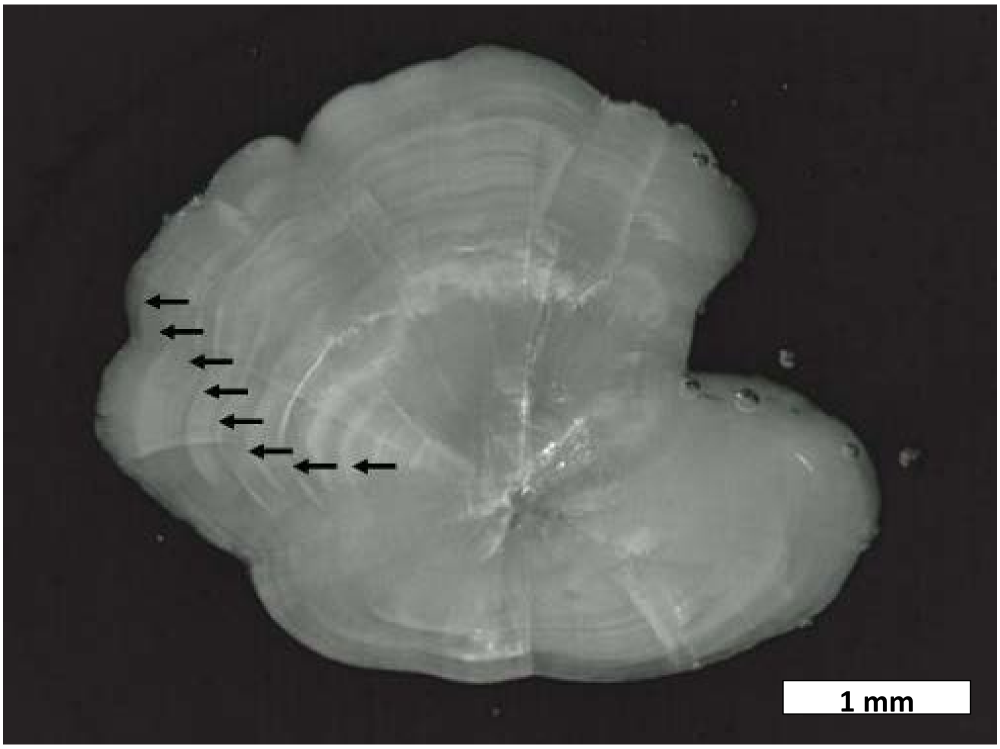

Figure 3. Sagittal section of otolith from *Chaenocephalus aceratus* estimated to be 8 years old, showing the annulation pattern made up of thin translucent (arrows) and opaque zones.

To assess growth, TL was plotted against age and the Von Bertalanffy growth function was fitted by region using the software GROWTH II and the Levenberg-Marquardt Method for non-linear regression. The Von Bertalanffy growth parameters (L_∞, k and t_0) were estimated for SOI and SSI, excluding fish for which the sex remained undetermined, and then compared between regions applying the likelihood ratio test (Kimura 1980). The growth performance index (Φ’) was also calculated as $\Phi' = 2 \log L_\infty + \log k$ (Munro and Pauly 1983) to compare growth between the two regions. We compared maximum ages, and hence the relative number of cohorts between regions, using the 99th percentile of the age distribution to avoid undue volatility as a result of outliers. As this stabilized the number of cohorts, and growth analyses corroborated isolation between regions after settlement, we estimated mortality rates (Z) using the method of Royce (1972) and compared them between distributions.

Size and age at first spawning

The size at first spawning was calculated as the length at which 50% of fish spawn for the first time (TL_{50}). We considered spawners as females with ovaries at macroscopic stages 3 - 5 (developing, gravid, spent) and males with testes at macroscopic stages 3 - 5 (developed, ripe, spent) (Kock and Kellerman, 1991). For each sex from each area, TL_{50} was estimated by fitting a logistic curve to the percentage of spawning specimens per size class. All statistical analyses were performed using STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA) software.

Results

Sampling and preliminary analyses

A total of 1862 specimens were sampled, 1057 from the SOI (538 females, 515 males and 4 undetermined) ranging from 18 cm to 66 cm TL, and 805 from the SSI (333 females, 393 males and 79 undetermined) with TL range of 12.5 - 68.5 cm. Sex ratios were not significantly different from 1 : 1 in both regions (1 : 1.05 in SOI, $\chi^2 = 0.27$, df = 1, p = 0.601 and 1 : 0.85 in SSI, $\chi^2 = 2.48$, df = 1, p = 0.115). Mean TL was larger for SOI than SSI (SOI TL = 43.4 ± 10.6 cm; SSI: TL = 32.1 ± 14 cm; pseudo-F = 443.95, df = 1,1775, p = 0.0001), while no significant differences were found within each sex in different areas (pseudo-F = 21.4, df = 1,1775, p = 0.327). The length-frequency distributions were very different between regions: relatively few younger fish were caught at the SOI, and the mode occurred at 46 cm TL. Individual fish <18 cm were only caught in the SSI. Moreover, a relatively larger proportion of SOI fish were larger than 50 cm, although the maximum age for the two regions was similar (Fig. 4).

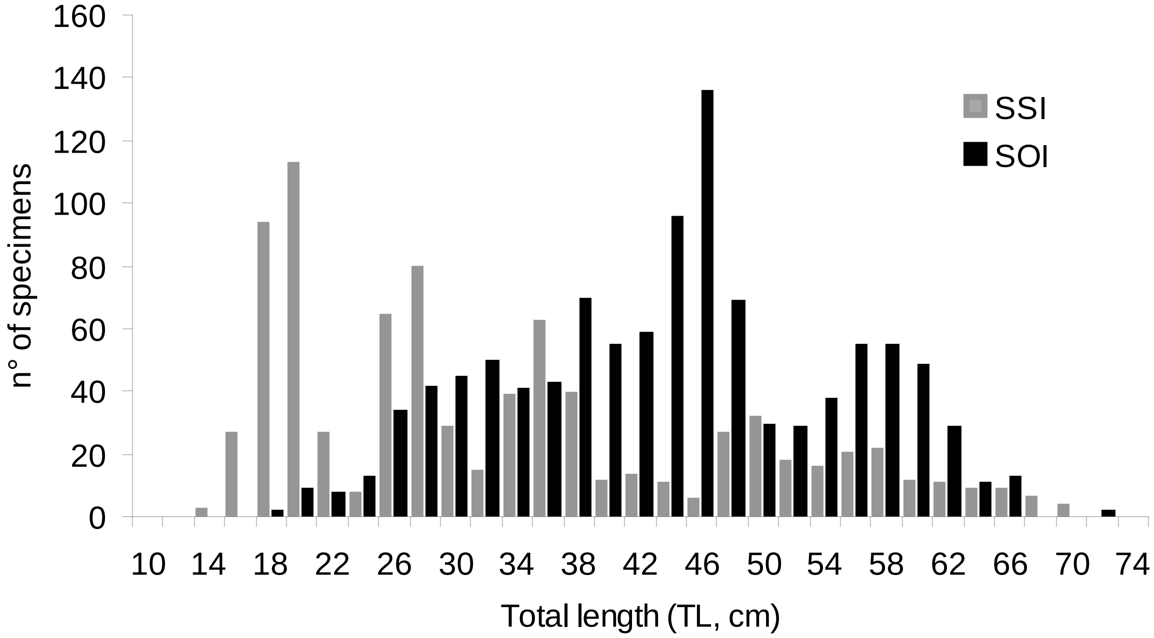

Figure 4. Fish length frequency distributions of *Chaenocephalus aceratus* collected off the South Orkney Islands (SOI) and South Shetland Islands (SSI). n = number of specimens.

Length-weight relationships showed significant differences in slopes (b) between sexes for each region (SOI: F = 25.14, df = 1,1049, p < 0.001; SSI: F = 8.17, df = 1,722, p < 0.005) and between regions, with SOI significantly higher than SSI (F = 93.89, df = 1,1857, p < 0.001). Comparing sexes between populations, the slope in females and males was significantly higher for SOI than SSI (F = 31.01, df = 1,867, p < 0.001; F = 127.79, df = 1,904, p < 0.001) (Fig. 5). All the relationships exhibited positive allometric growth (b > 3) (Bagenal and Tesch 1978).

Mean GSI for developing females (macroscopic stage 3) was 9.0 ± 3.6 (n = 30) for SOI, and 14.3 ± 4.2 (n = 30) for SSI. Female mean gonad weight was significantly lower for SOI than SSI when body weight was taken into account (ANCOVA: F = 5.08, df =1,57, p = 0.028). Mean GSI for developed males (macroscopic stage 3) was 1.5 ± 0.3 (n = 27) for SOI and 1.8 ± 0.6 (n = 20) for SSI. Male mean gonad weight did not differ between regions after body weight was taken into account (ANCOVA: F = 0.11, df =1,44, p = 0.743). The histological analyses confirmed the macroscopic maturity stage assignment in samples from both areas. They also showed that three specimens for which no sex could be determined were males with immature testes (microscopic stage I).

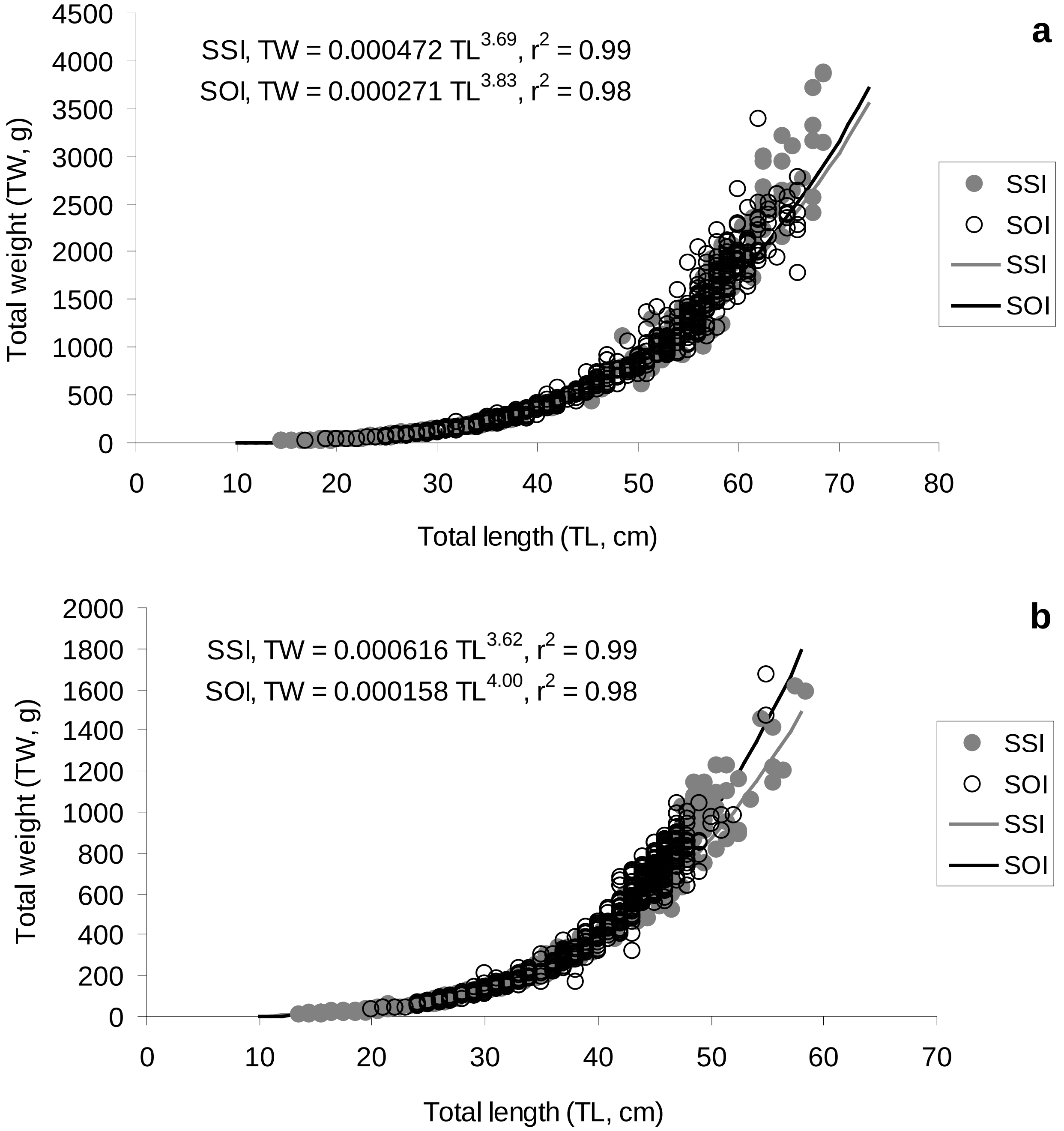

Figure 5. Fish length-weight relationships of females (**a**) and males (**b**) of *Chaenocephalus aceratus* from the South Orkney Islands (SOI) and South Shetland Islands (SSI). n = number of specimens.

Gonad morphology and maturity

A total of 24 ovaries were histologically analysed, revealing the presence of oocytes at different developmental stages. In both populations, immature females (macroscopic stage 1) presented previtellogenic oocytes and only few oocytes at early cortical alveoli stage (histological stage II and III) (Fig. 6a). In maturing females (macroscopic stage 2), ovaries were filled with cortical alveoli oocytes and a few oocytes at early vitellogenic stage were present in four out of the six analysed females (Fig. 6b). Developing females (macroscopic stage 3) exhibited mature ovaries with oocytes in advanced exogenous vitellogenesis stage, completely filled with fused yolk granules and oocytes at the cortical alveoli stage (Fig. 6c). No post-ovulatory follicles or atretic oocytes were found in all developing females analysed.

Histological analyses were performed on 25 males, which showed lobular testes of the unrestricted spermatogonial type (Grier *et al*. 1980). In both populations, males at macroscopic stage 1 had compact testes, uniformly occupied by spermatogonia (Fig. 6d). In eight maturing males analysed (macroscopic stage 2) lobule lumina were small, abundant spermatogonia and few spermatocytes, and few spermatids distributed mainly in the proximal part (Fig. 6e). Developed males (macroscopic stage 3) presented abundant spermatozoa in the lobule lumina. Spermatogonia were present only in the distal part, in the walls of lobules (Fig. 6f).

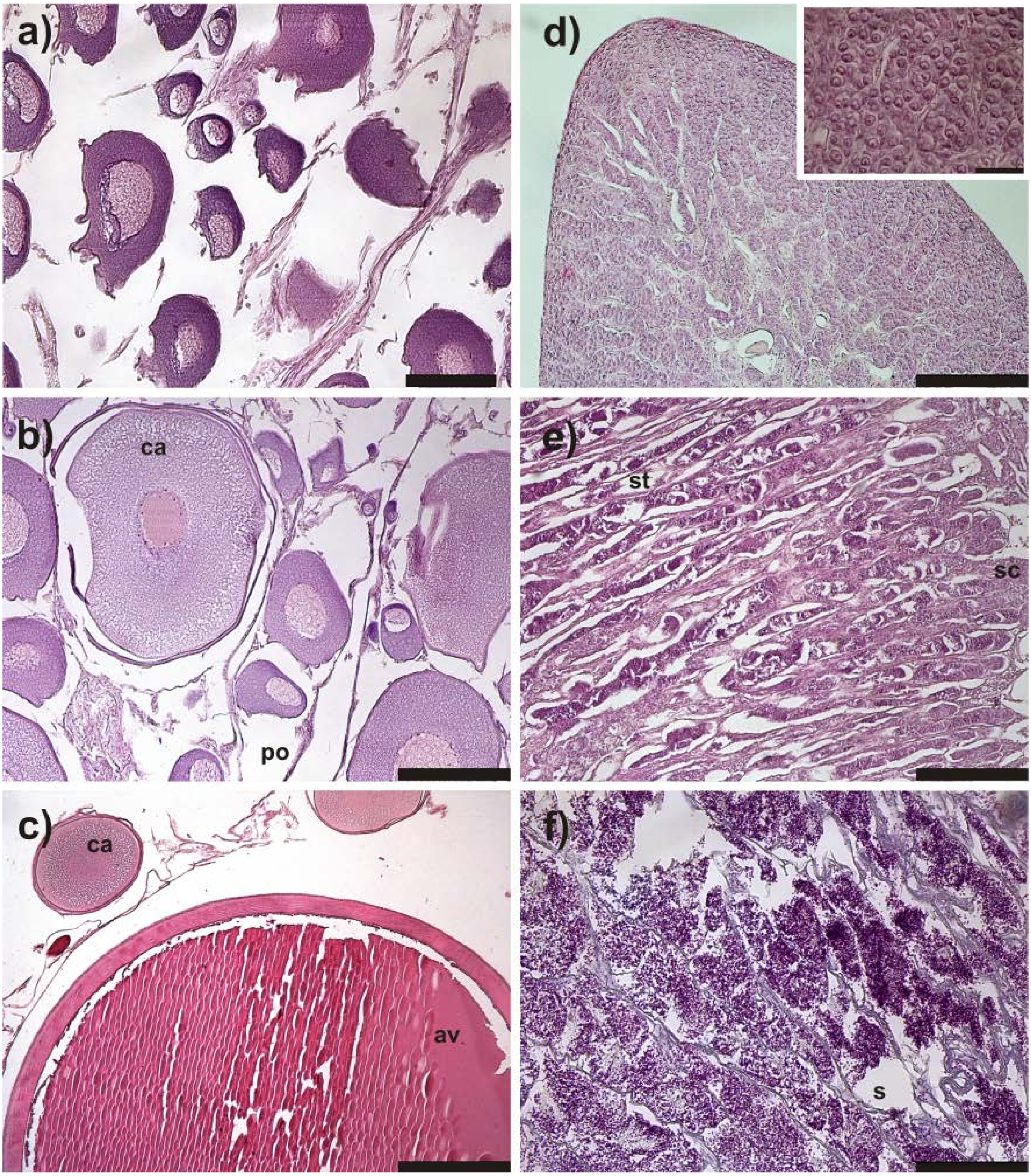

Figure 6. Histological gonad sections of *Chaenocephalus aceratus* at different maturity stages: **a)** immature female (macroscopic stage 1) with previtellogenic oocytes of two different sizes (scale bar = 100 µm); **b)** maturing female (macroscopic stage 2) ovaries filled with cortical alveoli oocytes (ca) and few previtellogenic oocytes (po) (scale bar = 200 µm); **c)** mature ovaries (macroscopic stage 3) with oocytes in advanced exogenous vitellogenesis stage completely filled with fused yolk granules (av) and oocytes at the cortical alveoli stage (ca) (scale bar = 500 µm); **d)** immature testis (macroscopic stage 1) presenting only spermatogonia (scale bar = 200 µm), inset: enlargement of lobule wall (scale bar = 40 µm); **e)** maturing males (macroscopic stage 2) with spermatocytes (sc) and spermatids (st) (scale bar = 200 µm). **f)** developed males (macroscopic stage 3) with abundant spermatozoa (s) in the lobule lumina (scale bar = 100 µm).

Estimating fecundity

Absolute fecundity (F_{abs}) was estimated as 9309 ± 1742 eggs/individual in SOI females (n = 30), and 17205 ± 4372 eggs/individual in SSI females (n = 30). The total number of vitellogenic oocytes was positively related to total body weight in both populations (SOI: $F_{abs} = 116.6\ TW^{0.588}$, $r^2 = 0.66$, F = 54.3, df = 28, $p < 0.001$; SSI: $F_{abs} = 49.31\ TW^{0.7507}$, $r^2 = 0.73$, F = 76.1, df = 28, $p < 0.001$), with significant differences in the relationships between SSI and SOI females (Wald statistic = 106.8, df = 1, $P < 0.001$; Fig. 7). Relative fecundity (F_{rel}) was significantly lower for SOI than SSI (SOI: 6.1 ± 0.8 eggs/g, SSI: 8.4 ± 1.3 eggs/g; t = 8.3, df = 58; $p < 0.001$), while oocyte mean diameter was slightly but not significantly lower in SOI females compared to SSI females (SOI: 2.8 ± 0.5 mm; SSI: 3.0 ± 0.4 mm; t = 1.5, df = 58; p = 0.136).

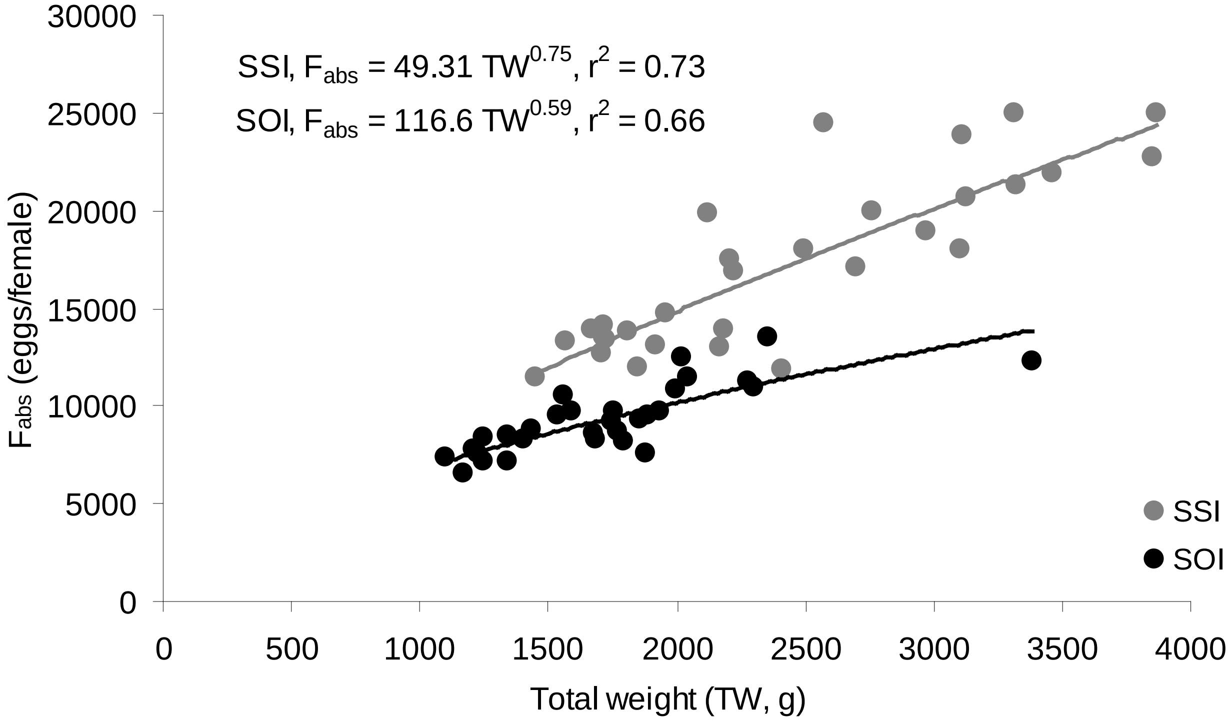

Figure 7. Relationships between fish total weight and absolute fecundity (Fabs) in *Chaenocephalus aceratus* from the South Orkney Islands (SOI) and South Shetland Islands (SSI).

Age, growth and mortality

Age was estimated in 223 individuals from SOI and 234 from SSI. APE and CV were reasonable for both regions (SOI: 5.1% and 7.2% respectively; SSI: 4.1% and 5.8%), indicating the reliability of the ageing procedure and reproducibility between readings across age classes. Estimates of von Bertalanffy growth parameters showed that L_∞ was lower for SOI than SSI whereas k was higher (Tab. 1, Fig. 8). The likelihood-ratio test indicated that the estimated growth curves of SOI and SSI differed significantly ($\chi^2 = 14.6$, df = 3, $p < 0.005$) when L_∞, K and t_0 were unconstrained; the regions did not differ significantly from each other when parameters were constrained (Tab. 1). The growth performance Φ' was 2.73 for SOI and 2.76 for SSI. Maximum age was 15 years for SOI and 17 years for SSI; mortality rate based on Royce's (1972) method was Z = 0.31 for SOI and Z = 0.27 for SSI.

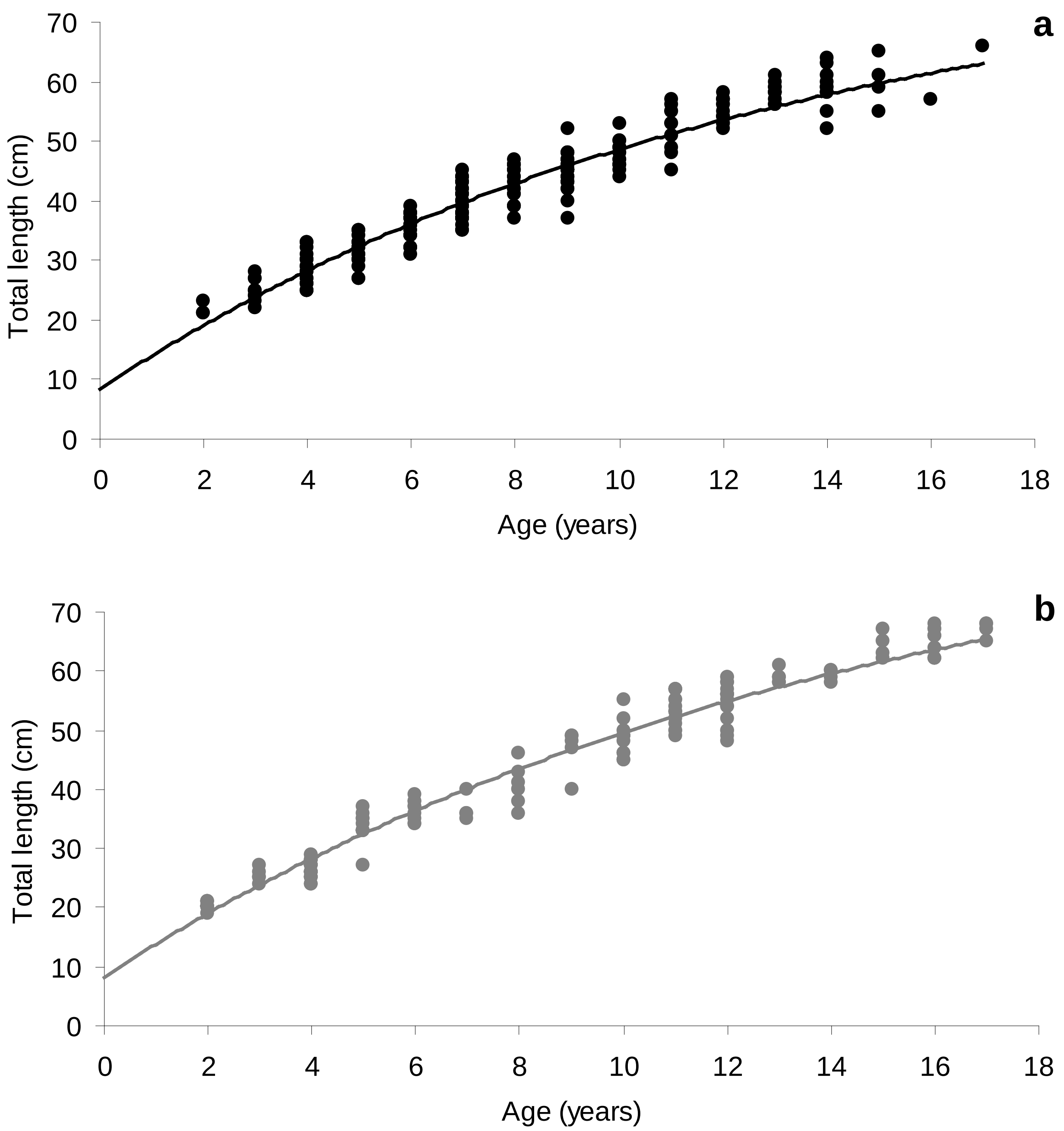

Figure 8. Von Bertalanffy growth curves fitted to the estimated age-length data pairs of *Chaenocephalus aceratus* from the South Orkney Islands (**a**) and South Shetland Islands (**b**).

Table 1. Likelihood ratio test comparing von Bertalanffy parameter estimates for both populations of *Chaenocephalus aceratus*. Statistics are based on null hypotheses, assuming that each parameter or a combination of them do not differ between areas. RSS = residual sum of squares; df = degree of freedom; * significant; ns = not significant.

Parameter	**South Orkney**			**South Shetland**							
	L_∞	k	t_0	L_∞	k	t_0	**RSS**	χ^2	df	P	
Hypothesis											
Independent	82.99	0.08	-1.37	90.82	0.07	-1.38	3149.50				
H_0:=L_∞; =k; =t_0	88.16	0.07	-1.40	88.16	0.07	-1.40	3242.92	14.61	3	0.0001	*
H_0:=L_∞	87.04	0.07	-1.48	87.04	0.08	-1.28	3173.35	3.77	1	0.0521	ns
H_0:=k	85.49	0.07	-1.45	88.12	0.07	-1.30	3160.40	1.73	1	0.1887	ns
H_0:=t_0	83.08	0.08	-1.38	90.71	0.07	-1.38	3149.52	0.00	1	0.9526	ns

Size and age at first spawning

Size at first spawning (TL_{50}) was 57.1 cm (CI: 56.7 - 57.5) for females (n = 91, $r^2 = 0.99$, $p < 0.0001$) and 43.9 cm (CI: 43.1 - 44.7) for males (n = 52, $r^2 = 0.99$, $p < 0.0001$) from SOI. From SSI, it was 57.7 cm (confidence interval CI: 57.1 - 58.3) for females (n = 142, $r^2 = 0.99$, $p < 0.0001$) and 45.4 cm (CI: 44.9 - 46.0) for males (n = 69, $r^2 = 0.99$, $p < 0.0001$) (Fig. 9). Based on the von Bertalanffy models, age at first spawning in females was 14 years for SOI and 13 years for SSI. Allowing for the maximum ages for each region, this indicated only two female spawning cohorts at SOI and five at SSI.

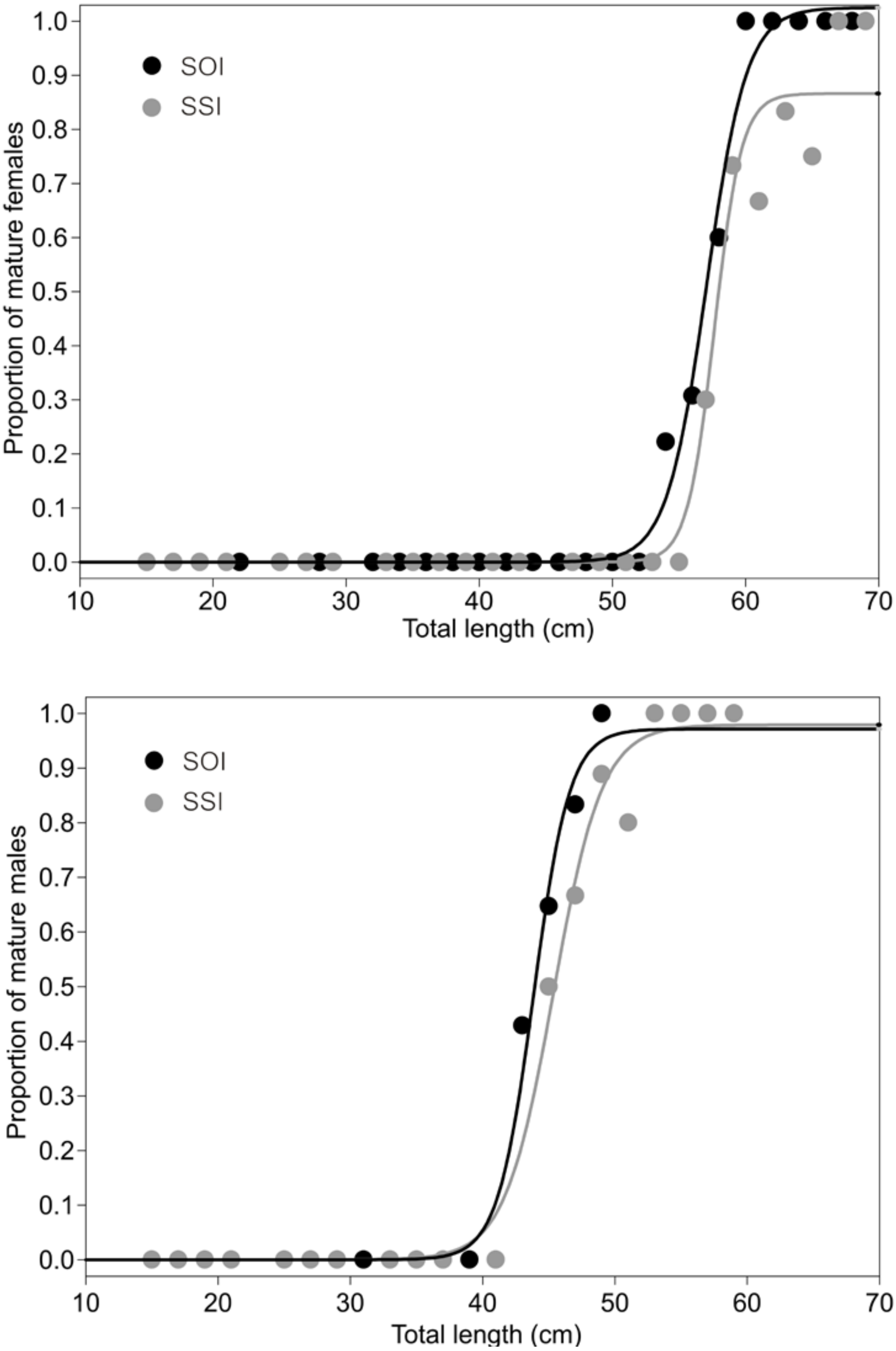

Figure 9. Logistic curves fitted to the proportion of spawner individuals of *Chaenocephalus aceratus* from the South Orkney Islands (SOI) and South Shetland Islands (SSI).

Discussion

Spatial differences in life history strategy

Examining the reproductive biology and life history traits of *C. aceratus* caught off SOI and SSI, we found evidence of similar patterns of sexual dimorphism, gametogenesis and stages of maturity. In samples from both areas, histological analyses confirmed that females have group synchronous ovaries (Wallace and Selman 1981), with a bimodal oocyte size distribution (Lisovenko 1988). The occurrence of late vitellogenic oocytes together with others at the cortical alveoli stage supported a prolonged oogenesis, likely lasting more than one year (Lisovenko 1988). The pronounced bimodal distribution confirmed *C. aceratus* as a total spawner. In males, spermatogenesis appeared synchronous as well. Developing males presented lobule lumina completely filled with sperm and few cysts of early stages of spermatogenesis only in the distal portions of the lobules, indicating that spermatogenesis is complete at the beginning of the spawning period. The completion of spermatogenesis before the spawning period has been reported in other notothenioid fish (e.g. Russo *et al*. 2000; La Mesa *et al*. 2003, 2006) and is likely related to the long time required for spermatogenesis at low temperature and the high reproductive investment. The similarities in the gametogenic stage of developing males and females supported a synchronous breeding period between the two regions (Kock and Jones 2005).

Nevertheless, we found strong spatial differences. Overall, the mean size of fish caught off SOI was larger and the length frequencies differed sharply, with relatively few smaller fish caught. Moreover, weight increased faster with length for SOI, whereas female gonad weight and GSI in both sexes were lower. Notably, absolute fecundity was 46% lower than for SSI, and increased much more slowly with size; the proportion of female spawning cohorts was lower for the SOI as well. There was evidence of significant differences in growth between regions, with L_∞ lower and k higher for SOI. These results corroborated regional separation after settlement, and suggested distinct life history strategies, in which fish from SOI invested much less in reproduction and somewhat more in somatic growth earlier in their life history.

Confounding biases between regions were unlikely to have caused the differences. Gear selectivity may introduce some differences between RV *Yuzhmorgeologiya* and RV *Polarstern*. However, the regional disparity in length frequencies was much larger than between vessels during previous surveys of the SSI (e.g. Jones *et al*. 2003; Lucassen *et al*. 2012), and sampling from the RV *Yuzhmorgeologiya* at the SOI used the same equipment and nets as the vessel's earlier surveys. Alternative explanations for the lack of smaller fish around SOI were that they followed a clumped spatial distribution or were not available to the gear. However, a previous survey of the SOI in 1999 found similar length distributions, and sampling covered the same range of depths as for the SSI; instead, smaller fish may have occupied depths shallower than 50m at the SOI. Additionally, because there has been no age validation for *C. aceratus*, there might have been biases in the age data. However, the readings were made for both regions by the same reader trained using the same criteria. The consistency between readings was reasonable for both regions and any biases in interpreting growth increments would have applied to both regions. As a result, although the data should be treated with caution as estimates of true age, regional comparisons were appropriate and unlikely to have been confounded by biases during reading. Finally, because sampling was not synoptic, inter-annual and seasonal variability might have confounded measures of life history traits. In particular, the slightly larger egg size in SSI might have reflected sampling one month later in the season. However, the seasonal discrepancy was too small to have much effect on most measures, and the regional differences in relative and absolute fecundity were consistent with observations made during previous studies (Kock and Kellermann 1991; Kock and Jones 2005; Tab. 2). It is therefore unlikely that temporal variability could account for the large spatial differences we found.

Instead, environmental variation may be involved. The influence of water from the Weddell Sea is considerable over the South Orkney shelf and decreases northwards (Kock *et al*. 2000), and the seasonal effect of pack ice is longer and more intense with more and deeper benthic scouring by icebergs. Food availability may have been important: *C. aceratus* feeds mainly on krill during its juvenile phase and on fish as adults, and there is evidence of differences in krill abundance between regions with greater biomass at SSI in some years (Nicol *et al*. 2000) to support locally abundant juveniles. In this environmental context, SSI fish could take advantage of the warmer shelf waters and higher food availability to invest more in reproduction. Phenotypic differences have been documented previously between regions in growth parameters and parasite infestation (Sosinski and Janusz 2000; La Mesa *et al*. 2004), and life history traits are considered to be phenotypic expressions of the interaction between genotypic and environmental effects (Begg 2005). In *C. aceratus*, however, gene flow via larval dispersal is likely to counteract genetic drift (Papetti *et al*. 2009; Damerau *et al*. 2012) and, rather than genetic differentiation, the differences highlighted in this study are likely to be more the result of a phenotypic response to the prevailing environmental and ecological conditions. Instead of the latitudinal trend often seen elsewhere (e.g. Ashton 2001; Meiri and Dayan 2003; Laptikhowsky 2006; Fisher *et al*. 2010; Huston and Wolverton 2011), the conditions were in turn related to regional hydrography. Size at sexual maturity was only slightly different between regions, and the response appeared strongest in mature individuals related to reproductive investment.

Table 2. Reproductive characteristics of *Chaenocephalus aceratus* females from different areas. F_{abs} = absolute fecundity; F_{rel} = relative fecundity; GSI = gonadosomatic index; - =not available. Source: 1 = Kock and Kellermann 1991; 2 = Kock 2005; 3 = Kock 1989.

Area	Max egg size (mm)	Size at spawning (TL_{50}, cm)	F_{abs}	F_{rel}	Lenght range (cm)	Max GSI (female)	Source
South Georgia	4.4	47 ♂, 58 ♀	7358 - 22626	5 - 9	48 - 73	27	1, 2
South Georgia	--	--	3082 - 22626	3.9 – 9.2	48 - 72	--	1, 2
South Georgia	--	--	5898 - 18039	4 - 6.8	58 - 75	--	1
South Orkney Is.	4.9	--	4499 - 15174	3.9 - 6.8	52 - 67	28	1, 2
South Orkney Is.	4.8	44 ♂, 57 ♀	6534 - 13529	4 - 7.4	52 - 66	15	present study
Elephant Is.	4.7	46 ♂, 57 ♀	6711 - 19672	4.9 - 9.2	51 - 66	25	3
South Shetland Is.	--	--	7375 - 12450	4.6 - 8.2	53 - 62	--	3
South Shetland Is.	4.3	45 ♂, 58 ♀	11493 - 25012	5.2 - 12.2	56 - 68	23	present study

Life history strategies and population connectivity

Lower reproductive capacity around the SOI suggests that, reinforced by strong eastward flow along the southern ACC and flows from the Weddell Sea around the Bransfield Strait and Powell Basin, the SSI may be more important in influencing distributions and abundance along the southern Scotia Ridge. Nevertheless, westward gene flow (Papetti *et al*. 2009) implies a corresponding dispersal mechanism. Ashford *et al*. (2010) suggested the possibility of counter-current movement by an active post-larval stage prior to settlement; alternatively, the origin of the westward-flowing Polar Current remains unclear, and the genetic data suggest it may reach as far eastward as the SOI. A larger proportion of sexually mature fish at the SOI may also help counteract low fecundity. Even so, higher fecundity and a larger number of spawning cohorts combined with the much larger volume of eastward flow, suggest that the SSI have a much greater influence on the SOI than vice versa.

Variation in the spatial delivery of life stages between habitats can impact abundance and distribution over large areas. Although currents that connect disparate areas create opportunities for succeeding stages during the life history (e.g. Harden Jones 1968), adult *C. aceratus* appear highly constrained spatially: fish dispersed downstream in the ACC appear unable to maintain population membership (Sinclair 1988) by returning to the areas where they were spawned. Fish that are entrained in life history trajectories that do not allow closure become non-breeding vagrants (Sinclair 1988), among which abundance is determined by immigration and local mortality. Alternatively, dispersed fish can eventually join another population and spawn: abundance in the new population is then determined by immigration as well as self-recruitment and mortality. Our results showed that almost all fish in the largest size classes were spawners, suggesting that any fish arriving at SOI or SSI were migrants that joined locally self-recruiting populations.

These considerations have implications for fisheries management. Migrants can help maintain sink populations in which self-recruitment is insufficient to offset local mortality (Pulliam 1988; Polachek 1990). Low reproductive capacity at SOI, and higher mortality compared to SSI, suggest that immigration may be relatively important. *C. aceratus* was an important target species from 1976 onwards: early on, it was caught around South Georgia, and then successively around SOI and SSI. Data on yearly catch of *C. aceratus* are incomplete and uncertain (Kompowski 1994; Kock and Jones 2005), but those available show that catches reached ca. 2000 tons between the 1970s and the 1980s (CCAMLR 1990; Kompowski 1990; Kock *et al*. 2000), declining suddenly to 40% of the previous biomass (Kock 1991). Length and age composition changed, and catches consisted mostly of immature fish (Kompowski 1990). Longer residence times for females in the fishery grounds (Kock 1990), and parental care by males may have increased availability and vulnerability to fishing. Moreover, maximum size and age, and size and age at maturity, are considered important indicators of vulnerability to fishing in marine species (Jennings *et al*. 1998; Dulvy *et al*. 2003). In *C. aceratus*, the late maturity relative to maximum age suggests that caution is advisable when considering future exploitation. Moreover, the presence of fewer spawning cohorts, combined with geographical isolation after settlement, imply that the population at SOI is more vulnerable to natural and anthropogenic impacts than SSI. For comparison, we calculate based on Royce's (1972) approach that raising mortality to = 0.35 from = 0.31 would juvenesce the population at SOI below the age at sexual maturity, whereas an increase from = 0.27 to = 0.38

would be necessary at the SSI. Interruptions to migration can lead to extinctions of sink populations, even without local changes in births and deaths; under these conditions, connectivity from the SSI may be important in maintaining abundances at SOI. A recent proposal for a marine protected area (MPA) around the SOI can help preserve local self-recruitment and protect the population from increases in mortality due to fishing, but further protection of source populations is necessary if persistence depends on immigration from outside the MPA.

C. aceratus shares common features in life history traits with other icefish species. Evidence of large female reproductive effort includes high gonadosomatic indices (GSI), gonad weights ranging from 20 to 30% of the total body weight, and production of eggs that are often more than 4 mm of diameter (Kock 2005). Absolute fecundity is generally lower than for *C. aceratus*, from less than 1000 eggs in small-sized species (e.g. *Pagetopsis maculatus*) to only rarely more than 20000 in some of the larger species (e.g. *Cryodraco antarcticus*) (Kock and Kellermann 1991). Relative fecundity is less than 15 eggs per gram of body weight (Kock 2005). Larval and juvenile phases are pelagic in most icefish and may last for 2-4 years, resulting in a high potential for dispersal along transport pathways but also in considerable vulnerability of early life history stages (Kock 2005), especially to advective mortality. Slow somatic growth and late sexual maturity (around 5-8 years in several species) are other common features of icefish life history. Parental care has been documented in six species of channichthyids (Detrich *et al*. 2005; Kock 2005; Kock *et al*. 2006, 2008; Ferrando *et al*. 2014; Knust and Schröder 2014), and nest guarding is suspected in other icefish (e.g. *Chionodraco* spp.), influencing the timing and release of larvae and hence the direction of connectivity (Ashford *et al*. 2010). Intra-specific variability is unlikely to be restricted to *C. aceratus*: *Champsocephalus gunnari* and to a lesser extent *Gobionotothen gibberifrons* and *Notothenia coriiceps* showed evidence of similar regional differences (Kock and Jones 2005).

Better understanding of the interplay between population connectivity and life history strategy, and the physical-biological interactions involved, can help managers maintain the distribution and abundance of icefish species along the southern Scotia Ridge and around the Antarctic.

References

Anderson M.J., Gorley R.N., Clarke K.R. (2008). PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth UK.

Ashford J.R., Arkhipkin A.I., Jones C.M. (2006). Can the chemistry of otolith nuclei determine population structure of Patagonian toothfish *Dissostichus eleginoides*? J. Fish. Biol. 69:708-721.

Ashford J.R., Jones C.M., Hofmann E.E., Everson I., Moreno C.A., Duhamel G., Williams R. (2008). Otolith chemistry indicates population structuring by the Antarctic Circumpolar Current. Can. J. Fish. Aquat. Sci. 65: 35-146.

Ashford J.R., La Mesa M., Fach B.A., Jones C.M., Everson I. (2010). Testing early life connectivity using otolith chemistry and particle-tracking simulations. Canadian Journal of Fisheries and Aquatic Sciences 67: 1303-1315.

Ashford J.R., Dinniman M., Brooks C., Andrews A.H., Hofmann E., Cailliet G., Jones C.D., Ramanna N. (2012). Does large-scale ocean circulation structure life history connectivity in Antarctic toothfish (*Dissostichus mawsoni*)? Canadian Journal of Fisheries and Aquatic Sciences 69: 1903-1919.

Ashton K.G. (2001). Are ecological and evolutionary rules being dismissed prematurely? Diversity Distribution 7: 289-295.

Bagenal T.B., Tesch F.W. (1978). Age and growth. In: Bagenal T.B. (eds) Methods for assessment of fish production in fresh waters, 3rd edn. IBP Handbook No. 3, Blackwell Science Publications, Oxford, 101-136.

Beamish R.J., Fournier D.A. (1981). A method of comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences 38: 982-983.

Begg G.A. (2005). Life history parameters. In: Cadrin S.X., Friedland K.D., Waldman J.R. (eds) Stock Identification Methods. Applications in Fishery Science, Elsevier, Amsterdam, 119-150.

Billard R. (1986). Spermatogenesis and spermatology of some teleost fish species. Reproduction Nutrition Development 26: 877-920.

Campana S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197-242.

CCAMLR (1990). Statistical Bulletin 1. CCAMLR, Hobart, Australia.

Chang W.Y.B. (1982). A statistical method for evaluating the reproducibility of age determination. Canadian Journal of Fisheries and Aquatic Sciences 39: 1208-1210.

Cowen R.K., Sponaugle S. (2009). Larval dispersal and marine population connectivity. Annual Review of Marine Science 1: 443-466.

Damerau M., Matschiner M., Salzburger W., Hanel R. (2012). Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica. Polar Biology 35: 1073-1086.

Detrich H.W., Jones C.D., Kim S., North A.W., Thurber A., Vacchi M. (2005). Nesting behaviour of the icefish *Chaenocephalus aceratus* at Bouvetøya Island, Southern Ocean. Polar Biology 28: 828-832.

Dulvy N., Sadovy Y., Reynolds J.D. (2003). Extinction vulnerability in marine populations. Fish and Fisheries 4: 25-64.

Eastman J.T. (1993). Antarctic fish biology: Evolution in a unique environment. Academic Press, San Diego, 322 pp.

Eastman J.T., Sidell B.D. (2002). Measurements of buoyancy for some Antarctic notothenioid fishes from the South Shetland Islands. Polar Biology 25: 753-760.

Everson I. (1980). Antarctic fish age determination methods. BIOMASS Handbook 8: 1-24.

Fach B.A., Klinck J.M. (2006). Transport of Antarctic krill (*Euphausia superba*) across the Scotia Sea. Part I: Circulation and particle tracking simulations. Deep Sea Research Part I 53: 987-1010.

Ferrando S., Castellano L., Gallus L., Ghigliotti L., Masini M.A., Pisano E., Vacchi M. (2014). A demonstration of nesting in two Antarctic icefish (genus *Chionodraco*) using a fin dimorphism analysis and *ex situ* videos. PLoS ONE 9: e90512.

Fisher J.A.D., Frank K.T., Leggett W.C. (2010). Global variation in marine fish body size and its role in biodiversity–ecosystem functioning. Marine Ecology Progress Series 405: 1-13.

Gordon A.L., Visbeck M., Huber B. (2001). Export of Weddell Sea Deep and Bottom Water. Journal of Geophysical Research 106(C5): 9005-9018.

Grier H.J., Linton J.R., Leatherland J.F., de Vlaming V.L. (1980). Structural evidence for two different testicular types in teleost fishes. The American Journal of Anatomy 159: 331-345.

Gubsch G. (1980). Untersuchungen zur Alterbestimmung und zum Wachstum beim Eisfisch *Chaenocephalus aceratus* (Lönnberg). Fischerei-Forschung 18: 7-10.

Hanchet S.M., Rickard G.J., Fenaughty J.M., Dunn A., Williams M.J. (2008). A hypothetical life cycle for Antarctic toothfish *Dissostichus mawsoni* in Antarctic waters of CCAMLR Statistical Area 88. CCAMLR Science 15: 35-54.

Harden Jones F.R. (1968). Fish migration. Edward Arnold, London, 325 pp.

Heywood K.J., Naveira Garabato A.C., Stevens D.P., Muench R.D. (2004). On the fate of the Antarctic Slope Front and the origin of the Weddell Front. Journal of Geophysical Research 109: C06021.

Hofmann E.E., Klinck J.M., Locarnini R.A., Fach B., Murphy E. (1998). Krill transport in the Scotia Sea and environs. Antarctic Science 10: 406-415.

Hunter J.R., Lo N.C.H., Leong R.J.H. (1985). Batch fecundity in multiple spawning fishes. NOAA Technical Report, NMFS 36: 67-77.

Huston M.A., Wolverton S. (2011). Regulation of animal size by eNPP, Bergmann's rule, and related phenomena. Ecological Monographs 81: 349-405.

Ihssen P.H., Booke H.E., Casselman J.M., McGlade J.M., Payne N.R., Utter F.M. (1981). Stock identification: materials and methods. Canadian Journal of Fisheries and Aquatic Sciences 38: 1838-1855.

Iwami T., Kock K.H. (1990). Channichthyidae. In: Gon O., Hemstra P.C. (eds) Fishes of the Southern Ocean. J.L.B. Smith Institute of Ichthyology, Grahamstown, 381–399.

Jennings S., Reynolds J.D., Mills S.C. (1998). Life history correlates of responses to fisheries exploitation. Proceedings of the Royal Society B: Biological Sciences 265: 333-339.

Jones C.D., Kock K.H., Ashford J.R., DeVries A., Dietrich K., Hanchet S., Near T.J., Turk T., Wilhelms S. (2003). Standing stock, biology, diet and spatial distribution of demersal finfish from the 2003 US AMLR bottom trawl survey of the South Shetland Islands (Subarea 48.1). Document WG-FSA-03/38, CCAMLR, Hobart, Australia.

Jones C.D., Kock K.H. (2009). Standing stock, spatial distribution, and biological features of demersal finfish from the 2009 US AMLR bottom trawl survey of the South Orkney Islands (Subarea 48.2). Document WG-FSA-09/19, CCAMLR, Hobart, Australia.

Kellermann A. (1986). Geographical distribution and abundance of postlarval and juvenile *Pleuragramma antarctica* (Pisces, Notothenioidei) off the Antarctic Peninsula. Polar Biology 6: 111-119.

Kimura D.K. (1980). Likelihood methods for the von Bertalanffy growth curve. Fishery Bulletin 77: 765-776.

Knust R., Schröder M. (2014). The Expedition PS82 of the Research Vessel POLARSTERN to the southern Weddell Sea in 2013/2014 . Berichte zur Polar- und Meeresforschung = Reports on polar and marine research. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, 680: 1-155.

Kock K.H. (1981). Fischereibiologische Untersuchungen an drei antarktischen Fischarten: *Champsocephalus gunnari* Lonnberg 1905, *Chaenocephalus aceratus* (Lonnberg, 1906) und *Pseudochaenichthys georgianus* Norman, 1937 (Notothenioidei, Channichthyidae). Mitt. Inst. Seefisch. Hamburg 32: 1-226.

Kock K.H. (1989). Reproduction in fish around Elephant Island. Archiv für Fischereiwissenschaft 39: 171-210.

Kock K.H. (1990). Reproduction of the mackerel icefish *Champsocephalus gunnari* and its implications for fisheries management in the Atlantic sector of the Southern Ocean. SC-CAMLR Select Scientific Papers 1989, 51-68.

Kock K.H. (1991). The state of exploited fish stocks in the Southern Ocean-A review. Archiv für Fischereiwissenschaft 41: 1-66.

Kock K.H. (2005). Antarctic icefishes (Channichthyidae): a unique family of fishes. A review, Part I. Polar Biology 28: 862-895.

Kock K.H., Kellermann A. (1991). Reproduction in Antarctic notothenioid fish. Antarctic Science 3: 125-150.

Kock K.H., Jones C.D., Wilhelms S. (2000). Biological characteristics of Antarctic fish stocks in the southern Scotia Arc region. CCAMLR Science 7: 1-42.

Kock K.H., Stransky C. (2000). The composition of the coastal fish fauna around Elephant Island (South Shetland Islands, Antarctica). Polar Biology 23: 825-832.

Kock K.H., Jones C.D. (2005). Fish stocks in the southern Scotia Arc region-a review and prospects for future research. Reviews in Fisheries Science 13: 75-108.

Kock K.H., Pshenichnov L.K., Devries A.L. (2006). Evidence for egg brooding and parental care in icefish and other notothenioids in the Southern Ocean. Antarctic Science 18: 223-227.

Kock K.H., Pshenichnov L., Jones C.D., Gröger J., Riehl R. (2008). The biology of the spiny icefish (*Chaenodraco wilsoni* Regan, 1914). Polar Biology 31: 381-393.

Kompowski A. (1990). Biological characteristics of Scotia Sea icefish *Chaenocephalus aceratus* (Lönnberg, 1906) from South Georgia area. Rep. Sea Fish. Inst. Gdynia 22: 49-71.

Kompowski A. (1994). Changes in the blackfin icefish stock structure (*Chaenocephalus aceratus* (Lönnberg, 1906), Pisces, Notothenioidei, Chanichthyidae) of South Georgia within 1975–1992. Acta Ichthyologica et Piscatoria 24: 53-60.

La Mesa M., Vacchi M., Iwami T., Eastman J.T. (2002). Taxonomic studies of the Antarctic icefish genus *Cryodraco* Dollo, 1900 (Notothenioidei: Channichthyidae). Polar Biology 25: 384-390.

La Mesa M., Caputo V., Rampa R., Vacchi M. (2003). Macroscopic and histological analyses of gonads during the spawning season of *Chionodraco hamatus* (Pisces, Channichthyidae) off Terra Nova Bay, Ross Sea, Southern Ocean. Polar Biology 26: 621-628.

La Mesa M., Ashford J.R., Larson E., Vacchi M. (2004). Age and growth of Scotia Sea icefish, *Chaenocephalus aceratus*, from the South Shetland Islands. Antarctic Science 16: 253–262.

La Mesa M., Caputo V., Eastman J.T. (2006). Gametogenesis and reproductive strategies in some species of the Antarctic fish genus *Trematomus* (Nototheniidae) from Terra Nova Bay, Ross Sea. Polar Biology 29: 963-970.

La Mesa M., Ashford J.R. (2008). Age and early life history of juvenile Scotia Sea icefish, *Chaenocephalus aceratus*, from Elephant Island and the South Shetland Islands. Polar Biology 31: 221-228.

La Mesa M., Riginella E., Mazzoldi C., Ashford J.R. (2014). Reproductive resilience of ice-dependant Antarctic silverfish in a rapidly changing system along the Western Antarctic Peninsula. Marine Ecology. doi:10.1111/maec.12140.

La Mesa M., Piñones A., Catalano B., Ashford J.R. (2015) Predicting early life connectivity of Antarctic silverfish, an important forage species along the Antarctic Peninsula. Fish. Oceanogr. 24: 150-161.

Laptikhovsky V. (2006). Latitudinal and bathymetric trends in egg size variation: a new look at Thorson's and Rass's rules. Marine Ecology 27: 7-14.

Lisovenko L.A. (1988). Some new information on the reproduction of *Chaenocephalus aceratus* (Fam. Channichthyidae) of the region of the Island of South Georgia. Journal of Ichthyology 28: 130-135.

Lucassen M. (2012). The expedition of the research vessel "Polarstern" to the Antarctic in 2012 (ANT-XXVIII/4). Berichte zur Polar- und Meeresforschung = Reports on polar and marine research. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, 652: 1-90.

Meiri S., Dayan T. (2003). On the validity of Bergmann's rule. Journal of Biogeography 30: 331-351.

Munro J.L., Pauly D. (1983). A simple method for comparing the growth of fishes and invertebrates. ICLARM Fishbyte 1: 5-6.

Murua H., Kraus G., Saborido-Rey F., Witthames P.R., Thorsen A., Junquera S. (2003). Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal of Northwest Atlantic Fishery Science 33: 33-54.

Nagahama Y. (1983). The functional morphology of teleost gonads. In: Randall D.J., Hoar W.S., Donaldson E.M. (eds) Fish physiology. Academic, London, 223-275.

Nicol S., Constable A., Pauly T. (2000). Estimates of circumpolar abundance of Antarctic krill based on recent acoustic density measurements. CCAMLR Science 7: 87-99.

North A.W. (1988). Age of Antarctic fish: validation of the timing of annuli formation in otoliths and scales. Cybium 12: 107-114.

Orsi A.H., Whitworth III T., Nowlin J.W.D. (1995). On the meridional extent and fronts of the Antarctic Circumpolar Current. Deep Sea Research 42: 641-673.

Papetti C., Susana E., La Mesa M., Kock K.H., Patarnello T., Zane L. (2007). Microsatellite analysis reveals genetic differentiation between year-classes in the icefish *Chaenocephalus aceratus* at South Shetlands and Elephant Island. Polar Biology 30: 1605-1613.

Papetti C., Susana E., Patarnello T., Zane L. (2009). Spatial and temporal boundaries to gene flow between *Chaenocephalus aceratus* populations at South Orkney and South Shetlands. Marine Ecology Progress Series 376: 269-281.

Pearse A.G.E. (1985). Histochemistry, Theoretical and Applied Analytical Technology. Churchill Livingstone, Edinburgh, 624 pp. doi:10.1002/path.1711470319.

Polachek T. (1990). Year round closed areas as a management tool. Natural Resource Modeling 4: 327-354.

Pulliam H.R. (1988). Sources, sinks and population dynamics. The American Naturalist 132: 652-661.

Royce W.F. (1972). Introduction to the fishery sciences. Academic Press, New York, 351 pp.

Russo A., Angelini F., Carotenuto R., Guarino F.M., Falugi C., Campanella C. (2000). Spermatogenesis in some Antarctic teleosts from the Ross Sea: histological organisation of the testis and localisation of bFGF. Polar Biology 23: 279-287.

Shaw P.W., Arkhipkin A.I., Al-Khairulla H. (2004). Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep water troughs as barriers to genetic exchange. Molecular Ecology 13: 3293-3303.

Siegel V. (1980). Quantitative investigations on parasites of Antarctic channichthyid and notothenioid fishes. Meeresforschung, Reports on Marine Research 28: 146-156.

Sinclair M. (1988). Marine populations: an essay on population regulation and speciation. University of Washington Press, Seattle, 252 pp.

Smith D.A., Hoffmann E.E., Klinck J.M., Lascara C.M. (1999). Hydrography and circulation of the West Antarctic Peninsula Continental Shelf. Deep Sea Research Part I 46: 925-949.

Sokal R.R., Rohlf F.J. (1995). Biometry: the principle and practice of statistics in biology research. Freeman and Co., New York, 887 pp.

Sosinski J., Janusz J. (2000). Infection variability of the parasitic copepod *Eubrachiella antarctica* (Quidor, 1906) on fishes in the Atlantic sector of the Antarctic. Bulletin of the Sea Fisheries Institute 2: 25-41.

Thompson A.F., Heywood K.J., Thorpe S.E., Renner A.H.H., Trasvina A. (2009). Surface Circulation at the tip of the Antarctic Peninsula from drifters. Journal of Physical Oceanography 39: 3-26.

Thorpe S.E., Murphy E.J., Watkins J.L. (2007). Circumpolar connections between Antarctic krill (*Euphausia superba* Dana) populations: Investigating the roles of ocean and sea ice transport. Deep Sea Research Part I 54: 792-810.

Wallace R.A., Selman K. (1981). Cellular and dynamic aspects of oocyte growth in teleost. American Zoologist 21: 325-343.

Watson J.R., Kendall B.E., Siegel D.A., Mitarai S. (2012). Changing seascapes, stochastic connectivity, and marine metapopulation dynamics. The American Naturalist 180: 99-112.

West G. (1990). Methods of assessing ovarian development in fishes: a review. Australian Journal of Marine and Freshwater Research 41: 199-222.

White M.G. (1991). Age determination in antarctic fish. In: di Prisco G., Maresca B., Tota B. (eds) Biology of Antarctic fish. Springer Science & Business Media, Berlin Heidelberg New York, 87-100.

PAPER IV

Life history strategies of *Notothenia rossii* and *N. coriiceps* along the Southern Scotia Arc

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Abstract

Notothenia rossii and *N. coriiceps* are two nototheniid species inhabiting the Antarctic continental-shelf and surrounding islands. This study provides insights on some aspects of the reproductive biology and age structure in *N. coriiceps* (from South Shetland Islands - SS) and in *N. rossii*, in which we compared life history traits between fish sampled off the SS and around South Georgia (SG). Histological analyses provided new description of gonadal maturation in both males and females. Absolute fecundity estimations ranged from 8300 to 52000 eggs/female in *N. coriiceps* and from 6700 to 68000 eggs/female in *N. rossii*, in both cases with fecundity positively related to size. Age estimations highlighted that *N. coriiceps* can live up to 18 years, while *N. rossii* up to 24 years, a value highest than the maximum age previously estimated. At intraspecific level, no differences in life history traits emerged between the two study areas in *N. rossii*. Comparing the two species, this study highlighted high similarities in life history traits, despite their differences in adult ecology.

Introduction

The Nototheniidae represents the most diverse family in the Suborder Notothenioidea in terms of size, body shape and ecological diversification (Eastman 1993; Rutschmann *et al.* 2011; Dettai *et al.* 2012). Nototheniids species are distributed mainly around Antarctica (34), while few ones are widespread along New Zealand and South America coasts (Eastman 1993). The family includes 12 genera, accounting for 49 species (*Aethotaxis*, *Cryothenia*, *Dissostichus*, *Gobionotothen*, *Gvozdarus*, *Indonototheni*a, *Lepidonotothen*, *Notothenia*, *Paranotothenia*, *Patagonotothen*, *Pleuragramma, Trematomus*) (Eastman and Eakin 2000; Dettai *et al.* 2012; Near *et al.* 2012). From the evolutionary aspect, ancestors presented benthic habits, while some derived species occupied other habitats, such as cryopelagic, pelagic, semipelagic and epibenthic ones (Eastman 1993). Among nototheniids, the genus *Notothenia* includes 5 species, two of these (*N. angustata* and *N. microlepidota*) are distributed in cool temperate waters, whereas *N. rossii, N. coriiceps* and *N. cyanobrancha* live in Antarctic waters (beyond the Antarctic Circumpolar Current 45°-60°S). These species are typically part of the benthic community in the shallow coastal waters, except for the adult of *N. rossii*, which are considered bentho-pelagic (Cheng *et al.*2003; Eastman *et al.* 2011).

Fish life history traits are known to show variability at interspecific, even between closely related species, and intra-specific level, in relation to environmental conditions and species habits (Begon *et al.* 1990; Roff 2002; Young *et al.* 2015). The genus *Notothenia* represents a good study group to investigate the influence of environmental conditions and species habits on life history traits, as it is composed of species with different ecological features and some of them show wide Antarctic distributions, inhabiting areas with different environmental conditions (Eastman *et al.* 2011).

The black rockcod, *Notothenia coriiceps* Richardson, 1884, and the marbled rockcod, *N. rossii* Richardson, 1884, present wide Antarctic distributions, particularly in shelf areas of the Scotia Arc

(DeWitt *et al.* 1990). However while *N. rossii* is distributed mainly at low latitudes, *N. coriiceps* is also presents at higher latitudes, close to the Antarctic continent. *N. rossii* was one of the first Antarctic fish species overexploited by the commercial finfish fishery, which began in the Scotia sea around 1970s (Kock 1992). With the establishment of the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) in 1982, a series of conservation measures were enforced to promote the recovery of this and other overexploited fish species in many Antarctic zones. Nevertheless, even if finfishing was banned in the South Shetlands Islands and Antarctic Peninsula since 1991, the catches of this species remained very low, but with signals of slow recovery in the latest surveys (Barrera-Oro and Marschoff 2007; Marschoff *et al.* 2012; Kock and Jones 2012). *N. coriiceps* was not commercially exploited in these last decades, but was considered as an accessory catch in the fishing activity (Kock 1992). Therefore, this species did not show the same population decline of his congeneric and both have been used as reference species in monitoring programs of overexploited species (Barrera-Oro *et al.* 2000; Barrera-Oro and Marschoff 2007; Marschoff *et al.* 2012).

The life cycles of these two species are rather well known and unique among notothenioids. Marbled and black rockcod share similar reproductive features, such as an remarkably high investment in reproductive effort, represented by high gonadosomatic index (GSI) both in males (up to 40% of body weight in *N. rossii*) and females (up to 50 % of body weight in *N. rossii*) (Kock and Kellermann 1991). Spawning period occurs in autumn, with a latitudinal temporal variation (Casaux *et al.* 1990). As already described for many notothenioids, females spawn large eggs (4-5 mm in diameter), but differently from most of the other species these eggs have a positive buoyancy, being localized on the surface layer of water column (Kock and Kellermann 1991; White *et al.* 1996). Absolute fecundity in *N. coriiceps* and *N. rossii* is high, especially considering their egg size, with values from few thousands to more than 100000 eggs/female (Kock and Kellermann 1991). The incubation time exceeds 100 days in both species and after hatching, the so called fingerlings have a pelagic life for at least 6-8 months. At later stage, they become demersal and settle in macroalgae beds in coastal waters (White *et al.* 1982; Sapota 1999; Barrera-Oro *et al.* 2014). Demersal juveniles develop in shallow coastal areas until they reach the sexual maturity at 6-9 years (Casaux *et al.* 1990; Eastman *et al.* 2011). Once sexually matured adult individuals of *N. coriiceps*, demersal and sedentary, remain in coastal waters. Instead, those of *N. rossii*, bentho-pelagic and “migratory”, move offshore, joining the adults (Casaux *et al.* 1990; Eastman *et al.* 2011). As *N. coriiceps* lives in close relationship with the bottom, it feeds generally on a wide range of benthic organisms, whereas *N. rossii*, especially adult individuals, feeds on the water column preying mainly krill and fish (Barrera-Oro *et al.* 2002; Eastman *et al.* 2011). The available data on the reproductive biology of these species are referred to several areas but an analysis on its variability between or within species has not been attempted.

This study aims to: i) estimate reproductive investment in terms of Gonadosomatic Index (GSI), absolute and relative fecundity, oocyte diameter, length/age at maturity in the two *Notothenia* species from South Shetlands Islands (SS) and in *N. rossii* also from South Georgia (SG); ii) compare some biological traits at interspecific level, between the two congeneric species, and at intraspecific level, evaluating potential differences in SS and SG *N. rossii* populations.

Materials and methods

Samples were collected during two *RV Polarstern* expeditions, ANT-XXVII/3 and ANT-XXVIII/4, conducted in the Atlantic sector of Southern Ocean in February-April 2011 and in March-April 2012 respectively (Knust *et al.* 2012; Lucassen 2012). Samples of *Notothenia rossii* were caught in two different areas, SG and SS, while *N. coriiceps* samples were collected in SS, in both cases between 50 and 500 meters of depth (Fig. 1). Sampling was carried out using a 130 feet bottom trawl with a codend mesh size of 10 mm, hauled for 30 minutes (Lucassen 2012).

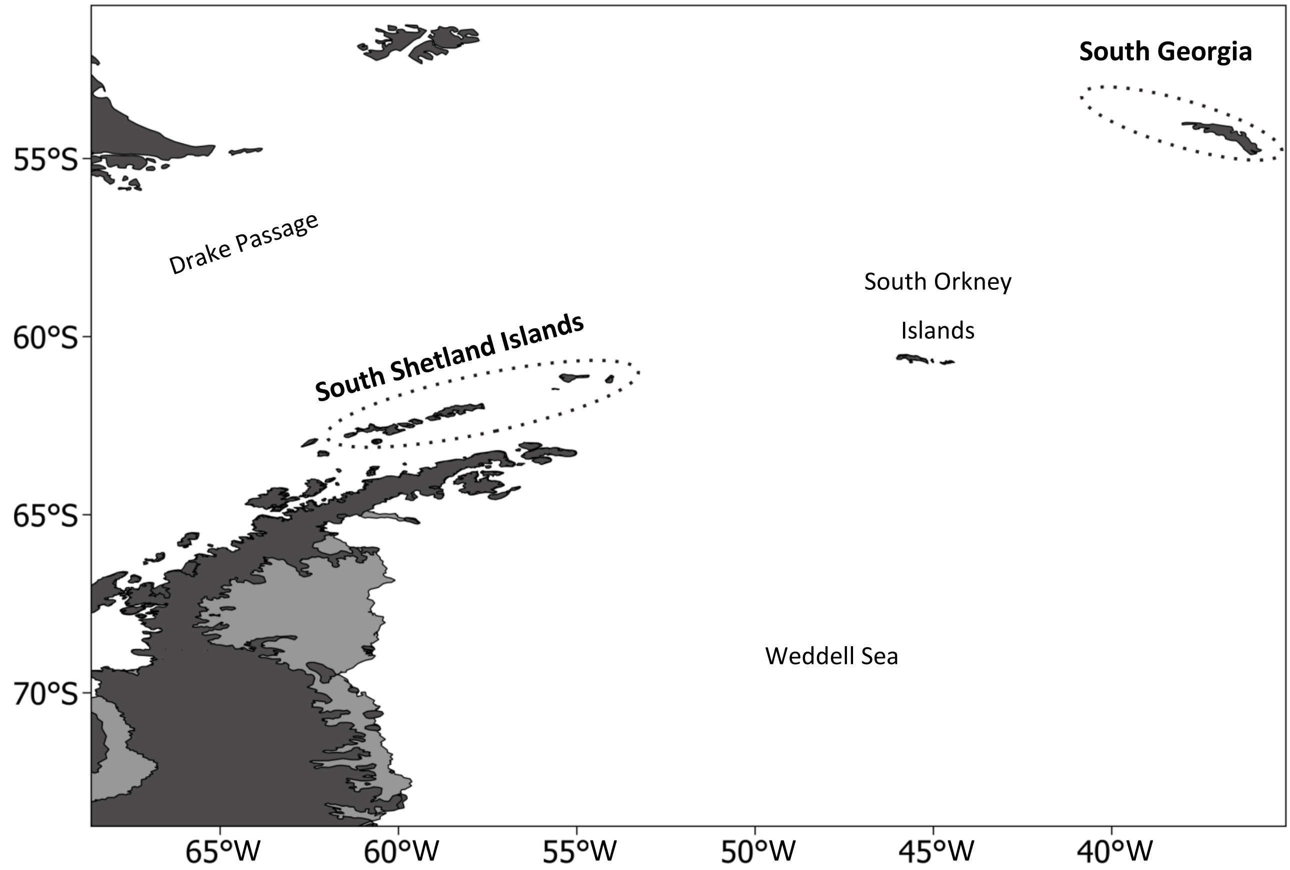

Figure 1. Map of the Southern Scotia Arch, showing the sampling area of *Notothenia rossii* (South Shetland Islands and South Georgia) and *N. coriiceps* (South Shetland Islands).

Fish were sorted from the catches, identified to species level and measured to the nearest lower half centimetre (total length, TL). Total weight (TW, g) and gonad weight (GW, 0.1 g) were also recorded for each specimen. In samples collected in SG, TW and GW were not recorded, therefore TW was estimated by applying the length–weight relationship calculated from SS samples. Sex was assigned macroscopically and gonad maturity stage was evaluated using the five point scale for notothenioids proposed by Kock and Kellermann (1991). To assess the reproductive investment in gonads, GW was used to calculate the gonadosomatic index (GSI= GW / (TW – GW) *100). A subsample of each gonad was weighed and fixed in 10% seawater formaldehyde or Dietrich solutions (Gray 1954), for fecundity estimation or histological analysis respectively. Sagittal otoliths were removed, cleaned and stored dry for ageing purposes. Minimum size at first spawning was estimated considering the smallest male or female at the macroscopic stage of maturity 3 (developing stage in females, developed stage in males).

Gonad morphology and maturity

The histological analyses were conducted on a subsample of 39 specimens, including both sexes in the two species, representing the 20% of the total sample, in order to describe gonad morphology and gametogenesis and to validate the gonad maturity stage attributed macroscopically. Furthermore, the presences of post-ovulatory follicles and/or atretic oocytes were investigate in females. Gonad samples, preserved in Dietrich solution for at least 20 days, were dehydrated, embedded in paraplast, cut in a series of transverse sections (7 µm) and mounted on slides. Slides were then stained with Mayer's haematoxylin and eosin (Pearse 1985). The histological sections were examined under a light microscope (LEICA DM LB).

Based on histological analyses, gonad developmental stages were assigned according to Wallace and Selman (1981), in females, and Billard (1986), in males. Ovaries were classified using five stages of development, based on the oogenesis stage: I – immature (nucleolar and perinucleolar chromatin oocytes); II – early maturation, endogenous vitellogenesis (yolk vesicle formation/cortical alveoli oocytes) ; III – late maturation, exogenous vitellogenesis (vitellogenic oocytes); IV – mature (ripe); V – post-reproduction (post-ovulatory follicles). Similarly, testes were classified using five stages of development, based on the spermatogenesis stage as: I – immature (spermatogonia); II – early maturation (spermatocytes I and II); III – late maturation (spermatids); IV– mature (spermatozoa); V – post-reproduction (residual spermatozoa in collapsed lobules). Maturity stage was attributed considering the most advanced stage observed in ovarian or testicular sections (La Mesa *et al.* 2014).

Fecundity estimation

Fecundity was estimated in developing and ripe females (macroscopic stages 3-4; Kock and Kellermann 1991), using the gravimetric method, therefore counting the number of most advanced oocytes in a weighed subsample (Murua *et al.* 2003). The gonad subsamples represented from 2% to 10% of the GW. To verify the presence of differences in oocyte density across the ovary, in 6 specimens three subsamples were taken from different portions of the ovary (anterior, median and posterior) and compared. As no significant differences among portions were found (Friedman test, χ^2= 2.33, p= 0.311, df= 2), the portion of the ovary for fecundity estimation was randomly chosen. To estimate fecundity, oocytes were immersed in filtered seawater in a Petri dish, manually separated and photographed under a stereomicroscope using a photo camera (LEICA DFC 420). In 4 females per species, the oocytes in the subsample were measured and the frequency distribution of oocyte diameters described. In the remaining samples, only the oocytes of the larger (second) mode, above the threshold size chosen according to the size frequency distributions (see results), were manually counted and a subsample of 170 oocytes were measured using an image software (IMAQ VISION BUILDER 6 software, National Instrument Corporation, Austin, TX, USA) (Meneghesso *et al.* 2013; La Mesa *et al.* 2014). Absolute fecundity (F_{abs}), defined as the standing stock of vitellogenic oocytes (Hunter *et al.* 1992), was estimated applying the follow relationship:

F_{abs} = (n/sw) GW,

where n is the number of vitellogenic oocytes in the subsample and sw is the weight of the subsample. Relative fecundity (F_{rel}) was then calculated as the number of vitellogenic oocytes per gram of body weight (TW). The relationship between fish size and absolute fecundity was assessed applying the least-squares regression analysis to $\log_{10}$-transformed data. Normality and homogeneity of variances were tested by the Shapiro-Wilk and F-ratio tests. To compare the mean size of vitellogenic oocytes between the two sampling areas in *N. rossii*, the t-test for independent data was applied. Analysis of covariance (ANCOVA) was used to test differences in absolute fecundity (log transformed) between samples from different areas, using TL (log transformed) as covariate and the area as fixed factor.

Age estimation

The age was estimated using a randomly selected otolith per specimen. Otoliths of *N. rossii* and *N. coriiceps* have a dense calcareous structure and are opaque, as observed in several notothenioids (Everson 1980; White 1991; La Mesa *et al.* 2009). In order to estimate age, otoliths were burned in an oven at 350° C (to enhance the contrast between ialine and opaque rings), embedded in resin (Crystalbond 509 Amber, Aremo products, inc.), grinded using an abrasive paper and polished on a lapping film (with 0.05 µm alumina powder) to reveal the annulation pattern. Transverse sections across the nucleus of the otoliths were obtained, placed on glass sides using resin and then read immersed in fresh water. The readings were performed, under reflected light, in the region from the nucleus to the proximal side of cross section. Under reflected light, the nucleus and the opaque zones appeared as light rings and the translucent zones as dark rings (Fig. 2). As generally reported in notothenioids (Everson 1980), each pair of opaque and subsequent translucent zones was considered to form an annulus. Assuming that every year the deposition of a single annulus is completed, the age of fish was estimated by counting all translucent or opaque zones.

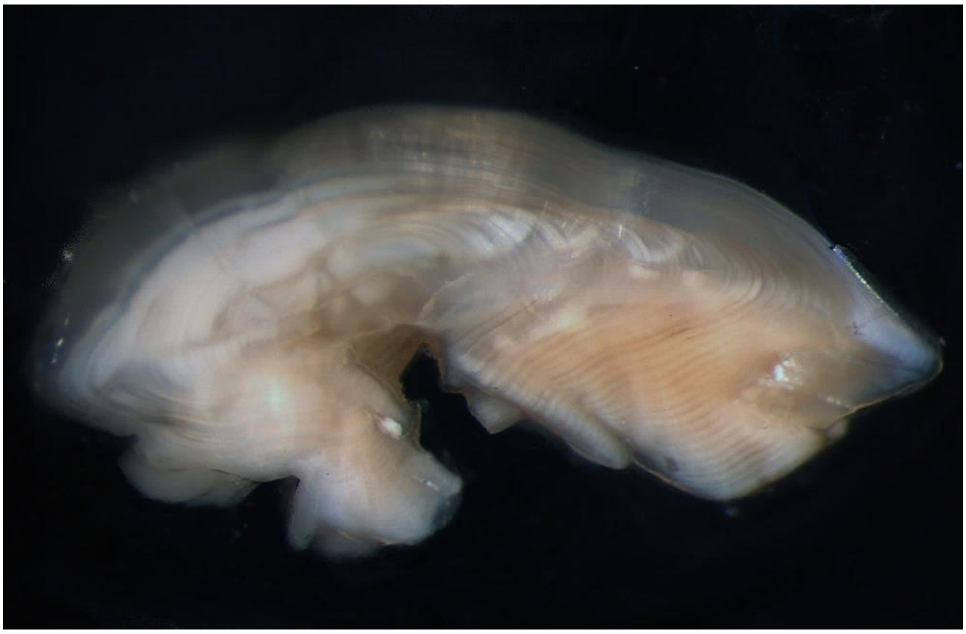

Figure 2. Grinded and polished sagittal otolith of *Notothenia rossii* from South Georgia, showing the annulation pattern of translucent and opaque zones.

Otoliths were read twice by two different readers, blindly respect to fish length. When readings differed by more than one year, a third reading was performed. When the readings differed by more than two years, the otolith was discarded. To evaluate the ageing precision (Campana 2001), the index of average percent error (APE) (Beamish and Fournier 1981) and the mean coefficient of variation (CV) (Chang 1982) were calculated comparing readings within each reader and readings from two readers. To test for differences in the total length-age relationship between specimens of *N. rossii* from different areas, a t test for dependent data was applied.

Results

A total of 137 specimens of marbled rockcod and 44 specimens of black rockcod were analysed (Tab. 1). In *Notothenia rossii* mean total length (TL) in South Georgia (SG) specimens was significantly higher than South Shetland Islands (SS) ones (t= 5.40, p= < 0.001, df= 134).

Table 1. Available samples of *Notothenia rossii* and *N. coriiceps*, in the sampled areas.

	Site	Sex	n	Tot n
N. rossii	South Shetland Is.	♀	50	
		♂	21	71
	South Georgia	♀	33	
		♂	26	
		NA	7	66
N. coriiceps	South Shetland Is.	♀	32	
		♂	12	44

The length-frequency distribution showed an abundance of small and large specimens in SS and the presence of only large-sized specimens in SG (Fig. 3). Whereas *N. coriiceps* sample was represented exclusively by large specimens (total length > 26 cm; Fig 4).

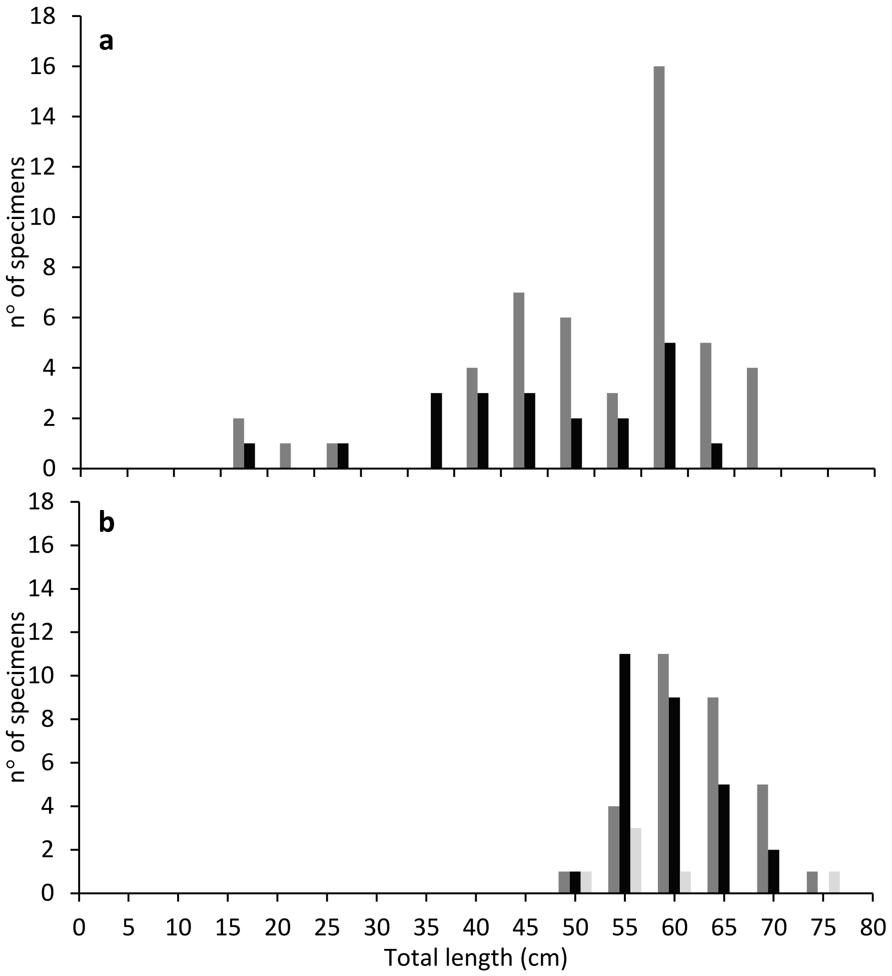

Figure 3. Length-frequency distributions of *Notothenia rossii* from South Shetland Islands (**a**) and from South Georgia (**b**). Black bars= males; dark grey bars= females; light grey bars= sex not determined.

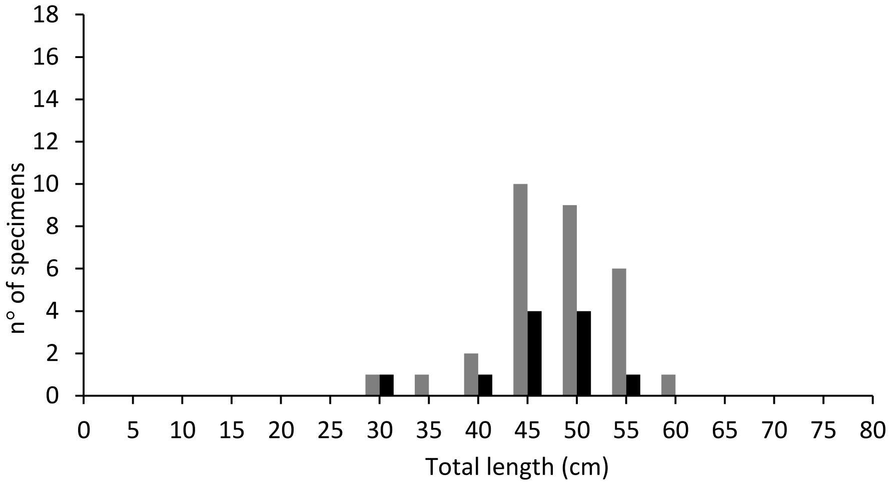

Figure 4. Length-frequency distributions in *Notothenia coriiceps* from South Shetland Islands (black bars=males; dark grey bars=females).

Length-weight relationship and the fitted curves were calculated in both species (Fig. 5 and 6). Gonadosomatic index (GSI) of *N. rossii* developing (macroscopic stage 3) females from SS was 20.2 ± 6.3 % (ranging from 6.5 to 28.8%), whereas in developed males (macroscopic stage 3) was 33.4 ± 16.6 % (ranging from 8.6 to 51.4%). GSI in *N. coriiceps* developing females was 12.6 ± 2.6 % (ranging from 5.5 to 18.6%), whereas in developed males 16.7 ± 4.5 % (ranging from 6.8 to 23.8%). The minimum size of a sexually mature specimen was 50 cm TL in *N. rossii* females and 44 cm TL in males. In *N. coriiceps*, the smallest sexually mature specimen was 39 cm TL for females and 36 cm TL for males.

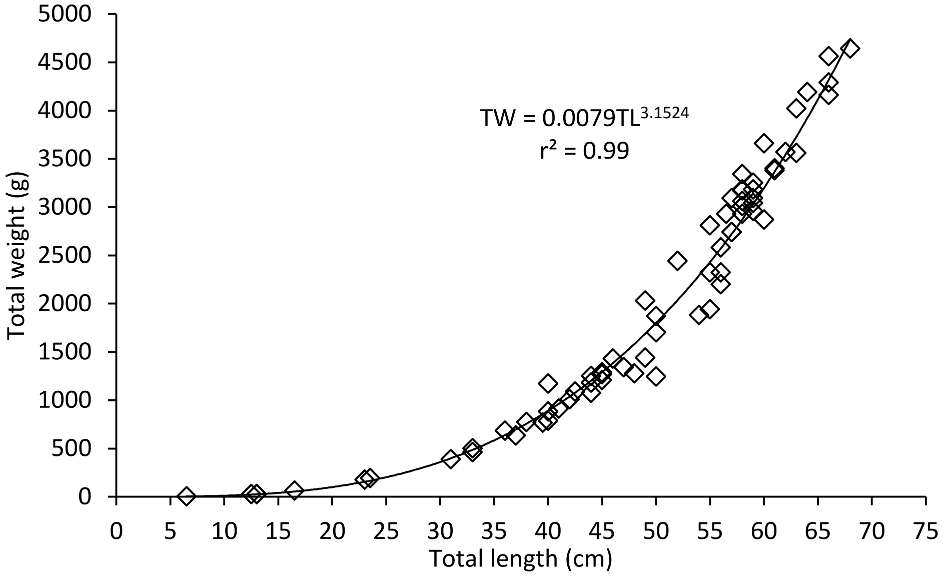

Figure 5. Length-weight relationship in *Notothenia rossii* from South Shetland Islands.

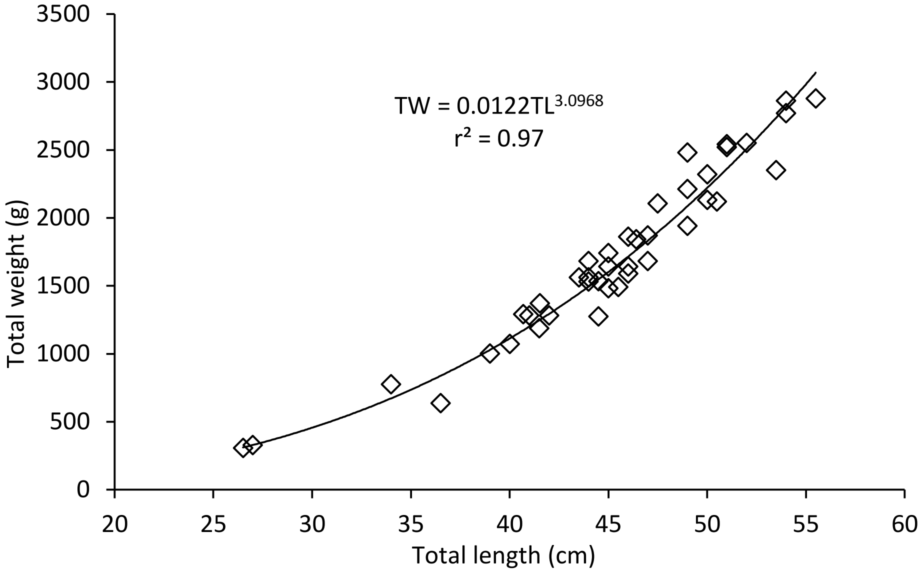

Figure 6. Length–weight relationships in *Notothenia coriiceps* from South Shetland Islands.

Gonad morphology and maturity

The histological analyses confirmed the maturity stage macroscopically attributed in 35 out of 39 samples analysed (22 females and 17 males). The individuals with different microscopic attribution of maturity stage were in *N. rossii*: one female, macroscopically classified as stage 1 in which oocytes at "cortical alveoli" stage were observed (microscopic stage II); one male, macroscopically classified as stage 1 in which spermatocytes were observed (stage II) and two males, macroscopically classified as stage 2, in which spermatids and spermatozoa were observed (stage III). A small specimen (6.5 cm TL) of *N. rossii*, macroscopically sexually not determined, resulted to be a female with immature ovaries. As the histological analyses revealed a similar gonads organization in the congeneric species, the description below refers to both species.

Microscopic observations of female gonads allowed to describe the ovary as being of the group synchronous type (Wallace and Selman 1981), with the presence of two discrete modes of oocyte diameters. Immature females (stage I) presented only small previtellogenic oocytes (Fig. 7a), maturing females (stage II) presented previtellogenic oocytes and few oocytes at cortical alveoli stage (Fig. 7b). In developing females (stage III) ovaries exhibited large oocytes (2-3 mm) in advanced vitellogenesis filled with yolk granules and few previtellogenic and cortical alveoli stage oocytes (Fig. 7c). Only two females of *N. rossii* were gravid (stage IV), showing some hydrated oocytes, large in diameter (4 mm) and with fused yolk granules (Fig. 7). Two developing females presented atretic oocytes, while post-ovulatory follicles were not observed. Histological analyses on males evidenced a lobular type testis, with an unrestricted organization, characterized by the distribution of spermatogonia along the entire length of the lobules (Grier 1981). Only one male was immature, showing spermatogonial cysts and small lobule lumina (Fig. 7). Developing males (stage II) presented spermatogonial cysts and spermatocytes, the last mainly close to the main testicular ducts (Fig. 7). In developed males (stage III) testis exhibited large lobule lumina, with spermatogonial cysts restricted to the distal portion of testes, while spermatocytes (type I-II), spermatids and spermatozoa occurred mainly in the proximal part (Fig. 7).

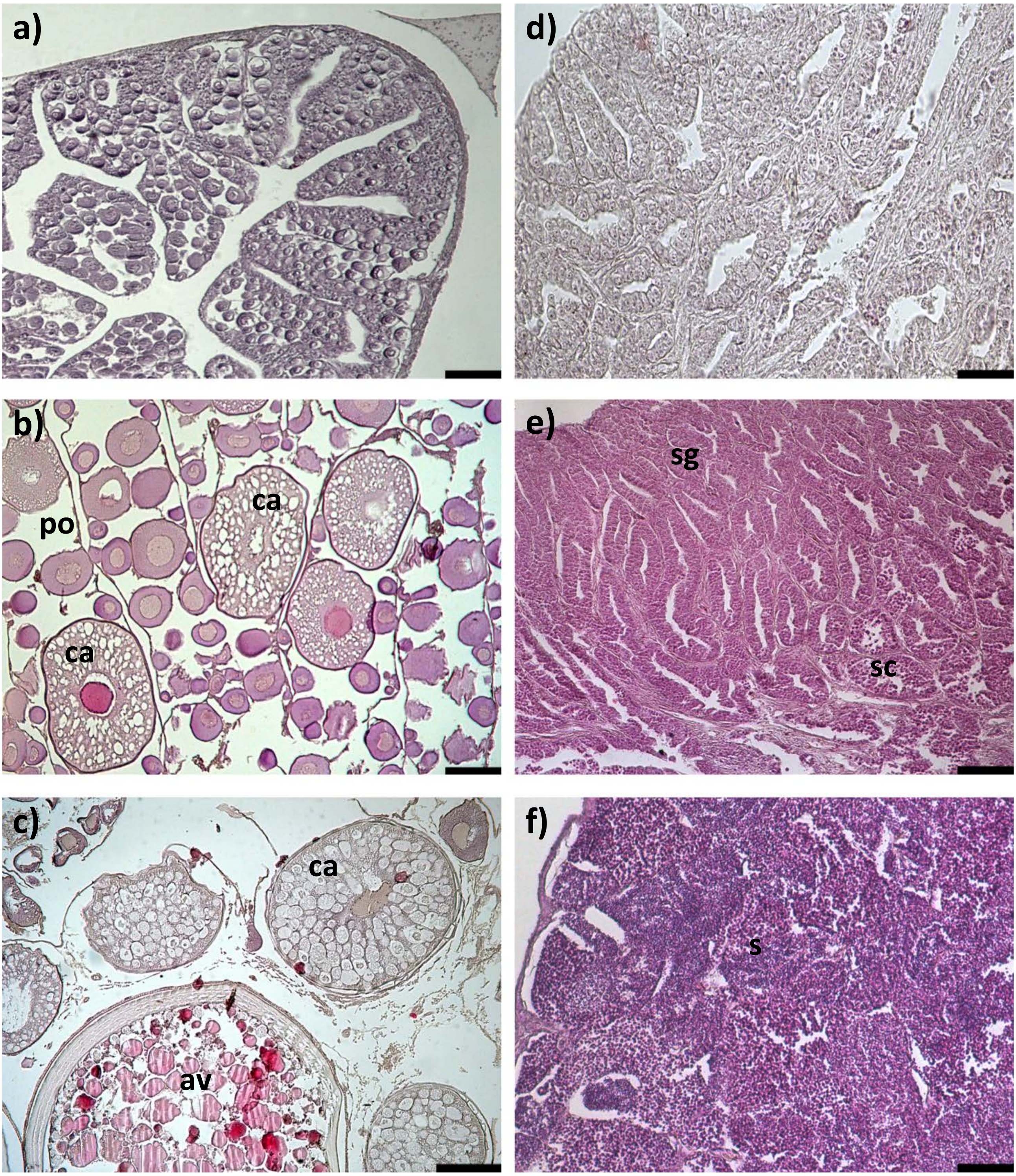

Figure 7. Histological sections of gonad of *Notothenia rossii and N. coriiceps* at different maturity stages: **a**) immature female (macroscopic stage 1) with previtellogenic oocytes (scale bar = 100 µm); **b**) maturing female (macroscopic stage 2) ovaries show cortical alveoli oocytes (ca) and previtellogenic oocytes (po) (scale bar = 200 µm); **c**) mature ovaries (macroscopic stage 3) filled of oocytes in advanced exogenous vitellogenesis stage with fused yolk granules (av) and oocytes at the cortical alveoli stage (ca) (scale bar = 300 µm); **d**) immature testis (macroscopic stage 1) showing only spermatogonial cysts (scale bar = 50 µm); **e**) developing males (macroscopic stage 2) with cysts of spermatogonia (sg) and spermatocytes (sc) (scale bar = 50 µm). **g**) developed males (macroscopic stage 3) showing spermatozoa (s) in the lobule lumina (scale bar = 50 µm).

Fecundity estimation

N. rossii

Oocytes presented a non-overlapping bimodal size frequency distribution (Fig. 8). The first mode corresponded to oocytes with a diameter between 0.2 and 1.3 mm (previtellogenic and cortical alveoli stage), the second one to oocytes with a diameter between 2 and 3.1 mm (vitellogenic oocytes). In two females from South Shetland Islands, hydrated oocytes were found (mean diameter 4.2 ± 0.3 mm and 4.8 ± 0.2 mm), representing 1 and 7% of the vitellogenic oocytes respectively. The size of females used for fecundity estimation did not differ between the different areas (t= 1.04, p= 0.301, df= 53). Absolute fecundity in SS females ranged from 6700 to 66000 eggs/female (36221 ± 13764 eggs/female), in SG females ranged from 25600 to 68800 eggs/female (42172 ± 11515 eggs/female). Relative fecundity for SS sample was estimated between 5.5 and 18.2 eggs/g (13.5 ± 3.4 eggs/g).

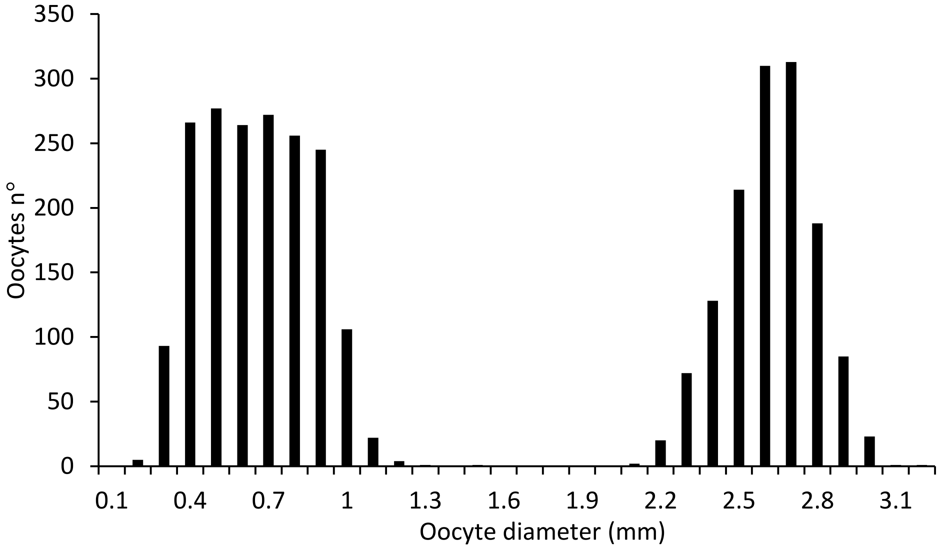

Figure 8. Size frequency distribution of oocytes in ovaries of a *Notothenia rossii* developing female (total length 59 cm).

The total number of vitellogenic oocytes was correlated to TL in both populations (F= 83, P< 0.001, df= 27 in SS and F= 47, P< 0.001, df= 26 in SG) (Fig. 9). No differences were found in absolute fecundity between the two populations, in relation to total length (ANCOVA: F=1.89, p= 0.175, df= 2, 49). Oocyte mean diameter in SG females was significantly lower than in SS females (t= 15.00, p< 0.001, df= 53), measuring 1.9 ± 0.2 mm in SG females and 2.6 ± 0.2 mm in SS females.

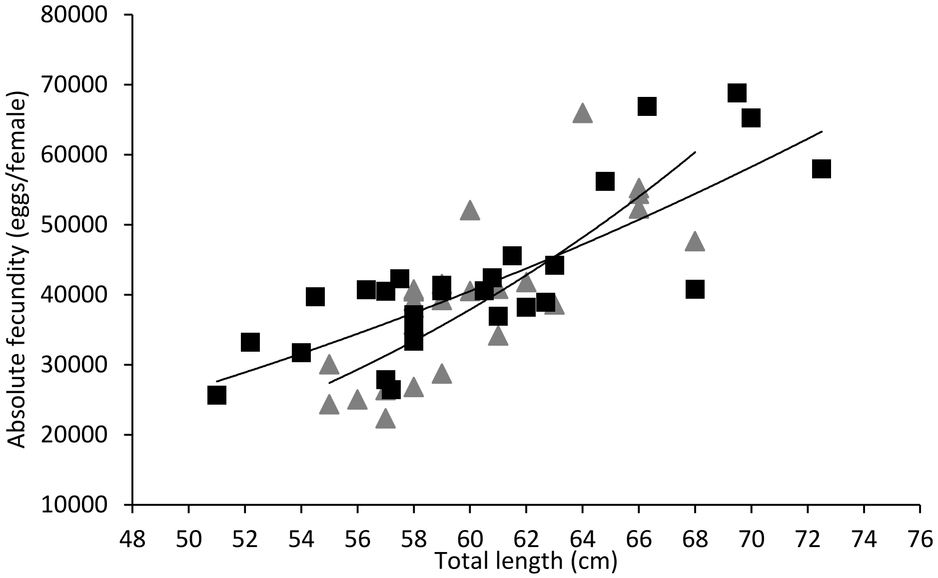

Figure 9. Relationships between total length and absolute fecundity in *Notothenia rossii* from South Shetland Islands (grey triangles) and from South Georgia (black squares).

N. coriiceps

Oocytes presented a non-overlapping bimodal size frequency distribution (Fig. 10). The first mode included oocytes with a diameter between 0.2 and 1 mm (previtellogenic and cortical alveoli stage), the second mode includes oocytes with a diameter between 1.5 and 2.5 mm (vitellogenic oocytes). In one female hydrated oocytes were found (mean diameter 3.6 ± 0.3 mm), representing 1.2 % of the vitellogenic oocytes.

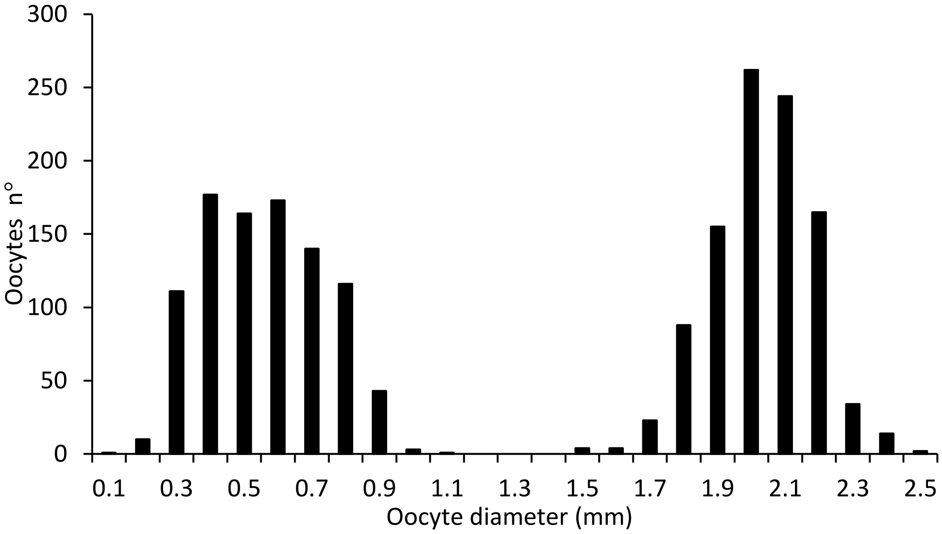

Figure 10. Size frequency distribution of oocytes in ovaries of a *Notothenia coriiceps* developing female (total length 51 cm).

The total number of vitellogenic oocytes was related to female TL (F= 141.64, P< 0.001, df= 27) (Fig. 11). Absolute fecundity estimations ranged from 8300 to 52000 eggs/female (28674 ± 11124 eggs/female). Relative fecundity was estimated between 8.8 and 23.3 eggs/g (16.7 ± 3 eggs/g). Oocyte mean diameter was 2.0 ± 0.1 mm.

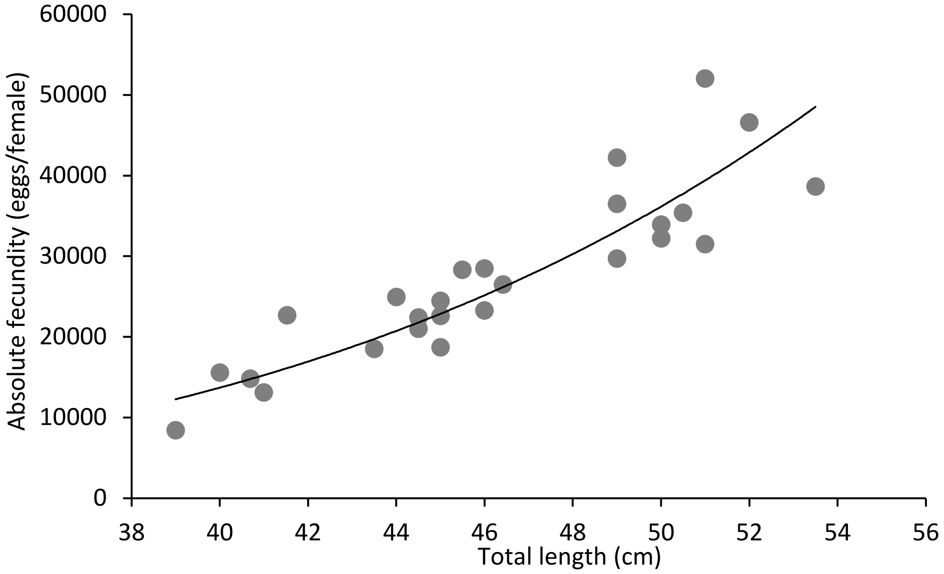

Figure 11. Relationships between total length and absolute fecundity in *Notothenia coriiceps* from South Shetland Islands.

Age estimation

In *N. rossii* 2 out of 132 otoliths were excluded because differences in age readings by more than two years emerged. Similarly, 4 out of 44 otoliths were discarded in *N. coriiceps*. Concerning ageing precision, the index of average percentage error (APE) and the mean coefficient of variation (CV mean) were both relatively low (3.6 and 5.1 % in *N. rossii*, 2.4 and 3.4 % in *N. coriiceps* respectively), indicating a good consistency between readings.

N. rossii

Specimens ranged in age between 2 and 16 years in SS and between 4 and 24 in SG, age-length key of both sexes were pooled together and summarized (Tab. 2 and 3). The youngest sexually mature specimen was 7 years old in females and 5 years old in males. No significant differences were found in the mean sizes at age between individuals in different areas (t= 1.15, p= 0.269, df= 12).

Table 2. Age-length key for *Notothenia rossii* from South Shetland Islands.

TL (cm)	**Age (years)**															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
16-17		1														
18-19																
20-21																
22-23			2													
24-25																
26-27																
28-29																
30-31					1											
32-33					2											
34-35																
36-37					2											
38-39			1			1										
40-41					2	2										
42-43						2										
44-45				1	2	3	1									
46-47						2										
48-49								1								
50-51							1	2								
52-53							1									
54-55								2	2							
56-57						1	1	1	1	2		1				
58-59								1	2	2	5		1			
60-61										2	2				1	
62-63										1	1					1
64-65														1		
66-67													2	1		
68-69															1	
N	0	1	3	1	9	11	4	7	5	7	8	1	3	2	2	1

Table 3. Age-length key for *Notothenia rossii* from South Georgia.

TL (cm)	Age (years)																				
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
40-41																					
42-43																					
44-45	1																				
46-47		1																			
48-49		1																			
50-51		1	1																		
52-53				6	3																
54-55					9																
56-57					2	4	3														
58-59							2	3	1	2											
60-61								1	2	1	2	1	1								
62-63												1	2								
64-65									1			1	1		1						1
66-67												1		1							
68-69												1	1	1			1				
70-71															1						
72-73																1					
74-75																1					
N	1	3	1	6	14	4	5	4	4	3	2	5	5	2	2	2	1	0	0	0	1

Table 4. Age–length key for *Notothenia coriiceps* from South Shetland Islands.

TL (cm)	Age (years)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
26-27	1	1											
28-29													
30-31													
32-33													
34-35	1												
36-37		1											
38-39													
40-41				2	2	1							
42-43						1		1					
44-45					2	1	3	1		1			
46-47						3		3	1				
48-49								1	1	1			
50-51								1	1	3			
52-53									1				1
54-55													3
N	2	3	0	2	4	6	3	7	4	5			4

N. coriiceps

The age range was between 6 and 18 years in both sexes. The youngest sexually mature specimen was 7 years old for both females and males. Mean sizes (TL) for age class were reported (Tab. 4).

Discussion

The results of this study provided new data on some reproductive traits and age of both marbled rockcod, *Notothenia rossii*, and black rockcod, *N. coriiceps*, from South Shetland Islands (SS) and South Georgia (SG).

Samples of *N. rossii* collected in SS and SG are mainly adult (sexually mature) individuals. The low abundance of juveniles is related to the use of different habitats, with immature individuals living in nearshore waters, while adults migrate offshore after sexual maturation (Burchett 1983; Barrera-Oro *et al.* 2014). Most of the sampled individuals exhibited gonads in developing stage, in accordance with an autumn spawning period (White *et al.* 1982; Kock 1989). The histological analyses provided description of the gonadal maturation cycle in *N. rossii*. In particular, we described four maturity stages (I, II, III, IV) in females and three (I, II, III) in males. Histological analyses revealed that ovaries are of the group synchronous type, with a bimodal oocytes size distribution (Wallace and Selman 1981). This ovarian type is typical of notothenioid species (Kock and Kellermann 1991; Shandikov and Faleeva 1992; La Mesa *et al.* 2006, 2014), and indicate that the marbled rockcod is a total spawners, therefore releasing all eggs within one season in a short period, likely in one batch. In males, spermatogenesis appeared synchronous as well, with developing males presenting lobule lumina completely filled with sperm and few cysts of early stages of spermatogenesis only in the distal portions of the lobules, indicating that males are ready to spawn before the spawning period and before females. The completion of spermatogenesis before the spawning period is reported also in other notothenioid fish (Russo *et al.* 2000; La Mesa *et al.* 2003, 2006, 2008), and is probably related to the long time required for the spermatogenesis. Two females showed oocyte resorption processes (atresia), that generally can be observed before or during the spawning period in response to environmental stress (Wallace and Selman 1981). This process has been observed in several species of notothenioids and put in relationship to poor trophic conditions (Calvo *et al.* 1999; La Mesa *et al.* 2003; Van der Molen and Matallanas 2003, 2004; Vanella *et al.* 2005) and considered to allow optimizing the allocation of food resources in the developing of the most advanced oocytes (Rae and Calvo 1996). The occurrence of phenomena of atresia indicates also that the absolute fecundity estimates must be considered a measure of the potential fecundity of this species and that the realized fecundity (that is to say the number of eggs actually laid by a female; Hunter *et al.* 1992), might be quite lower than the potential one. Taking this point into considerations, this study provided estimates of potential fecundity slightly lower than the ones reported in literature for the same areas as well as other areas. For SS, also relative fecundity estimates were slightly lower (see Tab. 5 for a comparisons with previous studies). Considering the clear separations of modes in the frequency distribution of oocyte diameters showed by this species, fecundity estimate constitutes an easy task, making quite reliable the comparisons among studies. The observed lower value of fecundity might be related, in some cases, to the slightly smaller sizes of the females sampled in this study, in comparison with previous one, but also to temporal and/or spatial variability in fecundity related to environmental conditions and/or food availability (Calvo *et al.* 1999; La Mesa *et al.* 2003; Van der Molen and Matallanas 2003, 2004; Vanella *et al.* 2005).

In this paper we provided also new data on age of large-size specimens (> 60 cm TL), estimated using otoliths (sagittae). Previous studies attempted to estimate age in large individuals (up to 84 cm TL) using scales, admitting the inaccuracy of readings, because scales can be replaced several times or can be damaged (Freytag 1980; Kock 1992; Tankevich 1990, 1994). The method we used to process otoliths for age readings allowed to contrast dark and light rings also in large sized individuals, therefore achieving a good repeatability. Differences among our age estimates and literature data, especially regarding the estimates of large fish, emerged (Freytag 1980; Kock and Kellermann 1991) and, considering the methodological issues related to the use of scales, likely indicate an underestimation of age in previous studies. Finally, the size and age at sexual maturity were consistent with previous data (Camus and Duhamel 1985; Kock and Kellermann 1991).

The comparisons of samples collected in the two study areas did not highlight consistent differences in life history traits. Comparing fecundity estimates, in relation to female body size, between SS and SG females, no significant differences emerged, even if higher fecundity values are reported from previous studies in SG females (Kock and Kellermann 1991; Tab. 5). Mean oocyte size in mature females resulted lower in SG than in SS. The observed differences likely reflects the temporal variability in sampling, being one month later in the season in SS, rather than to a real difference in the egg size between the two populations. Similarly to the other traits, comparing the age estimates in the two populations, we did not find any difference in the relationship between size and age in specimens from SS and SG. Although SS and SG are located in the path of the Antarctic Circumpolar Current (ACC), the shelf waters adjacent to SG often show properties that are markedly different from the open ocean waters beyond (Brandon *et al.* 1999, 2000; Meredith *et al.* 2005). Although much of the Southern Ocean is characterized by high-nutrient and low-chlorophyll conditions, the waters around SG are highly productive and support significant international fisheries (Hogg *et al.* 2011, Young *et al.* 2012). Consequently, it is reasonable to hypothesize that the different environmental conditions can induce different phenotypic response or a microevolutive process (leading to distinct populations). Some studies showed phenotypic differences (growth parameters, parasite infestation, allozyme data) at intraspecific level in notothenioid species from different areas (Freytag 1980; Carvalho and Lloyd-Evans 1990; Sosinski and Janusz 2000; Riginella *et al.* unpubl.) and life history traits are considered to be phenotypic expressions of the interaction between genotypic and environmental effects (Begg 2005). However, we did not find any significant difference in the life history traits analysed, between SS and SG population, probably in relation to the peculiar life history traits of this species. The prolonged pelagic phase of eggs and larvae creates high potential of larval dispersal, which ensures a certain level of interaction and gene flow between populations of different areas preventing genetic divergence (Young *et al.* 2015). Nevertheless, considering the distance between SS and SG (approximately 700 NM) and the hydrography at regional scale (Fach and Klinck 2006), we would have expected at least some phenotypic divergence in relation to environmental features, as reported for other notothenioids (Freytag 1980; Riginella *et al.* unpubl.).

The marbled rockcod was heavily exploited by fishery, leading to a strong stock depletion. Several data available on life history traits refer to the 1970s - 1980s, when fishery on this species was allowed but possibly before the stock collapse. The larger sizes of fish (and consequently higher fecundities) reported in these literature data, compared to our study (see Tab. 5), may reveal a decrease in maximum size, a phenomenon well documented in overexploited stocks (Pauly *et al.*

2005). The slow recovery of this species in abundance (Barrera-Oro and Marschoff 2007; Marschoff *et al.* 2012; Kock and Jones 2012) and, possibly, in maximum size, observed after the ban of fishery highlight how vulnerable and low resilient can be species, like *N. rossii*, characterized by high age at maturity and slow growth.

Table 5. Reproductive characteristics of *Notothenia rossii* and *N. coriiceps* from different areas. TL_{mat}= total length at first maturity; F_{abs}= absolute fecundity; F_{rel}= relative fecundity; GSI= gonadosomatic index. a= Kock and Kellermann 1991; b= Everson 1970; c= Sapota 1999; d= White *et al*. 1982; e= Burchett *et al*. 1982;

f= Kock 1989; g= Tankevich 1994; h= Freytag 1980; i= Tankevich 1990; j= Camus and Duhamel 1985.

	Area	**Max egg size (mm)**	**TL_{mat}**	**Age_{mat}**	**F_{abs}**	**F_{rel}**	**Lenght range (cm)**	**Max GSI ♀**	**Max age**	**Source**
N. rossii	South Shetland Is.	--	--	--	19271-99330	6.1-22.6	51-78	--	13	a, h
	South Shetland Is.	5	44♂, 50♀	5♂, 7♀	6700-66000	5.5-18.2	50-68	28.8	16	Present study
	Kerguelen Is.	5	--	--	12200-109710	9.9-20.4	47-77	50	14	a, i
	Kerguelen Is.	5	43♂, 48.5♀	5♂, 6♀	--	--	--	--	--	j
	South Georgia	5	--	--	--	--	--	36.5	16	a, h
	South Georgia	--	--	--	20137-94146	8-16	50-83	--	--	a
	South Georgia	--	--	--	20100-130000	12-24	52-84	--	--	a
	South Georgia	3.7	--	--	25600-68800	--	51-72	--	24	Present study
	Ob Bank	--	--	--	--	--	--	--	16	g
N. coriiceps	South Orkney	4	30 ♀♂	7-8	8000-35000	8-15	--	16	17	b
	King George Is.	4.6	29♂, 34♀	--	--	--	--	39	--	c
	Elephant Island	4.7	--	--	7000-41000	7-17	34-45	27	--	a
	South Shetland Is.	4.2	36♂, 39♀	7♀♂	8300-52000	8.8-23.3	39-55	18.6	18	Present study
	South Georgia	4.1	--	--	36000-48000	--	--	--	--	d, e
	South Sandwich Is.	--	--	--	7000-34000	7-14	--	--	--	f

In *N. coriiceps*, almost all the sampled individuals were large adults and exhibited gonads in developing stage, as expected considering that the spawning season occurs in autumn (Sapota 1999). Histological analyses allowed the description of the maturation cycle in this species, too, considering that only few information on female gonads were available in literature (Everson 1970). *N. rossii* and *N. coriiceps* present similar gametogenesis and gonadal structures, both in males and females, with the exception of atretic phenomena, not observed in this study, but reported by Everson (1970). Absolute fecundity estimates provide a maximum value (52000 eggs/female) in *N. coriiceps* in the studied area, higher than previously reported, likely due to the larger sizes of females included in this study respect to the previous one (Kock 1989). Finally size and age at sexual maturity were similar to those reported in literature (Everson 1970; Sapota 1999).

Despite the differences in adult ecology *N. rossii* and *N. coriiceps* share several common life history features and no marked differences emerged in this study. Indeed these species exhibit a different spatial distribution, that has been put in relation with different settlement requirements, in terms of habitats and available preys, of the two species (Barrera-Oro *et al.* 2014). *N. coriiceps* is widespread around the coasts of Antarctica while *N. rossii* is limited to some archipelagos of Southern Ocean (Scotia Arc, Prince Edward, Crozet, Kerguelen, Heard and Macquarie islands, and Ob' and Lena banks). Nonetheless, eggs and larvae of both species have a long pelagic life, in the surface layer of Antarctic water in the same areas (White *et al.* 1996, Barrera-Oro *et al.* 2014), spreading through the prevailing ocean currents along coastal waters of the Southern Ocean (Antarctic Circumpolar Current and Polar Current) (Turner *et al.* 2009). *N. rossii* and *N. coriiceps* present common and very rare features among nototheniids, laying pelagic eggs up to 5 mm in diameter. Generally, pelagic eggs are small in diameter and numerous, to maximize larvae dispersal and survival in the, usually, unpredictable pelagic environmental condition. Conversely, demersal eggs are usually large and larval survival increases as egg size increases (Duarte and Alcaraz 1989). Large pelagic eggs are reported also for other nototheniid species, *Dissostichus eleginoides* and, probably, *Dissostichus mawsoni* (Paper VI), suggesting that this feature may be related to ecological factors shared by these species. In particular, large eggs are linked to long development, leading to large larvae. Large larvae usually have better swimming performance than small ones with a consequently higher success in feeding and escaping from predators (Conover and Schultz 1997). Therefore the production of large pelagic eggs might be related to high levels of predation and/or competition occurring in Antarctic waters (Kock 1992). This hypothesis is also supported by the fact that the two other nototheniid species with pelagic eggs, *Eleginops maclovinus* and, probably, *Pseudaphritis urvillii*, produce much smaller eggs and inhabit Sub-Antarctic waters (Paper VI). *N. rossii* and *N. coriiceps* are characterized also by high fecundity, typical of pelagic spawners. However, considering the relative fecundity, the trade-off between egg size and fecundity (Smith and Fretwell 1974) emerges, indeed the relative fecundity is comparable, of even lower, to that of Antarctic species with demersal eggs (Paper VI).

Marbled and black rockcod females show high reproductive effort in gonads, with GSIs values between 40 and 50% (Kock and Kellermann 1991), in agreement with the large size and the high number of large eggs produced. *N. coriiceps* and *N. rossii* males show high reproductive effort in gonads, reaching 23.8% and 51.4% of body weight, respectively (the highest GSI male values among notothenioids). Such high values of male GSI, together with the abundance of sperm in testes, have been put in relation, in other teleost fish, to high levels of sperm competition occurring in spawning aggregations (Stockley *et al.* 1997), therefore suggesting this type of mating system also in these species. Considering that the occurrence of spawning aggregations make the species particularly vulnerable to fishery exploitation, this aspect deserves further investigations.

References

Barrera-Oro E. (2002). The role of fish in the Antarctic marine food web: differences between inshore and offshore waters in the southern Scotia Arc and west Antarctic Peninsula. Antarctic Science 14: 293-309.

Barrera-Oro E., Marschoff E.R., Casaux R.J. (2000). Trends in relative abundance of fjord *Notothenia rossii*, *Gobionotothen gibberifrons* and *Notothenia coriiceps* at Potter Cove, South Shetland Islands, after commercial fishing in the area. CCAMLR Science 7: 43-52.

Barrera-Oro E., Marschoff E. (2007). Information on the status of fjord *Notothenia rossii*, *Gobionotothen gibberifrons* and *Notothenia coriiceps* in the lower South Shetland Islands, derived from the 2000-2006 monitoring program at Potter Cove. CCAMLR Science 14:83-87.

Barrera-Oro E., La Mesa M., Moreira E. (2014). Early life history timings in marbled rockcod (*Notothenia rossii*) fingerlings from the South Shetland Islands as revealed by otolith microincrement. Polar Biology. doi: 10.1007/s00300-014-1503-0.

Beamish R.J., Fournier D.A. (1981). A method of comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences 38: 982-983.

Begg G.A. (2005). Life history parameters. In: Cadrin S.X., Friedland K.D., Waldman J.R. (eds) Stock Identification Methods. Applications in Fishery Science, Elsevier, Amsterdam, 119-150 pp.

Begon M., Harper J.L., Townsend C.R. (1990). Ecology - Individuals, Populations, Communities. Blackwell Scientific Publications, Oxford, England.

Billard R. (1986). Spermatogenesis and spermatology of some teleost fish species. Reproduction Nutrition Development 26: 877-920.

Brandon M.A., Murphy E.J., Whitehouse M.J., Trathan P.N., Murray A.W.A., Bone D.G., Priddle J. (1999). The shelf break front to the east of the sub-Antarctic island of South Georgia. Continental Shelf Research 19: 799-819.

Brandon M.A., Murphy E.J., Trathan P.N., Bone D.G. (2000). Physical oceanographic conditions to the northwest of the sub-Antarctic Island of South Georgia. Journal of Geophysical Research C 105: 23983-23996.

Burchett M.S. (1983). The life cycle of *Notothenia rossii* from South Georgia. British Antarctic Survey Bulletin 61: 71-73.

Calvo J., Morriconi E., Rae G.A. (1999). Reproductive biology of the icefish *Champsocephalus esox* (Günther, 1861) (Channichthyidae). Antarctic Science 11: 140-149.

Campana S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197-242.

Camus P., Duhamel G. (1985). Ponte et developpement embryonnaire de *Notothenia rossii rossii* (Richardson 1844). Nototheniidae des Iles Kerguelen. Cybium 9: 283-293.

Carvalho G.R., Lloyd-Evans D.P. (1990). Pilot study on electrophoretic variation and stock structure in the mackerel icefish, *Champsocephalus gunnari*, South Georgia waters. WG-FSA-90/10. CCAMLR, Hobart, Australia.

Casaux R., Mazzotta A., Barrera-Oro E. (1990). Seasonal aspects of the biology and diet of nearshore notothenioid fish at Potter Cove, South Shetland Islands, Antarctica. Polar Biology 11: 63-72.

Chang W.Y.B. (1982). A statistical method for evaluating the reproducibility of age determination. Canadian Journal of Fisheries and Aquatic Sciences 39: 1208-1210.

Cheng C.C., Chen L., Near T.J., Jin Y. (2003). Functional Antifreeze Glycoprotein Genes in Temperate-Water New Zealand Nototheniid Fish Infer an Antarctic Evolutionary Origin. Molecular Biology and Evolution 20(11): 1897-1908.

Dettai A., Berkani M., Lautredou A.C., Couloux A., Lecointre G., Ozouf-Costaz C., Gallut C. (2012). Tracking the elusive monophyly of nototheniid fishes (Teleostei) with multiple mitochondrial and nuclear markers. Marine Genomics 8: 49-58.

DeWitt H.H., Heemstra P.C., Gon O. (1990). Nototheniidae. In: Gon O., Heemstra P.C. (eds) Fishes of the Southern Ocean. J.L.B. Smith Institute of Ichthyology, Grahamstown 279-331.

Eastman J.T. (1993). Antarctic fish biology: evolution in a unique environment. Academic Press, San Diego.

Eastman J.T., Eakin R.R. (2015). Notothenioid classification and list of species. http://www.oucom.ohiou.edu/dbms-eastman.

Eastman J.T., Barrera-Oro E., Moreira E. (2011). Adaptive radiation at a low taxonomic level: divergence in buoyancy of the ecologically similar Antarctic fish *Notothenia coriiceps* and *N. rossii*. Marine Ecology Progress Series 438: 195-206.

Eastman J.T., Eakin R.R. (2000). An updated species list for notothenioid fish (Perciformes; Notothenioidei), with comments of Antarctic species. Archive of Fishery and Marine Research 48: 11-20.

Everson I. (1980). Antarctic fish age determination methods. BIOMASS published by: SCAR and SCOR, Scott Polar Research Institute, Cambridge, England, Handbook 8, 1-24 pp.

Everson I. (1977). The Living Resources of the Southern Ocean. GLO/SO/77/1 Rome 7-14.

Fach B.A., Klinck J.M. (2006). Transport of Antarctic krill (*Euphausia superba*) across the Scotia Sea. Part I: Circulation and particle tracking simulations. Deep Sea Research I 53:987-1010.

Freytag G. (1980). Length, age and growth of *Notothenia rossii marmorata* Fischer 1885 in the West Antarctic waters. Archiv für Fischereiwissenschaft 30: 39–66.

Gray P. (1954). The Microtomists' Formulary and Guide. Blakiston, New York, NY. 175 pp.

Hunter J.R., Macewicz B.J., Lo N.C.H., Kimbrell C.A. (1992). Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fishery Bulletin 90: 101-128.

Hogg O.T., Barnes D.K.A., Griffiths H.J. (2011). Highly Diverse, Poorly Studied and Uniquely Threatened by Climate Change: An Assessment of Marine Biodiversity on South Georgia's Continental Shelf. PLoS ONE 6(5): e19795. doi:10.1371/journal.pone.0019795.

Hunter J.R., Macewicz B.J., Lo N.C., Kimbrell C.A. (1992). Fecundity, spawning and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumption and precision. Fishery Bulletin. 90(1): 101-128.

Knust R., Gerdes D., Mintenbeck K. (2012). The expedition of the research vessel "Polarstern" to the Antarctic in 2011 (ANT-XXVII/3) (CAMBIO), Berichte zur Polar und Meeresforschung = Reports on polar and marine research, Bremerhaven, Alfred Wegener Institute for Polar and Marine Research 644: 1-202 pp.

Kock K.H. (1992). Antarctic fish and fisheries. Cambridge University Press, Cambridge.

Kock K.H. (1989). Reproduction in fish around Elephant Island. Archiv für Fischereiwissenschaft 39: 171-210.

Kock K.H., Jones C.D. (2012). The composition, abundance and reproductive characteristics of the demersal fish fauna in the Elephant Island-South Shetland Islands region and at the tip of the Antarctic Peninsula (CCAMLR Subarea 48.1) in March-early April 2012. WG-FSA-12/10.

Kock K.H., Kellermann A. (1991). Reproduction in Antarctic notothenioid fish. Antarctic Science 3(2): 125-150.

La Mesa M., Riginella E., Mazzoldi C., Ashford J. (2014). Reproductive resilience of ice-dependent Antarctic silverfish in a rapidly changing system along the Western Antarctic Peninsula. Marine Ecology 1-11. doi: 10.1111/maec.12140.

La Mesa M., De Felice A. (2009). Age and growth of spiny icefish (*Chaenodraco wilsoni* Regan, 1914) off Joinville-d'Urville islands (Antartic Peninsula). CCAMLR Science 16: 115-130.

La Mesa M., Caputo V., Eastman J.T. (2008). The reproductive biology of two epibenthic species of Antarctic nototheniid fish of the genus *Trematomus*. Antarctic Science 20(4): 355-364.

La Mesa M., Caputo V., Eastman J.T. (2006). Gametogenesis and reproductive strategies in some species of the Antarctic fish genus *Trematomus* (Nototheniidae) from Terra Nova Bay, Ross Sea. Polar Biology 29: 963-970.

La Mesa M., Caputo V., Rampa R., Vacchi M. (2003). Macroscopic and histological analyses of gonads during the spawning season of *Chionodraco hamatus* (Pisces, Channichthyidae) off Terra Nova Bay, Ross Sea, Southern Ocean. Polar Biology 26: 621-628.

Lisovenko L.A. (1988). Some new information on the reproduction of *Chaenocephalus aceratus* (Fam. Channichthyidae) of the region of the Island of South Georgia. Journal of Ichthyology 28(2): 130-135.

Lucassen M. (2012). The expedition of the research vessel "Polarstern" to the Antarctic in 2012 (ANT-XXVIII/4), Berichte zur Polar und Meeresforschung = Reports on polar and marine research, Bremerhaven, Alfred Wegener Institute for Polar and Marine Research 652: 1-89.

Marschoff E., Barrera-Oro E., Alescio N., Ainley D. (2012). Slow recovery of previously depleted demersal fish at the South Shetland Islands, 1983-2010. Fisheries Research 125: 206-213.

Meneghesso C., Riginella E., La Mesa M., Donato F., Mazzoldi C. (2013). Life-history traits and population decline of the Atlantic mackerel *Scomber scombrus* in the Adriatic Sea. Journal of Fish Biology. doi:10.1111/jfb.12223.

Meredith M.P., Brandon M.A., Murphy E.J., Trathan P.N., Torphe S.E., Bone D.G., Chernyshkov P.P., Sushin V.A. (2005). Variability in hydrographic conditions to the east and northwest of South Georgia, 1996-2001. Journal of Marine Systems 53: 143-167.

Murphy G.I. (1968). Pattern in life history and the environment. The American Naturalist 102(927): 391-403.

Murua H., Kraus G., Saborido-Rey F., Witthames P.R., Thorsen A., Junquera S. (2003). Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal of Northwest Atlantic Fishery Science 33: 33-54.

Near T.J., Dornburgb A., Kuhnb K.L., Eastman J.T., Penningtonb J.N., Patarnello T., Zane L., Fernández D.A., Jones C.D. (2012). Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. Proceedings of the National Academy of Sciences. doi/10.1073/pnas.1115169109.

Pauly D., Watson R., and Alder J. (2005). Global trends in world fisheries: impacts on marine ecosystems and food security. Philosophical Transactions of the Royal Society B: Biological Sciences 360(1453): 5-12.

Pearse A.G.E. (1985). Histochemistry, Theoretical and Applied Analytical Technology. Churchill Livingstone, Edinburgh, 624 pp.

Rae G.A., Calvo J. (1996). Histological analysis of gonadal development in *Patagonotothen tessellata* (Richardson 1845) (Nototheniidae: Pisces) from the Beagle Channel, Argentine. Journal of Applied Ichthyology 12: 31-38.

Riginella E., Mazzoldi C., Ashford J., Jones C.D., Morgan C., La Mesa M. Life history strategies of the Scotia Sea icefish, *Chaenocephalus aceratus*, along the Southern Scotia Ridge. Paper.

Roff D.A. (2002). Life history evolution. Sinauer, Sunderland, 1-527 pp.

Roff D.A. (1992). The evolution of life histories: theory and analysis. Chapman and Hall, New York, USA, 1-535 pp.

Russo A., Angelini F., Carotenuto R., Guarino F.M., Falugi C., Campanella C. (2000). Spermatogenesis in some Antarctic teleosts from the Ross Sea: histological organization of the testis and localization of bFGF. Polar Biology 23:279-287.

Rutschmann S., Matschiner M., Damerau M., Muschik M., Lehmann M.F., Hanel R., Salzburger W. (2011). Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptative radiation. Molecular Ecology 20: 4707-4721.

Sapota M.R. (1999). Gonad development and embryogenesis of *Notothenia coriiceps* from South Shetlands - Antarctica. Polar Biology 22: 164-168.

Shandikov G.A., Faleeva T.I., (1992). Features of gametogenesis and sexual cycles of six notothenioid fishes from East Antarctica. Polar Biology 11: 615-621.

Sosinski J., Janusz J. (2000). Infection variability of the parasitic copepod *Eubrachiella antarctica* (Quidor, 1906) on fishes in the Atlantic sector of the Antarctic. Bulletin of Sea Fisheries Institute 2(150): 25-41.

Stearns S.C. (1992). The evolution of life histories. Oxford University Press, New York, USA.

Tankevich P.B. (1994). Growth and Age of Antartic Cod, *Notothenia rossii rossii*, on the Ob Bank (Indian Ocean Sector of Antarctica). Journal of Ichthyology 34(4): 67-73.

Tankevich P.B. (1990). Growth, age and natural mortality of *Notothenia rossii rossii* in the Kerguelen islands area. Cybium 14(3): 269-276.

Tiedtke J.E., Kock K.H. (1989). Structure and composition of the demersal fish fauna around Elephant Island. Archiv für Fischereiwissenschaft 39: 143-169.

Turner J., Bindschadler R., Convey P., di Prisco G., Fahrbach E., Gutt J., Hodg-son D., Mayewski P., Summerhayes C. (2009). SCAR's Antarctic Climate Change and the Environment (ACCE) Review Report, XXXII Antarctic Treaty Consultative Meeting, Baltimore USA, (IP 5). 555 pp.

Van der Molen S., Matallanas J. (2004). Reproductive biology of female Antarctic spiny plunderfish *Harpagifer spinosus* (Notothenioidei: Harpagiferidae), from Iles Crozet. Antarctic Science 16: 99-105.

Van der Molen S., Matallanas J. (2003). Oocyte development and maturity classification of *Gerlachea australis* from the Weddell Sea, Antarctica. Polar Biology 26: 653-658.

Vanella F.A., Calvo J., Morriconi E.R., Aureliano D.R. (2005). Somatic energy content and histological analysis of the gonads in Antarctic fish from the Scotia Arc. Scientia Marina 69: 305-316.

Wallace R.A., Selman K. (1981). Cellular and dynamic aspects of oocyte growth in teleosts. American Zoologist 21: 325-343.

White M.G. (1991). Age determination in Antarctic fish. In: di Prisco G., Maresca B., Tota B. (eds) Biology of Antarctic fish. Springer, Berlin Heidelberg New York, 87-100 pp.

White M.G., Veit R.R., North A.W., Robinson K. (1996). Egg-shell morphology of the Antarctic fish, *Notothenia rossii* Richardson, and the distribution and abundance of pelagic eggs at South Georgia. Antarctic Science 8(3): 267-271.

White M.G., North A.W., Twelves E.L., Jones S. (1982). Early development of *Notothenia neglecta* from the Scotia Sea, Antarctica. Cybium 6: 43-51.

Young E.F., Belchier M., Hauser L., Horsburgh G.J., Meredith M.P., Murphy E.J., Pascoal S., Rock J., Tysklind N., Carvalho G.R. (2015). Oceanography and life history predict contrasting genetic population structure in two Antarctic fish species. Evolutionary Applications 8(5): 486-509.

Young E.F., Rock J., Meredith M.P., Belchier M., Murphy E.J., Carvalho G.R. (2012). Physical and behavioural influences on larval fish retention: contrasting patterns in two Antarctic fishes. Marine Ecology Progress Series 465: 201-215.

PAPER V

Life strategies of two Antarctic plunderfishes (Artedidraconidae) from the Weddell Sea

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Abstract

Artedidraco skottsbergi and *Dolloidraco longedorsalis* are two demersal species of the family Artedidraconidae inhabiting the continental-shelf of the High Antarctic Zone. Samples of both species were collected during the PS82 2014 expedition of the *RV Polarstern* and some of their life-history traits were investigated. Both species present a prolonged gametogenesis process and the production of few eggs. *A. skottsbergi* showed lower absolute (69 ± 7 oocytes per female) and relative (14.07 ± 6.68 oocytes$\cdot$g^{-1}) fecundities than *D. longedorsalis* (209 ± 73 oocytes per females and 15 ± 4.29 oocytes$\cdot$g^{-1}). Spawning likely occurs once a year. First spawning was estimated to occur at 9.5 cm in females, and 8 cm in males of *A. skottsbergi*, while at 11 cm in both sexes of *D longedorsalis*. Age estimates ranged from 6 to 14 years in *A. skottsbergi* and from 3 to 14 years in *D. longedorsalis*, with a high percentage agreement between readings and low values of counting variability indices APE (1.9 and 6.4%, respectively) and CV (2.7 and 9%, respectively) in both species. This paper provides for the first time information on some essential aspects of their reproductive biology and age-length relationship, with particular reference to the Weddell Sea populations.

Introduction

The demersal fauna of the Weddell Sea is composed of a variety of organisms highly adapted to the extreme conditions of the Southern Ocean, such as cold water temperatures and seasonality of environmental factors, like ice-cover and primary production. Nevertheless, demersal fish communities of the High Antarctic Zone are the most diverse and species-rich of Antarctica, a striking result of a long evolutionary process (Hubold 1992).

The plunderfishes are grouped within one of the five families which are endemic to the Southern Ocean, the Artedidraconidae (Eastman 1993), and together they represent an important component of the benthic fauna inhabiting the continental shelf and upper slope of the High Antarctic Zone, contributing to 20-23% of the fish diversity of the Ross Sea and Weddell Sea (Hubold 1992; Eastman and Hubold 1999). The four genera (*Artedidraco*, *Dolloidraco*, *Histiodraco* and *Pogonophryne*) and 25 species of this family (Eastman and Eakin 2000) are characterized by small to medium sizes, a sculpin-like appearance and the presence of a mental-barbel, a distinctive trait that was suggested to have either a lure or a tactile somatosensory function (Macdonald and Montgomery 1991; Janssen *et al*. 1993; Iwami *et al.* 1996; Balushkin and Eakin 1998; Eakin *et al*. 2001; Eastman and Eakin 2001; Eastman and Lannoo 2003). Most species of plunderfishes are found associated to habitats covered or in proximity of sponge beds (Gutt and Ekau 1996), where they seem to maintain sedentary habits. This characteristic is common to the majority of the Weddell Sea fish species, which are known to have a particularly ‘sluggish’ behaviour, probably to cope with low metabolic rates (Hubold 1991) and/or as a consequence of their diet, which is mainly based on actively moving polychaetes, caught with a typical “sit and wait” or “ambush” feeding strategy (Daniels 1982; Hubold 1992).

Overall scientific information on the biology of Artedidraconidae is still scattered and scarce, likely due to the difficult accessibility of specimens from the permanent pack-ice zone and their lack of importance from an economical point of view. However, despite they do not have any commercial value, plunderfishes play an important role in the High Antarctic trophic webs, representing one of the main food source for top predators, such as Emperor penguins and Weddell seals (Olaso *et al*. 2000). Knowledge of their biology is therefore essential and, only recently, has become subject to expanding research efforts.

While most studies on the Antarctic plunderfishes aimed at describing their feeding habits and the role and morphology of their peculiar mental barbel (Eastman and Eakin 2001; Macdonald and Montgomery 1991; Olaso *et al*. 2000; Schawrzbach 1988), only a few focused the attention on reproductive biology and age structure (Ekau 1991; Kock and Kellermann 1991; Hubold 1992; Duhamel *et al*. 1993; Morales-Nin *et al*. 2000; La Mesa *et al*. 2006), aspects which are crucial for deepen the knowledge of their life history strategies and understanding how they have successfully specialized to cope with the extreme conditions of their habitats. In general, Artedidraconidae are known to spawn once a year during spring or summer as most High Antarctic Zone species (Kock and Kellermann 1991). Their gonads present the same general features described for other notothenioids, such as a biennial process of gamete maturation, the production of large eggs and low fecundities (Everson 1984; North and White 1987; Kock and Kellermann 1991; Duhamel *et al*. 1993). The production of demersal eggs associated to parental care were also suggested in the genus *Artedidraco* (Ekau 1991; Kock and Kellermann 1991; Duhamel *et al*. 1993). Growth rate of these species appeared to be seasonal, possibly influenced by the different day-light lengths and shading effects of ice-cover throughout the year (Morales-Nin *et al*. 2000).

In the present study, we provide information on some aspects of the reproductive biology and age structure of two species of plunderfishes from the Weddell Sea, *Artedidraco skottsbergi* and *Dolloidraco longedorsalis*. In particular, their reproductive investment has been evaluated through the estimation of their gonadosomatic index (GSI), late vitellogenic oocytes sizes, absolute and relative fecundities and sizes at first spawning. Integrating different methodological approaches, we performed histological analyses of gonads to describe the gametogenetic processes of both sexes and to validate the macroscopic stages of maturity. Finally, age structure and growth rate were assessed through otolith reading.

Materials and methods

All specimens of *A. skottsbergi* and *D. longedorsalis* analysed in this study were sampled in the Antarctic summer 2014 (between January and February) during the *RV Polarstern* expedition PS82 in the Weddell Sea (Fig. 1). All specimens of *A. skottsbergi* were sampled by a bottom trawl hauled at 261 - 306 meters of depth, while *D. longedorsalis* were collected by bottom and Agassiz trawls hauled at 371 – 453 m and 473 – 572 m, respectively. Further details on sampling methods are available elsewhere (Lucassen 2012; Knust and Schröder 2014).

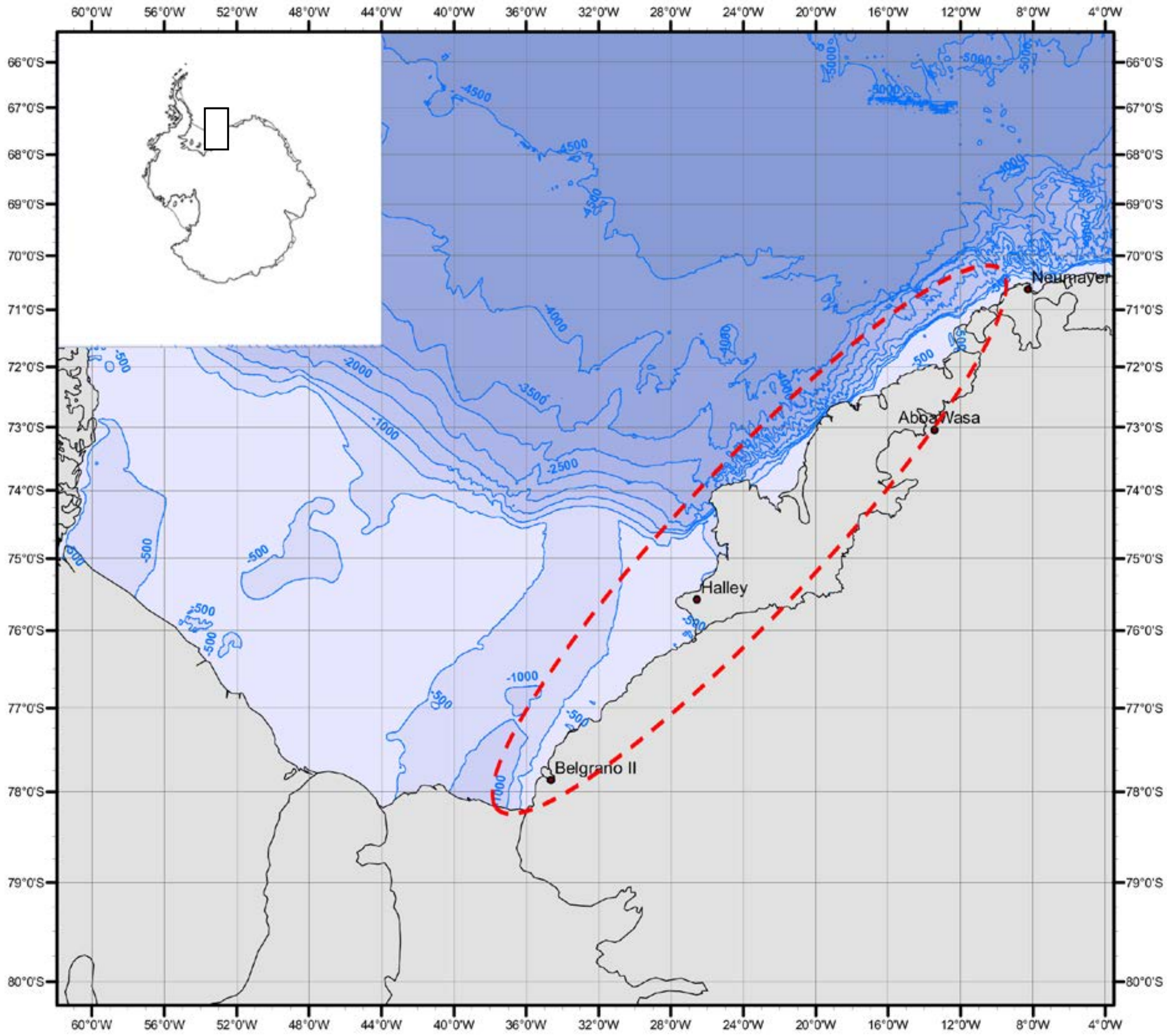

Figure 1. Map of the Weddell Sea showing bathymetry and sampling area of *Artedidraco skottsbergi* and *Dolloidraco longedorsalis* (red dashed circle).

Fish samples

For each specimen total length (TL, 0.1 mm), total weight (TW, g) and sex were recorded, sexual maturity stage was macroscopically assessed according to a five-point scale (1-5) used for notothenioids (Kock and Kellermann 1991). Sizes of *D. longedorsalis* (TL) sampled with the two different methods were compared with a t-test for two independent samples. Sex-ratios were calculated as the proportion of females on the total number of specimens caught and the values were compared to an hypothesis of balanced ratio (sex-ratio = 0.5) with a χ^2 test.

Reproductive investment

Reproductive investment was evaluated in terms of gonadosomatic index (GSI), fecundity and egg size (Duarte and Alcaraz 1989; Kamler 1992). For this purpose, gonads were excised and preserved in Dietrich's solution for histologic analyses.

Maturing ovaries (stage 3), were used for fecundity estimation, right and left gonads were separated and one of them randomly selected and preserved in formalin (7% in salt water) for oocytes size measures. Gonad weight was recorded in the lab on fixed samples with an accuracy of 0.001 g. GSI was calculated as the percentage of gonad weight to the total body weight of fish.

A sub-sample of 5 maturing ovaries (stage 3) per species was used for analysing the size-frequency distribution of oocytes. For this purpose, 5 ovaries fixed in formalin were randomly chosen and oocytes sizes (as maximum diameter) were estimated. Measurements were performed with a photo-editor software (IMAQ VISION BUILDER 6 National Instrument Corporation, Austins, TX, USA) from pictures of the oocytes spaced in a Petri dish filled with salt water. In both species, a distinct bimodal size frequency distribution was found, characterized by a group of smaller oocytes (in the pre-vitellogenetic or in the early vitellogenetic phases) and a group of larger oocytes in an advanced vitellogenesis phase (Fig. 2a and b, see also results of histological analyses). Considering that ova maturation in most Antarctic notothenioids is a biennial process (Kock and Kellermann 1991), we were confident to assume that the mode of larger oocytes represented the fecundity of the sampling year spawning season. Fecundity was therefore estimated through the counting of oocytes larger than 2.2 mm for *A. skottsbergi* and 1.6 mm for *D. longedorsalis*, thresholds established through the above mentioned size frequency distributions and set with the purpose of excluding the first mode of oocytes from counting. Absolute fecundity (F_{abs}) was calculated as the sum of the fecundities of both ovaries of each specimen, because, in both species, right and left gonads were clearly asymmetric. The total number of oocytes of the ovary fixed in formalin were counted, while only the oocytes of a weighed sub-sample of the ovary fixed in Dietrich's solution were considered, as the ovary remaining part was employed in histologic analysis. Therefore, the fecundity of the latter was assessed following the gravimetric method (Murua *et al*. 2003), estimating the total ovarian fecundity through the ratio between the sampled portion of the ovary in relation to the total ovarian weight. Relative fecundity (F_{rel}) was calculated as the number of eggs per gram of fish body weight without gonads (Witthames *et al*. 2009).

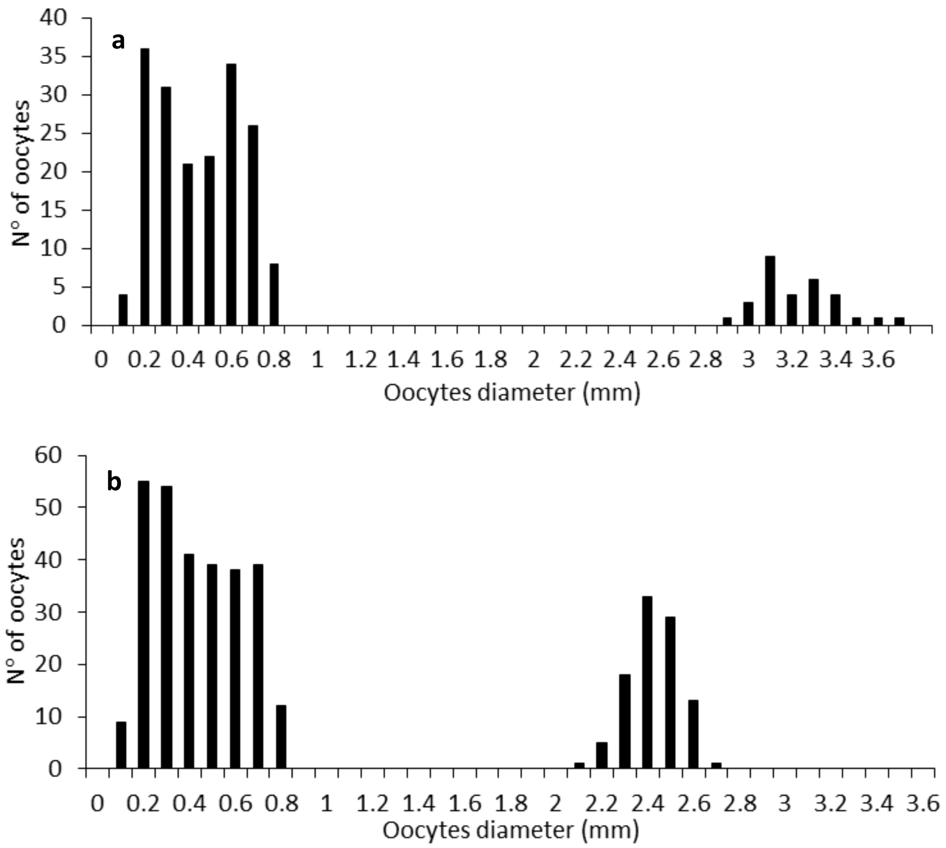

Figure 2. Size frequency distributions of oocytes diameters of ripe females of *Artedidraco skottsbergi*, (a; TL 10.3 cm) and *Dolloidraco longedorsalis* (b; TL 13.2 cm).

Histologic analyses

Histologic analysis of testes and ovaries were carried out for both species in order to study the gametogenetic processes and to either validate or solve doubts in the macroscopic attributions of gonad maturity stage. In the case of maturing ovaries used for fecundity estimations, histologic analyses aimed also at assessing the presence of post-ovulatory follicles (indicating the occurrence of recent spawning event) and/or phenomena of atresia (oocytes in reabsorption). Gonads were dehydrated in crescent concentrations of ethanol following an 8 hours protocol, embedded in paraplast, cut in transverse sections (7 µm) with a microtome and mounted on slides. Sections were then stained with Harrys' Haematoxylin and Eosin (Pearse 1985) and examined with a Leica DM LB light microscope at 5X - 100X magnifications. The number of analysed specimens for each maturity stage is reported in table 1. The reliability of macroscopic staging was assessed by evaluating the most advanced stage of development of gametocytes present in the sections (Billard 1986; West 1990).

Sizes at first spawning were estimated as the minimum sizes of specimens with gonads at macroscopic stages 3, 4 and 5 (maturing/developed, mature (gravid)/mature, spent), for each sex and species.

Table 1. Histologic analyses, number of samples (N) and gonadosomatic index (GSI) for each macroscopic stage of maturity (La Mesa *et al*. 2006).

			Stage			
	Sex		*1*	*2*	*2/3*	*3*
Artedidraco skottsbergi	*Females*	N	2	10	3	6
		GSI	-	1.1±0.6	1.2±0.2	16.4±7.5
	Males	N	0	1	0	2
		GSI	0	0.2	-	0.9±0.3
Dolloidraco longedorsalis	*Females*	N	5	10	0	12
		GSI	0.3±0.3	1.3±0.6	-	8±3.5
	Males	N	8	5	0	2
		GSI	0.2±0.2	0.9±0.2	-	1.3±0.7

Age estimation through otolith readings

Sagittal otoliths were removed, cleaned from adhering tissue with alcohol (70%) and preserved dry for ageing purposes. As otoliths showed a dense calcareous structure making them opaque, they were sectioned before reading (White 1991). Otoliths were embedded in resin (Crystalbond 509 Amber, Aremo products, inc.) on a glass slide and grounded to the core, where the sagittal section was clear enough to allow the reading of the growth structure. Sections were then polished with a decreasing grade lapping film and 0.05 µm alumina powder. Reading was performed under a Leica Mz95 Stereomicroscope at 25-40X, with a dark background and under a reflected light. Age was estimated by counting annuli, each formed by an adjacent opaque and translucent zones. Given the complexity of such structures in both species, counting was done comparing different otoliths axis, thus assuring no bias in the estimations. Estimations were performed by two readers. When ages estimates differed of more than 1 year between the two readers a third reading was done, discarding otoliths in case they still disagreed. The precision of readings was estimated through the calculation of the average percentage error (APE) and the coefficient of variation (CV) (Janssen *et al*. 1993).

A Von Bertalanffy growth curve was fitted to *D. longedorsalis* age-length data. Given the lack of individuals of small sizes, ageing data reported from the same area were included in the analysis (Voskoboinikova 2001). The few available specimens of *A. skottsbergi* did not allowed to fit a curve also for this species.

Age-length relationships keys were built for both species coupling both sexes, given the low number of males in the samples. A Kruskall-Wallis test was applied to data to test for differences between sizes of specimens among age classes.

All statistical analyses were performed with STATISTICA 8 software (StatSoft, Inc., Tulsa, OK, USA). Data normality was tested with Shapiro-Wilk tests. Mean values are showed with standard deviation.

Results

Fish samples

A total of 24 specimens (21 females, 3 males) of *A. skottsbergi* and 83 specimens (56 females and 27 males) of *D. longedorsalis* were sampled during the cruise. Samples of *D. longedorsalis* caught with different gear types did not show significant differences in sizes (bottom trawls: N = 59; Agassiz trawls: N = 24; t_{81} = 1.02, p = 0.31). In both species, a partial overlap in size between males and females was observed (*A. skottsbergi*: females TL = 6.2 – 10.3 cm; males TL = 7.8 – 8 cm; *D. longedorsalis*: females TL = 7.5 – 13.2 cm; males TL = 7 – 12cm). Due to sample size, differences in the total length between sexes were tested only for *D. longedorsalis* and were not significant (t_{81} = 1.41, df = 81, p = 0.16). Sex-ratio analysis revealed a significant majority of females in both *A. skottsbergi* (sex-ratio = 88%, $\chi 2$ = 7.85, p = 0.005) and *D. longedorsalis* (sex-ratio = 67.5%, $\chi 2$ = 5.23, p = 0.02).

Maturity stages and reproductive investment

Samples of both species were found to have gonads at macroscopic stages from 1 to 3 (Tab. 1).

In *A. skottbergi*, 25% of macroscopic stages did not match with the corresponding microscopic stages, evidencing a general underestimation in the attributions with the former method. Histologically maturing females (stage 3) had oocytes in pre-vitellogenesis and in early vitellogenesis (oocyte size range: 0.06 - 1.11 mm), but also in advanced vitellogenesis (Tab. 2). Absolute fecundity ranged between 60 and 76 oocytes/female, while relative fecundity between 6.4 and 16.3 oocytes/g (Tab. 2). Sizes at first spawning were 8 cm and 9.5 cm in males and females, respectively.

Table 2. Fecundity estimates of *Artedidraco skottsbergi* (N = 6). Diameter (Ø, Mean, Min: minimum; Max: maximum) of oocytes in advanced vitellogenesis for each female is reported.

	TL (cm)	*TW (g)*	*GSI (%)*	*F_{abs} (oocytes)*	*F_{rel} (oocytes/g)*	*Mean Ø. (mm)*	*Min Ø (mm)*	*Max Ø (mm)*
	9.5	7	10.9	76	12.2	2.7±0.1	2.4	2.9
	9.9	12	9.8	69	6.4	3.0±0.1	2.5	3.3
	9.9	5	23.3	63	16.3	2.9±0.2	2.2	3.1
	9.5	4	27.9	75	26.0	2.9±0.1	2.8	3.2
	9.5	6	11.2	60	11.3	2.9±0.2	2.6	3.2
	10.3	7	15.9	72	12.2	3.2±0.2	2.9	3.6
Mean±St.Dev.	9.8±0.3	7±3	16.5±7.5	69±7	14.1±6.6	2.9±0.2	2.6±0.3	3.2±0.2

In *D. longedorsalis*, 25.6% of macroscopic attributions were different from histologic observations and concerned mainly the ovarian staging. Maturing females (stage 3) showed oocytes in pre-vitellogenesis and in early vitellogenesis (oocyte size range: 0.04 - 1.14 mm), but also in advanced vitellogenesis (Tab. 2). Absolute fecundity ranged between 86 and 330 oocytes/female, while relative fecundity ranged between 7 and 20.5 oocytes/g (Tab. 3). Size at first spawning was 11 cm in both sexes.

Overall, the gametogenetic processes had similar characteristics in the two species. As gonad structure of *A. skottsbergi* has been recently described (La Mesa *et al*. 2006) and match with present results, only the histological characteristics of *D. longedorsalis* gonads at the different macroscopic developmental stages are reported.

Table 3. Fecundity estimates of *Dolloidraco longedorsalis* (N = 11). Diameter (Ø, Mean, Min: minimum; Max: maximum) of oocytes in advanced vitellogenesis for each female is reported.

	TL (cm)	*TW (g)*	*GSI(%)*	F_{abs} *(oocytes)*	F_{rel} *(oocytes/g)*	*Mean Ø (mm)*	*Min Ø (mm)*	*Max Ø (mm)*
	13.2	23	11.3	264	13.0	2.4±0.1	2.1	2.6
	11	14	8.2	184	14.4	2.1±0.1	1.8	2.3
	12	18	10.5	330	20.5	2.1±0.1	1.7	2.5
	12	16	12.1	270	19.2	2.1±0.1	1.6	2.4
	12	16	10.8	285	20.0	2.1±0.1	1.7	2.3
	11	14	9.6	231	18.3	2.2±0.1	1.7	2.6
	12	14	8.9	142	11.1	2.4±0.1	2.0	2.7
	12	13	8.7	148	12.4	2.4±0.2	2.1	2.8
	11	11	8.3	170	16.9	2.1±0.1	1.4	2.3
	11.5	13	4.6	86	7.0	2.3±0.1	1.8	2.7
	12	17	10.5	188	12.3	2.4±0.2	2.1	3.0
Mean± St. Dev.	11.8±0.7	15.4±3.2	9.4±2.0	209±73	15.0±4.3	2.2±0.2	1.8±0.2	2.6±0.2

Ovarian structure and oogenesis

The paired ovaries are clearly asymmetric in size, characterized by a torpedo-like shape and fused through a short oviduct. Internally, ovaries are filled with lamellae that lie perpendicularly to the lumen axis. Each lamella is filled with at least two types of oocytes at different developmental stages (‘group synchronous’ ovarian type), an organisation that is typical of species that spawn once a year over a brief spawning season (Wallace and Selman 1981).

Immature (stage 1; Fig. 3a): ovaries were filled with oogonia and proliferation was observed. Oogonia were characterized by a large spherical nucleus and a basophilic cytoplasm. Several nucleoli were present around the perinucleolar area. In some specimens, only some oogonia were recognizable, while the remaining germ cells appeared to be still in the differentiating phase.

Developing (stage 2; Fig. 3b): ovaries in this stage were characterized by the presence of two oocyte developmental stages, respectively oogonia and oocytes in the cortical alveoli phase. The latter phase was characterized by an increase in both their overall and nucleus sizes if compared to the former, and was mainly distinguishable for the conspicuous presence of chromophobic vesicles in the cytoplasm (endogenous vitellogenesis), that first appeared around the oocyte perinucleolar area and then extended to its periphery. This stage of maturity was also assigned to some specimens that exhibited a third group of oocytes of bigger sizes, that just started the exogenous vitellogenesis phase: the formation of yolk globules was observed in their cytoplasm, from the outer midcortical area towards the nucleus. Yolk globules differed from cortical alveoli for their marked Eosin staining. A thickening of the zona radiata was also observed.

Maturing (stage 3; Fig. 3c): very few oogonia and oocytes in the cortical alveoli phase were present. Ovaries were almost entirely filled with large oocytes that almost completed the exogenous vitellogenesis phase, being close to be ovulated. Larger oocytes were clearly distinguishable for their size almost doubled if compared to the other oocytes, and for the presence of dense and large yolk globules in the cytoplasm, some of which starting to fuse to form plates. The zona radiata was thicker than in the other stages. Occasional signs of atresia (1-2 atretic oocytes in 5 ovaries) were found (Fig. 3d). Atretic oocytes were characterized by a thick wall and clear signs of phagocytosis in the cytoplasm. All females used for fecundity estimation had gonads at this developmental stage, thus confirming the results evidenced in the size frequency distributions of oocytes and validating the method used for evaluating the fecundity of these species. Moreover, no signs of atresia and/or the presence of post-ovulatory follicles were observed in these samples.

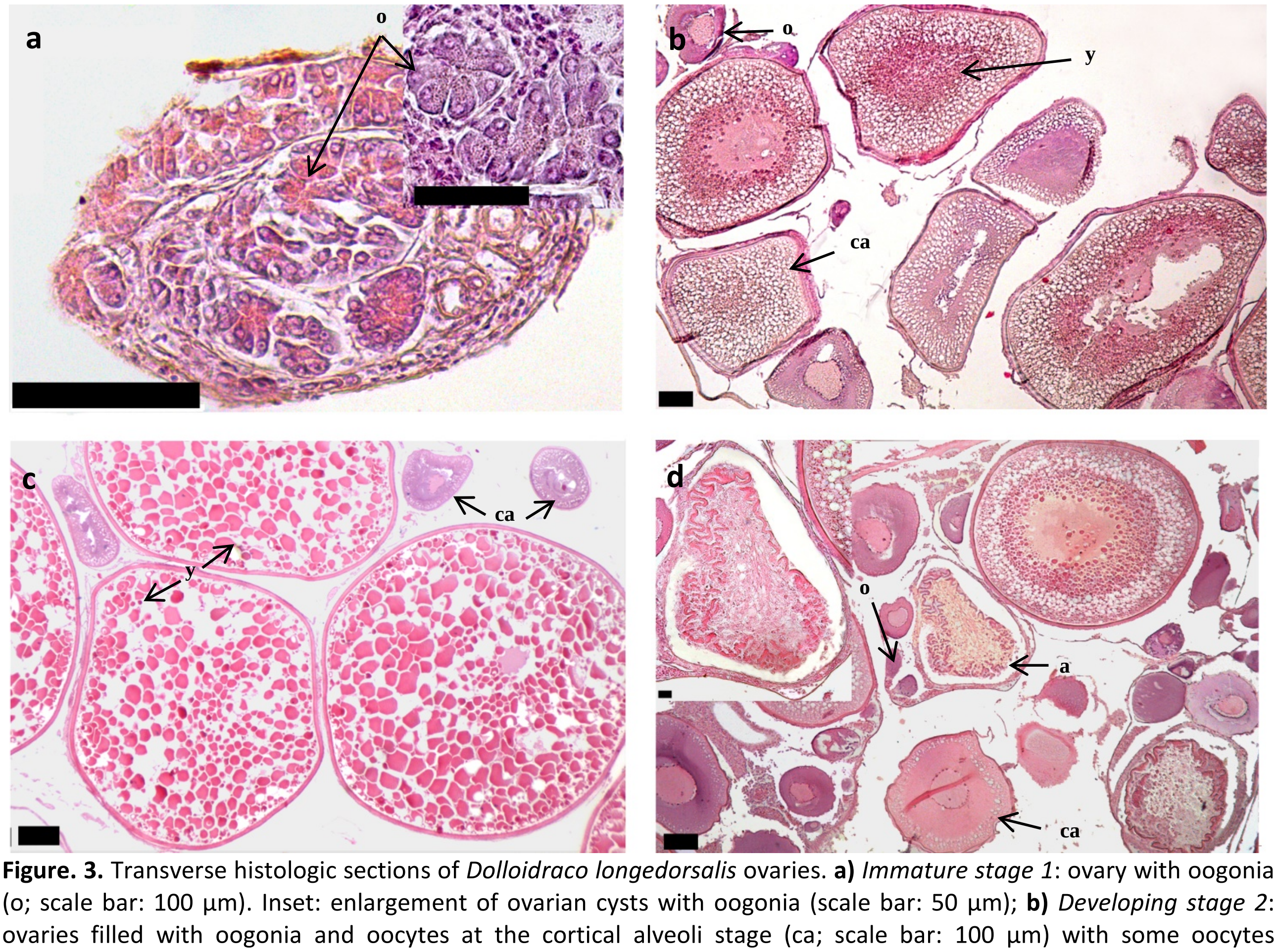

Figure. 3. Transverse histologic sections of *Dolloidraco longedorsalis* ovaries. **a)** *Immature stage 1*: ovary with oogonia (o; scale bar: 100 µm). Inset: enlargement of ovarian cysts with oogonia (scale bar: 50 µm); **b)** *Developing stage 2*: ovaries filled with oogonia and oocytes at the cortical alveoli stage (ca; scale bar: 100 µm) with some oocytes presenting yolk globules (y; initial exogenous vitellogenesis) in the cytoplasm; **c)** *Maturing stage 3*: ovary with large oocytes, characterized by dense and large yolk globules, and few oocytes at the cortical alveoli stage (ca; scale bar: 100 µm); **d)** *Atresia phenomena*: ovary with atretic oocytes, recognizable for the shrinking of the zona radiata and signs of phagocytosis within the cytoplasm; oocytes at the cortical alveoli stage (ca) and oogonia (o) are also present (scale bar: 100 µm). Inset: atretic oocyte (a; scale bar: 50 µm).

Testicular structure and spermatogenesis

Testes are paired elongated organs, fused posteriorly in the sperm duct. They are of the 'cystic lobular type' (Nagahama 1983), with finger-like lobules of different diameters and each of them characterized by a central, narrow lumen. Lobules are filled with several cysts of germ cells showing the same stage of development. Testes are of the 'unrestricted spermatogonial type' (Nagahama 1983), with spermatogonia randomly distributed along lobules. An histological description of testes was possible for macroscopic stages 1, 2 and 3.

Immature (stage 1; Fig. 4a): testes had a compact inner structure, organized into lobules and interstitial compartments filled with connective tissue. Lobules were filled with cysts with spermatogonia; lobules central lumen was barely visible; some evidence of occurring mitoses was present.

Developing (stage 2; Fig. 4b): testes were less compact if compared to stage 1 and lobules lumen were clearly visible; testes were undergoing spermatogenesis processes and cysts were filled with spermatogonia and spermatocytes.

Developed (stage 3; Fig. 4c): the lumen of the lobules was wider than in stage 2 testes; active spermatogenesis processes were occurring and it was possible to distinguish few spermatogonia cells, located mainly in the distal part of the lobules, from spermatocytes and spermatids.

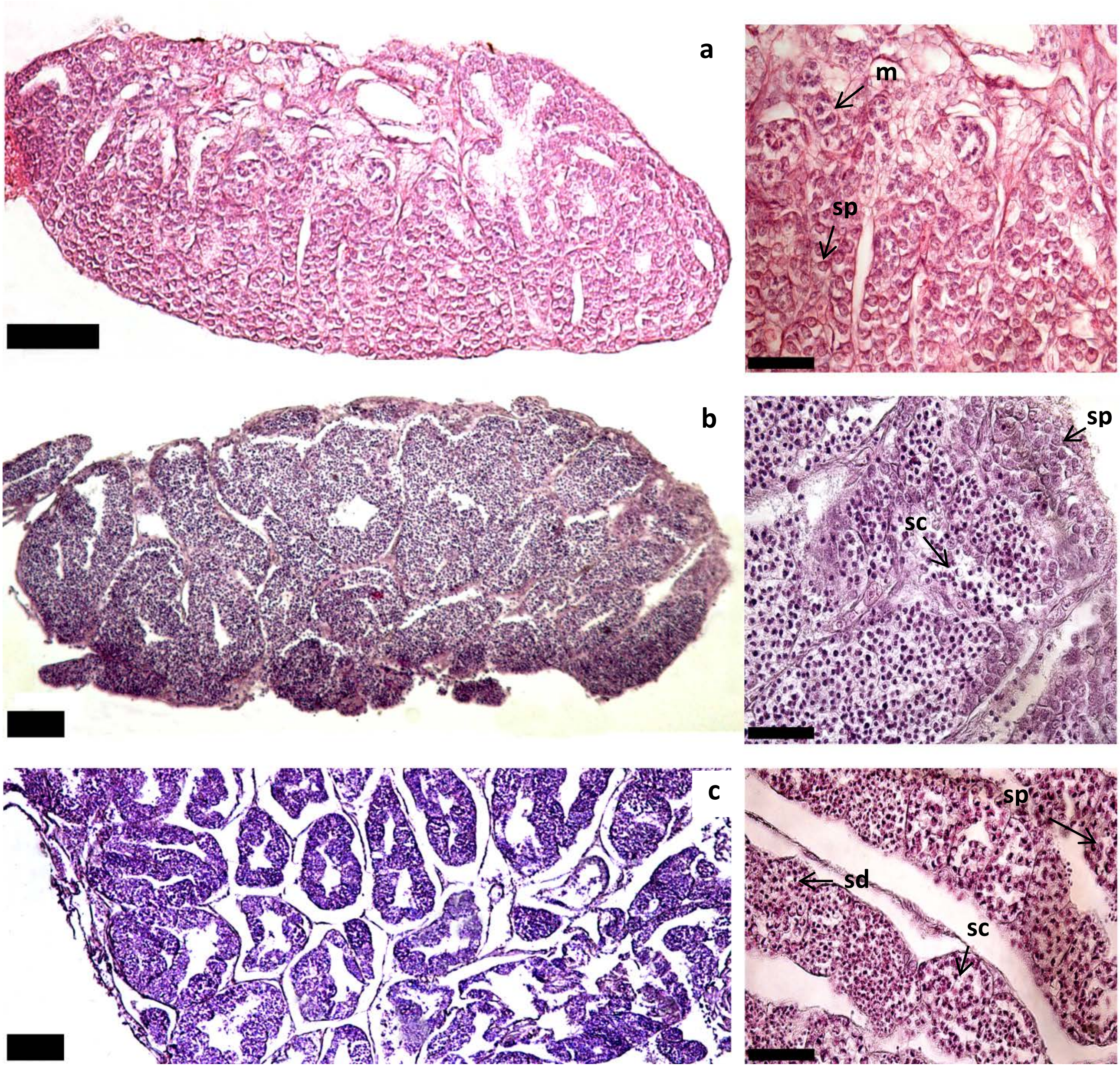

Figure. 4. Transverse histologic sections of *Dolloidraco longedorsalis* testes (scale bars of figures on the left: 100 µm; scale bars of enlargements on the right: 50 µm). **a)** *Immature stage 1*: testis filled with cysts of spermatogonia (sp); **b)** *Developing stage 2*: testis filled with cysts of spermatogonia and spermatocytes (sc); **c)** *Developed stage 3*: testis filled with cysts at 3 stages of development: spermatogonia, spermatocytes and spermatids (sd).

Age estimation through otolith readings

Otoliths of both species evidenced a complex growth structure (fig. 5a and b). Nevertheless, their readings were extremely precise when considering *A. skottsbergi* samples (APE = 1.9%; CV = 2.7%), less precise but still reliable in the case of *D. longedorsalis* (APE = 6.4%; CV = 9%). Specimens of *A. skottsbergi* (n = 22) ranged in age from 6 to 14 years (TL: Min = 7.8 cm; Max = 10.2 cm) while those of *D. longedorsalis* (n = 77) had 3 (TL = 7 cm) to 14 years (TL = 11.4 cm). The Von Bertalanffy growth-curve fitted for *D. longedorsalis* age-length data pairs is reported in figure 6. The estimated

Von Bertalanffy growth parameters were L_∞ = 12.5, K = 0.27, t_0 = -0.19 and the growth performance Φ' was 1.63.

In both species, ageing data estimated for each sex were pooled together and summarized in the age-length key (Tab. 4 and 5). The minimum age at sexual maturity was 5 and 7 years in *D. longedorsalis* and *A. skottsbergi*, respectively.

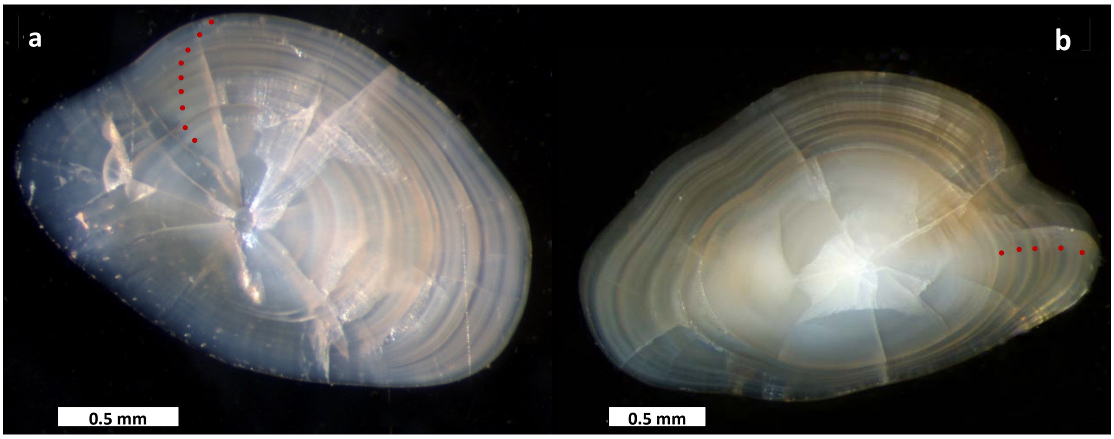

Figure 5. Sectioned sagittal otoliths of **a)** *Artedidraco skottsbergi* (TL = 8 cm; Age = 9 years) and **b)** *Dolloidraco longedorsalis* (TL = 11 cm; Age = 5 years).

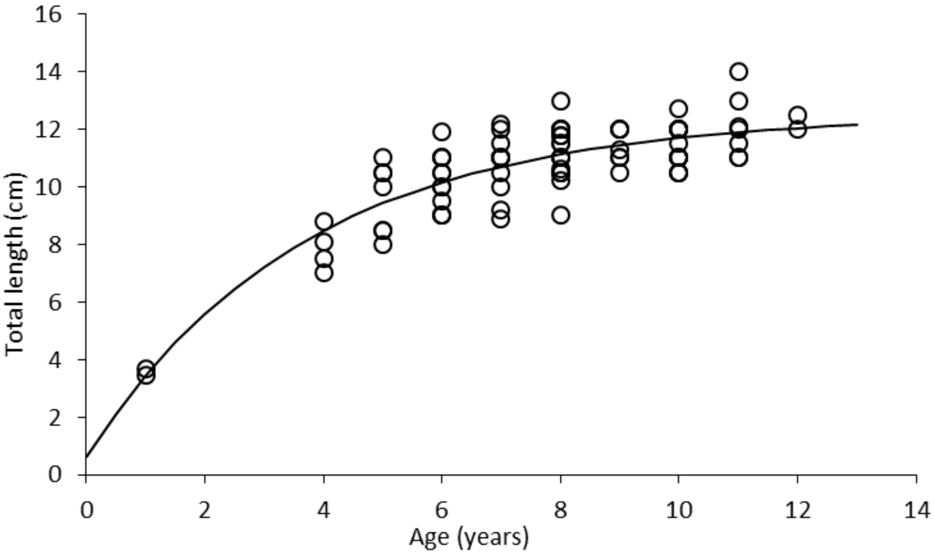

Figure. 6. Von-Bertalanffy growth-curve applied to *Dolloidraco longedorsalis* age data. Data of two specimens from Voskoboinikova (2001) were also used.

Table 4. Age-length key of *Artedidraco skottsbergi* (*N* number of specimens; *St. Dev.* standard deviation).

TL (cm)/Age (years)	6	7	8	9	10	11	12	13	14
6		1							
7	2								
8		1		3	2	1	1	1	1
9		1		1	2	1		1	1
10					1				1
11									
12									
13									
14									
N	2	3	0	4	5	2	1	2	3
Mean size	7.8	7.9		8.4	9.4	9.1		8.8	9.5
St. Dev.		1.6		0.8	0.8	0.2		0.4	0.9

Table 5. Age-length key of *Dolloidraco longedorsalis* (*N* number of specimens; *St. Dev.* standard deviation).

TL (cm)/Age (years)	3	4	5	6	7	8	9	10	11	12	13	14
7	2											
8		2	1	1	1							
9				3	1							
10			5		6	3	1	2	1			
11			4	2	5	5	4	3	3	1		1
12				1	2	4	4	2	4			
13					1	1						
14									1			
N	2	2	10	7	16	13	9	7	9	1	0	*1*
Mean size	7.3	8.5	10.6	9.9	10.8	11.5	11.5	11.2	11.8			
St. Dev.	0.4	0.5	0.96	1.4	1.1	0.8	0.6	0.6	1.0			

Discussion

This paper provides some new insights on the biology (reproduction and age) of *Artedidraco skottsbergi* and *Dolloidraco longedorsalis*, at the same time enforcing the few data available in literature. From the reproductive point of view, *A. skottsbergi* and *D. longedorsalis* exhibit the same features commonly reported for notothenioids. Both species produce a few (maximum 330 oocytes in advanced vitellogenesis) but large eggs. An investment in the production of large gametes is a characteristic shared by many Antarctic species (Ekau 1991) and has probably evolved to increase larvae survival in habitats characterized by harsh environmental conditions and strong competition which characterize the Southern Ocean (Conover *et al*. 1997; Bownds *et al*. 2010). The gametogenesis is suggested to be a biennial process (Duhamel *et al*. 1993), an hypothesis that is supported in this study by the observation that some of the smaller oocytes (first mode of the size frequency distribution of oocytes) already started the exogenous vitellogenesis phase at the time of sampling. Considering the closeness of the spawning season, which seems to take place late in summer according to present results and previous data (Kock and Kellermann 1991; La Mesa *et al*. 2006), it is likely that oocytes at the beginning of the exogenous vitellogenesis would not complete the maturation process before the following year. The presence of a clear separation between the two dimensional modes of oocytes in the size frequency distributions and the absence of post-ovulatory follicles in the histologic samples support the hypothesis that eggs are released during a single spawning event. Late vitellogenic oocytes of *A. skottsbergi* and *D. longedorsalis* are within the size range reported from other notothenioids in the Weddell Sea (2-4 mm). However, their absolute fecundity is 5 to 40 times lower than in other notothenioid families inhabiting the same area (Ekau 1991). The lower fecundity can be explained by the constraints related to their size (Duarte and Alcaraz 1989), being *A. skottsbergi* and *D. longedorsalis* smaller than almost all species of the above mentioned families.

Histologically, in both species, male testes are of the ‘unrestricted spermatogonial type’ (Nagahama 1983), with spermatogonia randomly distributed along the lobules, as previous described in *A. skottsbergi* (La Mesa *et al*. 2006).

Sex-ratios were significantly biased towards females in both species. Perhaps this result was due to a different catchability of the two sexes rather than to a real skewed sex-ratio, which was not previously reported for the same species (Morales-Nin *et al*. 2000; Eastman and Eakin 2001). The unbalanced sex-ratio evidenced in this study could be also related to a different spatial distribution of sexes, in concomitance with nest preparation undertaken generally by males before the spawning season. Adult males could therefore be more difficult to catch because hidden or aggregated in areas apart from trawlable grounds, an hypothesis enforced by the possible existence of males parental cares in these species, as already reported for some species of the genus *Artedidraco* (Daniels 1978; White and Burren 1992; Jones and Near 2012) and documented in closely related species of the genera *Harpagifer* and *Pogonophryne* (Ekau 1991; Kock and Kellermann 1991; Hubold 1992; Duhamel *et al*. 1993).

Although *A. skottsbergi* and *D. longedorsalis* shared the common reproductive features described in most notothenioids, they showed some differences in the allocation of energy towards reproduction. In particular, *A. skottsbergi* exhibited lower fecundities and produced larger oocytes than *D. longedorsalis*, consistently with a general trade-off between fecundity and egg size (Smith and Fretwell 1974; Stearns 1989; Stearns 1992). The difference in absolute fecundity between the two species could be explained by the larger sizes of *D. longedorsalis*.

In this study fecundity estimation in *A. skottsbergi* is consistence with previous one carried out in the same area (Duhamel *et al*. 1993). Differently, Ross Sea data show higher values (La Mesa *et al*. 2006). The differences in egg number investment in the two areas could be related to different environmental pressure or ecological constrains. (La Mesa *et al*. 2006). The factors influencing the observed differences remain to be investigated. Samples from the Ross Sea were found to have higher fecundities (both absolute and relative were estimated for *A. skottsbergi* from the Ross Sea: F_{abs} = 110-183 oocytes; F_{rel} = 17.2 – 22.6 oocytes/g TW; La Mesa *et al* 2006) than those from the Weddell Sea, analysed in this study and by Duhamel *et al*. (1993). Differences in female sizes and the low number of samples analysed in both La Mesa *et al*. (2006) and Duhamel *et al*. (1993) studies does not allow to draw conclusions on these differences.

This study highlighted some methodological issues. The first one refers to the discrepancy between the macroscopic staging of gonadal development of some samples and their histological features, as already highlighted in other notothenioids (Macchi and Barrera-Oro 1995; La Mesa *et al*. 2003). Histological analysis of samples should therefore always be coupled to such attributions in order to validate them (La Mesa *et al*. 2003).

The insights provided by this paper add key information for understanding the biology of *A. skottsbergi* and *D. longedorsalis*, in particular for the Weddell Sea populations. These information not only may contribute to the knowledge of the adaptations of notothenioids to the Antarctic environment in an evolutionary context, but are also essential in the perspective of evaluating the reproductive potential and thus the capacity of resilience or potential of recovery of these populations in response to environmental disturbances. A drop in their abundance could trigger a series of cascading effects in the Antarctic trophic chains, which are structured around ecological niches occupied by very specialized species, that could hardly be replaced.

References

Balushkin A., Eakin R.R. (1998). A new toad plunderfish *Pogonophryne fusca* sp. nova (fam. Artedidraconidae: Notothenioidei) with notes on species composition and species groups in the genus *Pogonophryne* Regan. Journal of Ichthyology 38: 574-579.

Billard R. (1986). Spermatogenesis and spermatology of some teleost fish species. Reproduction Nutrition Développement 26: 877-920.

Bownds C., Wilson R., Marshall D.J. (2010). Why do colder mothers produce larger eggs? An optimality approach. The Journal of Experimental Biology 213: 3796-3801.

Conover D.O., Brown J.J., Ehtisham A. (1997). Counter gradient variation in growth of young striped bass (*Morone saxatilis*) from different latitudes. Canadian Journal of Fisheries and Aquatic Sciences 54: 2401-2409.

Daniels R.A. (1978). Nesting behaviour of *Harpagifer bispinis* in Arthur Harbour, Antarctic Peninsula. Journal of Fish Biology 12: 465-474.

Daniels R.A. (1982). Feeding ecology of some fishes of the Antarctic Peninsula. Fishery Bulletin 80: 575-588.

Duarte C.M., Alcaraz M. (1989). To produce many small or few large eggs: a size-independent reproductive tactic of fish. Oecologia 80: 401-404.

Duhamel G., Kock K.H., Balguerias E., Hureau J.C. (1993). Reproduction in fish of the Weddell Sea. Polar Biology 13: 193-200.

Eakin R.R., Eastman J.T., Jones C.D. (2001). Mental barbel variation in *Pogonophryne scotti* Regan (Pisces: Perciformes: Artedidraconidae). Antarctic Science 13: 363-370.

Eastman J.T. (1993). Antarctic fish biology: evolution in a unique environment. Academic Press, San Diego, 322 pp.

Eastman J.T., Hubold G. (1999). The fish fauna of the Ross Sea, Antarctica. Antarctic Science 11: 293-304.

Eastman J.T., Eakin R.R. (2000). An updated species list for notothenioid fish (Perciformes; Notothenioidei), with comments on Antarctic species. Archive of Fishery and Marine Research 48: 11-20.

Eastman J.T., Eakin R.R. (2001). Mental barbel and meristic variation in the Antarctic notothenioid fish *Dolloidraco longedorsalis* (Perciformes: Artedidraconidae) from the Ross Sea. Polar Biology 24: 729-734.

Eastman J.T., Lannoo M.J. (2003). Diversification of brain and sense organ morphology in Antarctic dragonfishes (Perciformes: Notothenioidei: Bathydraconidae). Journal of Morphology 258: 130-150.

Ekau W. (1991). Reproduction in high Antarctic fishes (Notothenioidei). Berichte zur Polar- und Meeresforschung 33: 159-167.

Everson I. (1984). Fish biology. In: Laws R.M. (eds) Antarctic Ecology. Academic Press, London, 2: 491-532.

Gutt J., Ekau W. (1996). Habitat partitioning of dominant high Antarctic demersal fish in the Weddell Sea and Lazarev Sea. Journal of Experimental Marine Biology and Ecology 206: 25-37.

Hubold G. (1991). Ecology of notothenioid fish in the Weddell Sea. In: Di Prisco G., Maresca B., Tota B. (eds) Biology of Antarctic fish. Springer Science & Business Media, Berlin Heidelberg New York, 3-22.

Hubold G. (1992). Ecology of Weddell sea fishes. Berichte zur Polarforschung 103: 1-157.

Iwami T., Numanami H., Naito Y. (1996). Behavior of three species of the family Artedidraconidae (Pisces, Notothenioidei), with reference to feeding. Proceedings of the NIPR Symposium on Polar Biology 9: 225-230.

Janssen J., Jones W., Slattery M. (1993). Locomotion and feeding responses to mechanical stimuli in *Histiodraco velifer* (Artedidraconidae). Copeia 885-889.

Jones C.D., Near T.J. (2012). The reproductive behaviour of *Pogonophryne scotti* confirms widespread egg-guarding parental care among Antarctic notothenioids. Journal of Fish Biology 80: 2629-2635.

Kamler E. (1992). Early life history of fish: an energetics approach. Chapman and Hall, London, 267 pp.

Knust R., Schröder M. (2014). The Expedition PS82 of the Research Vessel POLARSTERN to the southern Weddell Sea in 2013/2014. Berichte zur Polar- und Meeresforschung = Reports on polar and marine research. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, 680: 1-155.

Kock K.H., Kellermann A. (1991). Reproduction in Antarctic notothenioid fish. Antarctic Science 3: 125-150.

La Mesa M., Caputo V., Rampa R., Vacchi M. (2003). Macroscopic and histological analyses of gonads during the spawning season of *Chionodraco hamatus* (Pisces, Channichthyidae) off Terra Nova Bay, Ross Sea, Southern Ocean. Polar Biology 26: 621-628.

La Mesa M., Caputo V., Rampa R., Eastman J.T. (2006). Gametogenesis in the Antarctic plunderfishes *Artedidraco lönnbergi* and *Artedidraco skottsbergi* (Pisces: Artedidraconidae) from the Ross Sea. Antarctic Science 18: 183-190.

Lucassen M. (2012). The expedition of the research vessel "Polarstern" to the Antarctic in 2012 (ANT-XXVIII/4). Berichte zur Polar- und Meeresforschung 652: 1-90.

Macchi G., Barrera-Oro E. (1995). Histological study on the ovarian development of mackerel icefish (*Champsocephalus gunnari*) from the South Georgia Islands. CCAMLR Science 2: 35-49.

Macdonald J., Montgomery J. (1991). The sensory biology of notothenioid fish. In: Di Prisco G., Maresca B., Tota B. (eds) Biology of Antarctic fish. Springer Science & Business Media, Berlin Heidelberg New York, 145-162.

Morales-Nin B., Moranta J., Balguerias E. (2000). Growth and age validation in high-Antarctic fish. Polar Biology 23: 626-634. doi:10.1007/s003000000132.

Murua H., Kraus G., Saborido-Rey F., Witthames P.R., Thorsen A., Junquera S. (2003). Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal of Northwest Atlantic Fishery Science 33: 33-54.

Nagahama Y. (1983). The functional morphology of teleost gonads. Fish Physiology 9: 223-275.

North A.W., White M.G. (1987). Reproductive strategies of Antarctic fish. In: KullanderSO, Fernholm B (eds) Proceedings of the V congress of the European Ichthyol, , Stockholm 1985. Swed Mus Mus Nat Hist 381-391 pp.

Olaso I., Rauschert M., De Broyer C. (2000). Trophic ecology of the family Artedidraconidae (Pisces: Osteichthyes) and its impact on the eastern Weddell Sea benthic system. Marine Ecology Progress Series 194: 143-158.

Pearse A.G.E. (1985). Histochemistry. Theoretical and applied Analytical technology. Churchill Livingstone, Edinburgh, 624 pp. doi:10.1002/path.1711470319.

Schawrzbach W. (1988). The demersal fish fauna of the eastern and southern Weddell Sea: Geographical distribution, feeding of fishes and their trophic position in the food web. Berichte zur Polarforschung 54: 1-93.

Smith C.C., Fretwell S.D. (1974). The optimal balance between size and number of offspring. The American Naturalist 108(962): 499-506.

Stearns S.C. (1989). Trade-offs in life-history evolution. Functional Ecology 3: 259-268.

Stearns S.C. (1992). The evolution of life histories. Oxford University Press, Oxford, 249 pp.

Voskoboinikova O. (2001). Evolutionary significance of heterochronies in the development of the bony skeleton in fishes of the suborder Notothenioidei (Perciformes). Journal of Ichthyology 41: 415-424.

Wallace R.A., Selman K. (1981). Cellular and dynamic aspects of oocyte growth in teleosts. American Zoologist 21: 325-343.

West G. (1990). Methods of assessing ovarian development in fishes: a review. Marine and Freshwater Research 41: 199-222.

White M.G. (1991). Age determination in antarctic fish. In: di Prisco G., Maresca B., Tota B. (eds) Biology of Antarctic fish. Springer Science & Business Media, Berlin Heidelberg New York, 87-100.

White M., Burren P. (1992). Reproduction and larval growth of *Harpagifer antarcticus* Nybelin (Pisces, Notothenioidei). Antarctic science 4: 421-430.

Witthames P.R., Thorsen A., Murua H., Saborido-Rey F., Greenwood L.N., Dominguez R., Korta M., Kjesbu O.S. (2009). Advances in methods for determining fecundity: application of the new methods to some marine fishes. Fishery Bulletin 107: 148-164.

PAPER VI

Evolution of life history traits in notothenioid fishes

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Abstract
Antarctic notothenioid fishes represent a striking example of adaptive radiation in marine environment, dominating the waters surrounding the Antarctic continent both in species number, with over 120 species (47%), and biomass (90-95%).

We investigated the evolution of some life history traits of notothenioids, such as: gonadal investment, represented by gonadosomatic index, fecundity and egg size, body maximum size in relation to environmental variables (sea water temperature, primary productivity, salinity, sea ice cover and latitude), using the independent contrasts as comparative method.

Controlling for phylogenetic relationships, maximum size was not related to any environmental factors. Even if egg size was positively related to maximum size, fish size appears to constrain, rather than to determine, the range of possible egg sizes. In fact, regardless body size, High-Antarctic species in general exhibited large eggs, while Sub-Antarctic smaller ones. Egg size resulted also negatively related to primary production, while relative fecundity was positively related to the water temperature, following the same trend highlighted in other taxa. Reproductive effort, in gonadal investment, showed a positive relationship with sea ice cover, probably due to the key role of the seasonal pack sea ice melting in the seasonal cycle of primary production.

Introduction

Teleost fish display a wide inter- and intraspecific variation in life history traits, reflecting the effects of evolutionary forces acting on them over time and across environmental conditions (Stearns 1992). Growth rate, age at sexual maturity, hatching size, egg size, fecundity and reproductive investment are largely influenced by temperature and food availability (Stearns 1977, 1992). Therefore temperature and food availability have been recognized as major drivers of variation in life history traits. Nonetheless, other factors, possibly related to the former, are known to influence life history traits, among them competition and predation (Roff 1992).

Fish reveal a wide range of fecundity and egg size, two key features of reproductive strategies, generally with a trade-off between them, leading to few large eggs (quality) or many small eggs (quantity). In fish, as in several other taxa, a bimodal distribution of egg size has been observed, with eggs being either relatively small or large, with few cases of intermediate size (Duarte and Alcaraz 1989; Laptikhovsky 2006). Egg size is generally species-specific, although within species it may vary between populations and even within a population, in relation to female size, food availability or spawning period (Laptikhovsky 2006). It has been stated that in marine fish large eggs are typically present in species with demersal eggs inhabiting estuarine and littoral or Antarctic environments (Dando 1984; Kock and Kellermann 1991). Some studies highlighted a positive relationship between egg size and parental care (Balon 1984; Kolm and Ahnesjö 2005). In general, a trend of increasing egg size with the decrease of temperature towards high latitudes has been documented in several taxa, including fish (Laptikhovsky 2006). In the tropics and in temperate latitudes, fish are represented by species producing both small and large eggs, whereas in polar regions only by species with large eggs (Kock and Kellermann 1991; Laptikhovsky 2006).

Antarctic notothenioid fish represent a striking example of adaptive radiation in marine environment, dominating the waters surrounding the Antarctic continent both in species number, with over 120 species (47%), and biomass (90-95%) (Eastman 2005). Since the mid-Miocene, they evolved an array of physiological and morphological adaptations in response to the peculiar Antarctic environment, occupying the unfilled ecological niches (Eastman 2005).

These fish present a common suite of characteristics (Andriashev 1965; Kock and Kellermann 1991). Indeed, compared to fish from other areas, notothenioids generally exhibit high reproductive investment, documented by high gonadosomatic index in females, low fecundity, large eggs, slow growth rate, late sexual maturity and, in some cases, prolonged parental care (Andriashev 1965; Kock and Kellermann 1991; Detrich *et al*. 2005; Ferrando *et al*. 2014). Species with large eggs are characterized by long embryonic development duration. Egg size strictly determines larval characteristics, showing a positive relationship with larval size at hatching (Duarte and Alcarez 1989; Kock and Kellermann 1991).

In notothenioids, eggs are generally released in one batch, as indicated by the group-synchronous ovarian type, with only one group of oocyte at the same maturity stage and of similar size (Wallace and Selman 1981; Kock and Kellermann 1991). Some species require about one year to complete the vitellogenesis, while others need longer time (Kock and Kellermann 1991; Everson 1994; Everson *et al*. 1996; Kock and Everson 1997). A general trend of increasing egg size and decreasing relative fecundity towards higher latitudes was described in nototheniids. Relative fecundity is typically higher in the Seasonal Pack-ice Zone and around the islands north of it than in the High-Antarctic Zone (Kock 1992), although a considerable variability among families is present (Kock and Kellermann 1991).

Generally life history traits do not provide independent trait value for comparative analyses, as species share characteristics through descendent from common ancestors (Harvey and Purvis 1991). The similarity in life histories among closely related species may be attributed to the phylogenetic relationships, in particular when the environments experienced by the considered species are not markedly different (Rochet 2000). When closely related populations, species or higher taxa show differences in life history traits under different environmental and ecological conditions, the investigation of the possible habitat-dependent evolutionary pattern is particularly interesting. Despite the availability of recent phylogenies of notothenioids (Near and Cheng 2008; Rutschmann *et al*. 2011; Near *et al*. 2012; Dettai *et al*. 2012), a comparative analysis (Harvey and Pagel, 1991) aimed at studying habitat dependent evolution of reproductive strategies has not yet been performed. Despite their common characteristics and even if limited in their distribution almost exclusively to the Antarctic and sub-Antarctic regions, notothenioids display wide variability in life history traits at both inter- and intra-specific levels. This taxon therefore represents a good study model to investigate the evolution of life history traits, considering their adaptive radiation, distribution at different latitudes, and therefore environment, in the Antarctic and Sub-Antarctic waters, and variability in reproductive features.

This study aims to investigate the evolution of some life history traits in notothenioids in relation to environmental variables: sea water temperature, water temperature variation (Δt = maximum temperature - minimum temperature), primary productivity, salinity and sea ice cover, using the independent contrasts as comparative method. In particular, we focused the attention on gonadal investment, represented by gonadosomatic index, fecundity and egg size, as well as body maximum size.

Considering the patterns highlighted for other taxa (Rass 1941, 1986; Angilletta and Dunham 2003; Laptikhovsky 2006; Fisher *et al.* 2010; Huston and Wolverton 2011), we expected: i. a negative relationship between egg size and sea water temperature and/or productivity, and a positive relationship between egg size and latitude; ii. as result of the trade-off egg size/number (Bell 1980), the opposite relationships between relative fecundity and sea water temperature, productivity and latitude; iii. no relationship between body size and latitude, temperature or productivity, since the well-known Bergmann's rule (occurrence of larger species at higher latitudes-lower temperature) has been hypothesized not to apply at high latitudes (Huston and Wolverton 2011); iv. a positive relationship between gonadal investment (GSI) and sea water temperature and/or productivity, and a positive relationship between GSI and latitude, as result of an overall higher reproductive investment expected in harsh conditions. These trends are expected to be confirmed also controlling for phylogeny.

This comparative study is interesting for evolutionary biology, given the adaptations of notothenioids to their peculiar environment, and for conservation biology, as the knowledge of reproductive traits of some exploited species is recognized as crucial for their sustainable management (Kock *et al*. 2007).

Materials and methods

Life history traits

The life history traits of notothenioid species were collected from literature (Annex 1) or estimated from samples collected during a series of trawl surveys (for detail see Knust *et al*. 2012; Lucassen 2012; Knust and Schröder 2014). Maximum fish size was included in the analysis, taking into account the largest size (total length) reported for each species (Gon and Heemstra 1990; Fisher and Hureau 1985; E. Riginella unpublished data). Available data on maximum egg size, relative fecundity (as mean number of eggs / total body weight of the female), maximum female gonadosomatic index (GSI) at spawning time, were included in the database, accounting for 57 out of the 139 notothenioid species described so far (41%) (Eastman and Eakin, 2015). When existing, data on egg type (demersal or pelagic), and the presence of parental care were registered as well.

For 17 species, belonging the eight notothenioid families, samples were processed to estimate fecundity, egg size and GSI. All fish samples were measured (total length, TL, to the nearest mm) and weighted (TW, in grams) and the maximum sizes were recorded and compared with those reported in literature. Sexual maturity stages were assigned to the gonads according to a five-point maturity scale (Kock and Kellermann 1991), gonads were excised and weighted (GW). GSI was calculated as the percentage of gonad weight to total somatic weight (TW - GW) of each specimen. Fecundity was estimated for mature females (developing or gravid) using the gravimetric method (Hunter *et al.* 1985). The samples were selected to include the entire size range of mature females. A subsample of the ovaries was excised from the middle portion and weighted (Murua *et al*. 2003). The size frequency distribution of the oocytes was evaluated by measuring and counting all the oocytes present in the subsample. The number of vitellogenic oocytes (stage assigned through histological analysis) was counted, and the total number of vitellogenic oocytes of the ovaries (absolute fecundity) estimated. Relative fecundity was calculated as the number of vitellogenic oocytes per gram of total body weight.

Environmental variables

Geographical distribution of each notothenioid species was downloaded from Fishbase (Froese and Pauly, 2013), which represents a compilation of entries made by the Global Biodiversity Information Facility (GBIF; http://www.gbif.org), the Ocean Biogeographic Information System (OBIS; http://www.iobis.org) and Fishbase itself. Environmental data were obtained from the AquaMaps and NOAA database (http://www.aquamaps.org; http://www.noaa.gov), the former designed for the prediction of global distributions of marine species (Kaschner *et al*. 2007, 2013; Ready *et al*. 2010; Colombo *et al*. 2015). These predictions are made on the basis of a characterization of the environmental preferences of each species, taking into account several environmental parameters, such as bottom depth, water temperature, salinity, primary production and sea ice cover, all parameters considered best suited to quantify the preferences of marine species (Kaschner *et al*. 2006; Colombo *et al*. 2015). Environmental factors show strong correlation with the mean latitude (all $p<0.01$, all $r>0.66$), which was therefore excluded from the analyses. Five environmental variables were selected: average water temperature, water temperature variation (Δt = maximum temperature - minimum temperature), average salinity, average primary production and average sea ice cover, as factors potentially related to life history traits' evolution. Mean values were calculated from the minimum and maximum available values. Minimum and maximum values of each environmental variable were initially considered, but they were then excluded being strongly correlated with the mean values (all $p<0.01$, all $r>0.1$), maintaining only variation in sea water temperature. In addition, the average salinity was excluded from the analyses considering its narrow range of variation (32 to 35.4 PSU, except for the catadromous *Pseudaphritis urvillii*) (Annex 1).

Comparative analyses

The role of environmental factors in determining the observed variation in life history traits was investigated using raw data and then accounting for the phylogenetic relationships among species, applying the Independent Contrast Method (Felsenstein 1985; Harvey and Pagel 1991; Garland *et al*. 1992). The analyses were performed using Mesquite (Mesquite version 2.75, Maddison and Maddison 2011) and STATISTICA 8 StatSoft software. The phylogenetic relationships and distances used in the analyses were based on the phylogeny of notothenioids provided by Near *et al.* (2012), resulting from combined nuclear and mitochondrial DNA genes.

Four different datasets and four corresponding sets of phylogenetic relationships were used for the life history traits, reflecting the availability of the data; overall, 50 species for relative fecundity, 53 species for egg size and 33 species for GSI. All the 57 species were considered for maximum size.

Relationships between variables were analysed firstly on raw data by means of Spearman rank correlations. Relationships between maximum size and the other life history traits and between raw data of each life history trait and environmental variables, were evaluated, applying the Benjamini–Hochberg FDR procedure to correct for multiple testing (Verhoeven *et al*. 2005). Taking into account phylogenetic relationship, contrasts among life history traits and environmental factors were analysed by simple regressions forced through the origin of the axes (as recommended by Garland *et al*. 2012); when more than one factor was significant, multiple regressions (again forced through the origin of the axes) were performed in order to highlight which factor/s better explained the interspecific variation of life history traits. To elucidate potential trends in strictly Antarctic notothenioids, the same analyses were also performed excluding Sub-Antarctic species.

From a zoogeographic point of view, three major ecological zones were considered in the notothenioid fish distribution, i.e. High, Low and Sub-Antarctic (Fig. 1). They are based on oceanographic frontal zones and ice cover extension: the High Antarctic zone is the area close to the continent which is covered by ice most of the year; the Low Antarctic zone extends between the northern limit of the pack ice in summer-autumn and the Antarctic Circumpolar Current (ACC); the Sub-Antarctic zone is located north of the ACC up to about 35° S of latitude (modified from Kock 1992).

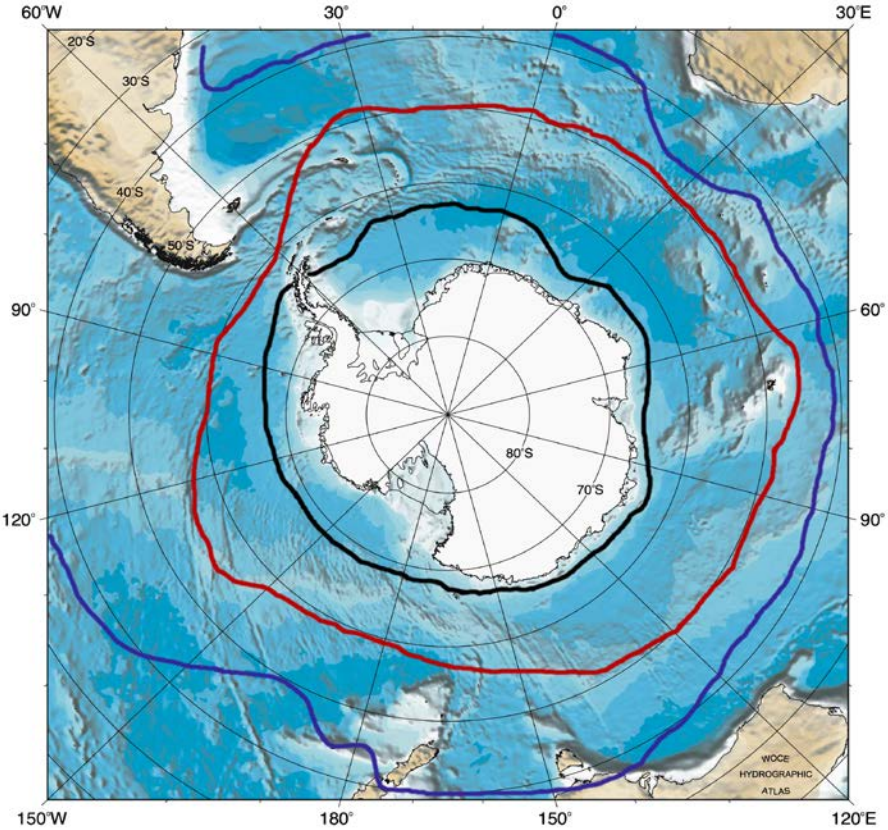

Figure 1. Ecological zonation considered for the species distribution. High Antarctic zone is between the Continent and the black line; Low Antarctic zone is the area between black and red lines and Sub-Antarctic zone is the area up to the blue line (modified from Kock 1992).

Results

The frequency distribution of maximum fish size highly overlapped among species occurring in different zones (High, Low and Sub-Antarctic) (Fig. 2a). High Antarctic species in general exhibit large eggs, while Sub-Antarctic smaller ones (Fig. 2b). Low values of relative fecundity are typical of High Antarctic species (3.3-123.9 eggs/g), while Low and Sub-Antarctic species show higher values and a wider range in the relative fecundity (Fig. 2c).

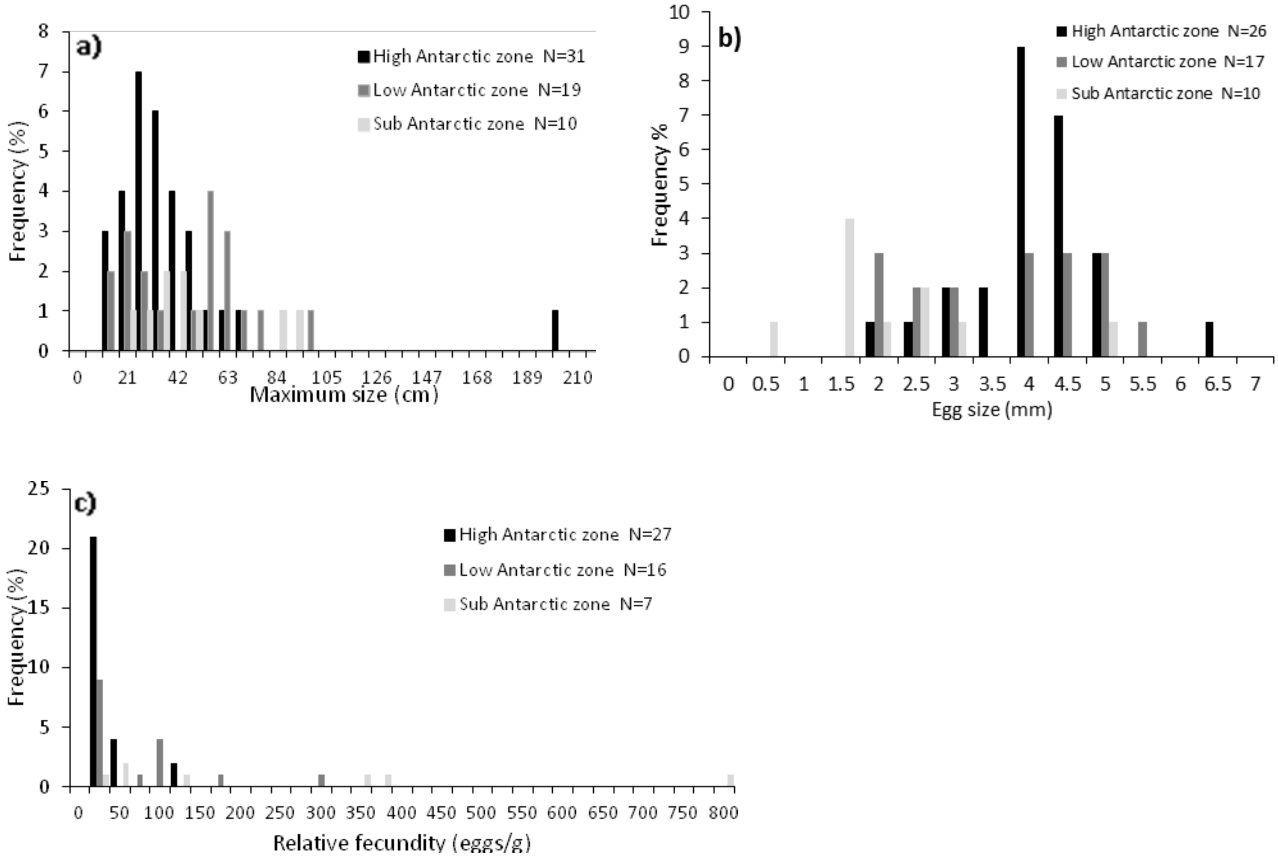

Figure 2. Frequency distribution in High, Low and Sub-Antarctic species of: **a)** maximum size; **b)** egg size and **c)** relative fecundity.

Using raw data, maximum size results positively related to egg size (n=53, r_s=0.43, p=0.001) and negatively to relative fecundity (n=50, r_s=-0.35, p=0.012). Significant correlations between raw data of each life history trait and different environmental variables are reported (Table 1).

Table 1. Spearman rank correlation results, between raw data of life history traits and environmental variables. P values and p values after the application of the Benjamini–Hochberg FDR are reported. Water temperature, primary production and sea ice cover are mean value; Δt = maximum temperature - minimum temperature; GSI = gonadosomatic index.

	N	r_s	p	p FDR
Maximum size vs Water temperature	57	0.27	0.041	0.060
Maximum size vs Δt	57	0.29	0.025	0.040*
Maximum size vs Primary Production	57	0.10	0.449	0.449
Maximum size vs Sea ice cover	57	-0.38	0.003	0.016*
Egg size vs Water temperature	53	-0.37	0.007	0.022*
Egg size vs Δt	53	-0.27	0.055	0.068
Egg size vs Primary Production	53	-0.41	0.002	0.016*
Egg size vs Sea ice cover	53	0.32	0.021	0.037*
Relative fecundity vs Water temperature	50	0.35	0.014	0.030*
Relative fecundity vs Δt	50	0.34	0.015	0.030*
Relative fecundity vs Primary Production	50	0.38	0.007	0.022*
Relative fecundity vs Sea ice cover	50	-0.28	0.049	0.065
GSI vs Water temperature	33	-0.43	0.012	0.030*
GSI vs Δt	33	-0.26	0.148	0.169
GSI vs Primary Production	33	-0.24	0.184	0.196
GSI vs Sea ice cover	33	0.50	0.003	0.016*

Independent contrasts

Controlling for phylogeny through independent contrasts, maximum size was not related to any environmental factor (Table 2).

Table 2. Relationship between the standardized independent contrasts of life history traits and environmental variables. Maximum size, egg size and gonadosomatic index (GSI) are maximum values; relative fecundity is mean value. Water temperature, primary production and sea ice cover are mean value; Δt = maximum temperature - minimum temperature.

	Maximum size (df =55)				**Egg size (df =51)**				**Relative fecundity (df =47)**				**GSI (df =30)**			
	b	**r^2**	**F**	**p**	**b**	**r^2**	**F**	**p**	**b**	**r^2**	**F**	**p**	**b**	**r^2**	**F**	**p**
Water temperature	1.37	0.03	1.39	0.243	-0.06	0.05	2.41	0.126	22.04	0.31	21.21	<0.001*	-1.47	0.12	3.99	0.055
Δt	2.52	0.05	3.1	0.084	-0.01	9.14	0.05	0.830	9.05	0.04	1.71	0.197	-0.72	0.02	0.72	0.401
Primary production	-0.01	0	0.15	0.697	-0.001	0.11	6.58	0.013*	0.22	0.18	10.21	0.002*	-0.01	0.04	1.24	0.275
Sea ice cover	-15.07	0.03	1.89	0.174	0.50	0.03	1.45	0.235	-37.09	0.01	0.32	0.571	35.82	0.27	11.07	0.002*
Maximum size					0.02	0.22	14.30	<0.001*	0.10	5.39	0.03	0.874	-0.04	0.01	0.30	0.587

Egg size was related to maximum size and primary production. When considered as independent variables in the multiple regression analysis, both factors were significant ($r^2=0.32$, $F_{2,50}=11.85$, $p<0.001$) and egg size was positively related to maximum size ($b=0.02$, $t_{50}=3.87$, $p<0.01$; Fig. 3a), negatively to primary production ($b=-0.001$, $t_{50}=2.87$, $p<0.01$; Fig. 3b). Relative fecundity was related to primary production and water temperature. When considered together (r^2=0.31, $F_{2,46}$=10.45, p<0.001), only water temperature was significant (b=23.93, t_{46}=2.99, p<0.01; Fig. 3c), while primary production was not (b=0.03, t_{46}=0.30, p=0.767). GSI was positively related to sea ice cover and negatively to water temperature. In the multiple regression analysis (r^2=0.27, $F_{2,29}$=5.45, p<0.01), only sea ice cover was significant (b=40.26, t_{29}=2.49, p=0.019; Fig. 3d), while the negative relationship with water temperature was not significant (b=0.37, t_{29}=0.37, p=0.711).

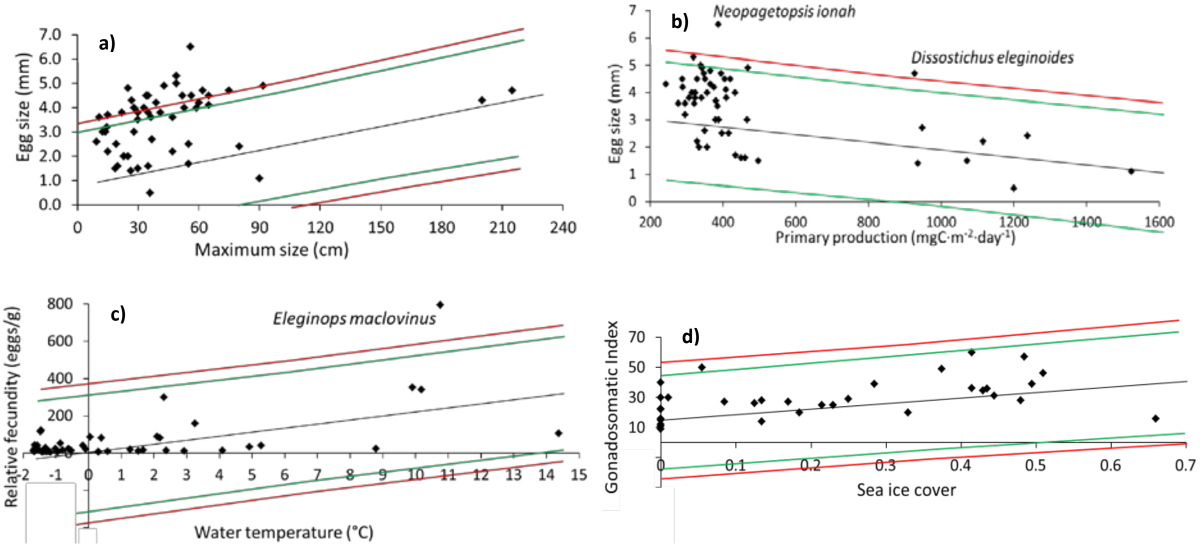

Figure 3. Linear regression of: **a)** egg size vs maximum size; **b)** egg size vs primary production; **c)** relative fecundity vs water temperature; **d)** Gonadosomatic Index vs sea ice cover. Total least square regression (black line) and associated 90% (green lines) and 95% (red lines) confidence intervals have been corrected for phylogenetic relationships and mapped on the original data space. The names of the species presenting values of the life history traits outside the 95% confidence interval are reported.

Excluding Sub-Antarctic species and using the independent contrasts, the absence of relationship between maximum size and the environmental variables was confirmed (all p>0.05). Egg size was, again, positively related to maximum size and negatively to primary production, in the multiple regression analyses (r^2=0.23, $F_{2,40}$=5.93, p=0.006) both independent variables were significant (b=0.01, t_{40}=2.29, p=0.028; b=0.003, t_{40}=2.34, p=0.025, respectively). Relative fecundity resulted positively related only to maximum size (b=-0.64, t_{40}=2.38, p=0.022), whereas gonadosomatic index (GSI) was related to sea ice cover (b=35.36, t_{23}=3.06, p=0.005).

Discussion

The results of the interspecific comparative analyses highlighted some correlation between environmental factors and life history traits, revealing potential evolutionary forces. Differently from the other biological traits considered in this study, the maximum size did not correlate with any environmental variable. Among general patterns, Bergmann's rule describes the occurrence of a positive relationship between maximum size and latitude, with smaller specimens found in warmer (lower latitudes) environments (Blackburn *et al*. 1999; Fisher *et al*. 2010). As already reported in taxa such as mammals and in general marine fish (Huston and Wolverton 2011), this study confirms that this pattern does not persist at high latitudes.

The relationships between life history traits and environmental variables highlighted differences in the results, using raw data or controlled for phylogeny ones, emphasizing the importance of taking into account the phylogenetic relationships to investigate on evolutionary processes. In particular, while several relationships lost significance, but maintained the general trend when phylogenetic relationships were taken into account, the analyses using raw data highlighted an unexpected positive relationships of maximum size with the range of temperature (Δt). This result could be explained by the occurrence of several small-sized species, phylogenetically related, in Antarctic waters, where Δt is lower. The discrepancies in the results between raw data and independent contrasts highlighted the fact that when raw data are considered, the observed results could be related to phylogenetic history of the species rather than to an actual evolutionary process.

In notothenioids egg size resulted negatively related to primary production. Primary production, in turn, is negatively related to latitude, and general theoretical models and relevant studies indicate a positive relationship between egg/offspring size and latitude (Mileykovsky 1971; Alekseev 1981; Levin and Bridges 2001; Laptikhovsky 2006). Both food availability and temperature have been claimed as factors driving these general trends of egg/offspring size with latitude (Laptikhovsky, 2006). Primary production can be considered a proxy of food availability, therefore in notothenioids food availability appears to be the main factor influencing egg size. In fish larval size is generally positively related to egg size, therefore the production of larger eggs imply the production of larger larvae (Duarte and Alcaraz 1989). Larger larvae have enhanced competitive abilities, improved capacity to feed on a wide size range of prey items and better starvation resistance, and therefore higher survival probabilities (Conover and Schultz 1997). These larval characteristics are expected to be particularly important in areas characterized by scarce food availability. Looking at the standardized independent contrasts, two species, *Neopagetopsis ionah* and *Dissostichus eleginoides*, did not follow the predictions, presenting eggs larger than expected. The duration of egg development is positively related to egg size (Duarte and Alcaraz 1989) and the glassfish *N. ionah* exhibits prolonged nest guarding, lasting up to death in some cases (Riginella personal observation). It is still unknown if *N. ionah* performs one or multiple breeding seasons, however large eggs and high mortality rates at nesting site might be related to an extremely high investment in reproduction and may suggest the occurrence of limited reproductive events. However, the factors driving the evolution of the large-sized eggs of the glassfish remain to be investigated. In *D. eleginoides*, the egg size larger than expected might be related to the large body size of this pelagic spawner, exceeding 2 meters in total length. In this study egg size was also related to the maximum parental body size, indicating a trend with larger fish laying larger eggs. In general, the variability in the allocation of reproductive effort to egg size and fecundity seems not to be influenced by body size (Ware 1975; Duarte and Alkaraz 1989) and in fact, large fishes are not more likely to have large eggs than small fish (Miller 1984; Hislop 1984; Duarte and Alcaraz 1989). Therefore, fish size appears to constrain, rather than to determine, the range of possible egg sizes. The wider spectrum of egg sizes possible for large fish implies that a greater range of tactics in the partition of reproductive effort between fecundity and egg size is available to them (Duarte and Alcaraz 1989). In Notothenioids, the selective pressures towards large eggs might explain the positive relationship between egg size and maximum size, with larger fish producing eggs as large as possible. Several species, small-size ones in particular, falling out the 95% of the confidence interval, present values of egg size higher than expected. These species are in particular High Antarctic ones, therefore reinforcing the role of environment in driving egg size.

The eggs size frequency distribution in High, Low and Sub-Antarctic fish confirm the trend described by Kock and Kellermann (1991). While Low Antarctic species produce small and large eggs, High Antarctic species present only large eggs, with the exception of the sea ice-dependent *Pleuragramma antarctica*. A theoretical optimization model suggests that pelagic spawners increase their fitness by maximizing eggs quantity (that implies small eggs), whereas demersal spawners produce larger eggs (that implies fewer eggs), maximizing the larval survival (Duarte and Alcaraz 1989). This general statement seems not to apply to notothenioid fish, where most pelagic spawners lay large eggs (reaching 5 mm in diameter, as in *Notothenia rossii* or *N. coriiceps*), with the exception of the Antarctic silverfish *P. antarctica*, that lays relative small eggs (about 2 mm) under the platelet seasonal pack sea ice.

Some studies reported a positive relationship between egg size and parental care (Balon 1984; Kolm and Ahnesjö 2005). Parental care is a quite common strategy among notothenioids and, in the last decade, many species were observed performing nest guarding. Unfortunately, data on the occurrence of parental care are still scanty and it was therefore not possible to include this factor in the analyses.

Considering the trade-off between egg size and number, a positive relationship between relative fecundity (i.e. the number of eggs in relation to body weight) and primary production was expected. This relationship was not found, while a positive relationship of relative fecundity and mean water temperature emerged. The influence of water temperature on relative fecundity therefore remains an issue to be further investigated. The relative fecundity of *Eleginops maclovinus* exhibits an unpredicted high value, exceeding the 95% confidence interval. This species presents several distinctive characteristics respect to other notothenioids. It has an exclusively non-polar distribution, inhabits estuarine waters and exhibits a proterandrous hermaphroditism sexual pattern (Brickle *et al*. 2005, Licandeo *et al*. 2006). In such a complex and dynamic scenario other factors, different than those considered in this study, may influence the high values of relative fecundity exhibited by this species.

The maximum female GSI, representing one component of the reproductive effort, showed a positive relationship with sea ice cover. GSI estimates reported in this study are in agreement with the general patterns observed in Antarctic notothenioids, which attain higher values than in temperate species and an increasing trend towards higher latitudes (Kock and Kellermann 1991), corresponding to areas with higher sea ice cover. In our study, the maximum female GSI, representing one component of the reproductive effort, showed a positive relationship with sea ice cover. Pack sea ice plays an important role in the Southern Ocean, being a key factor for the primary production. Indeed the seasonal pack ice melting, which occurs in summer at intermediate Antarctic latitudes, triggers phytoplankton blooms, resulting in seasonally increased primary production with a cascading effect on the pelagic food web. At higher latitudes, where maximum values of GSI have been detected, the sea ice cover is permanent through the year and the primary production likely remains low, being also influenced by the long and dark Antarctic winter. These extreme conditions may therefore trigger a higher investment in reproduction, including the investment in eggs (size and/or number) represented by the female GSI. This hypothesis needs seasonal values of environmental variables to be tested, as only mean annual estimates were used in the present study.

The analyses on a narrower latitudinal range, considering only Antarctic notothenioids (i.e. excluding Sub-Antarctic species), gave the same results emerged from the total data set, with the exception of relative fecundity. The consistency in the results strengthen the observed patterns, confirming the strong evolutionary pressure of environmental factors on life history traits. Relative fecundity in Low and High-Antarctic notothenioids was not related to water temperature any more, probably due to the more restricted temperature range. However relative fecundity was positively related to maximum size, underlining that the influence of fish-size in eggs production may be stronger in extreme environments and/or that positive allometric relationships between egg number and fish size occur.

In general, the observed trends in the different life history traits could be determined by other variables, different from those considered in this study but possibly related to them (Martins 2000). In the case of a correlation between ecological and environmental factors, resulting trends could be a hint of a stronger relationship involving unmeasured ecological variables. Factors such as competition for resources or predation, that have been hypothesized to influence the evolution of life history traits (Roff 1992), are often correlated with environmental variables, and their role in notothenioids remains to be investigated.

This paper produces first data on the evolution of some life history traits in notothenioids and, in a broader sense, new insights on the major factors that could drive the evolutionary processes occurring in the Antarctic environment. Notothenioids have proved to be an excellent model group for evolutionary studies however the observed patterns in the life history traits deserve further investigations in polar regions, including different marine taxa, as invertebrates, to highlight the generality of these results. In a more comprehensive framework, comprising several taxa and wider latitudinal range (implying a larger variation in environmental factors), more general statements on life history traits evolution could finally be traced.

The knowledge of life history traits of notothenioids is also crucial for conservation biology purposes, highlighting the high vulnerability of this taxon to potential impacts such as climate change and/or fishery. In fact the low fecundity and large egg size, the high reproductive effort in gonadal investment and in parental care, the low growth rate and the late sexual maturity in general, make this group of teleosts particularly sensitive to fishing. So notothenioids should be considered a very vulnerable taxon, especially at higher latitude where species vulnerability seems to increase, and caution and conservation policies are recommended when operating in a climate change and potential fishery re-opening scenario.

References

Alekseev F.E. (1981). Rass-Thorson-Marshall rule and biological structure of marine communities. In: G.G. Vinberg. 4th Congress of All-Union Hydrobiological Society. Theses of reports, Part I. Naukova Dumka, Kiev 4-6.

Alekseev E.I., Alekseev F.E., Konstantinov V.V., Boronin V.A. (1993). Reproductive Biology of Grenadiers, *Macrourus carainatus*, *M. whitsoni*, *Coelorinchus fasciatus* (Macrouridae), and *Patagonotothen guentheri shagensis* (Nototheniidae) and the Distribution of *M. carinatus*. Journal of Ichthyology 33(1): 71-84.

Andriashev P. (1965). A general review of the Antarctic fish fauna. In: Van Oye P., Van Menghem J. (eds) Biogeography and Ecology in Antarctica. Monographiae Biologicae 15: 491-550.

Arkhipkin A., Jurgens E., Howes P.N. (2013). Spawning, egg development and early ontogenesis in rock cod *Patagonotothen ramsayi* (Regan, 1913) caught on the Patagonian Shelf and maintained in captivity. Polar Biology 36: 1195-1204.

Arkhipkin A., Boucher E., Howes P.N. (2014). Spawning and early ontogenesis in channel bull blenny *Cottoperca gobio* (Notothenioidei, Perciformes) caught off the Falkland Islands and maintained in captivity. Polar Biology 38(2): 251-259.

Balon E.K. (1984). Reflections on some decisive events in the early life of fishes. Transactions of the American Fisheries Society 113: 178-185.

Barrera-Oro E.R., Lagger C. (2010). Egg-guarding behaviour in the Antarctic bathydraconid dragonfish *Parachaenichthys charcoti*. Polar biology 33(11): 1585-1587.

Bell G. (1980). The costs of reproduction and their consequences. The American Naturalist 116(1): 45-76.

Blackburn T.M., Gaston K.J., Loder N. (1999). Geographic gradients in body size: a clarification of Bergmann's rule. Diversity and Distributions 5: 165-174.

Bray D.J., Thompson V.J. (2011). Congolli, *Pseudaphritis urvillii*, in Fishes of Australia, accessed 01 Jul 2015. http://www.fishesofaustralia.net.au/home/species/403.

Brickle P., Laptikhovsky V., Arkhipkin A. (2005). Reproductive strategy of a primitive temperate notothenioid *Eleginops maclovinus*. Journal of Fish Biology 66(4): 1044-1059.

Brickle P., Laptikhovsky V., Arkhipkin A., Portela J. (2006). Reproductive biology of *Patagonotothen ramsayi* (Regan, 1913) (Pisces: Nototheniidae) around the Falkland Islands. Polar Biology 29: 570-580.

Burchett M.S., Sawyers P.J., North A.W., White M.G. (1983). Some biological aspects of the nearshore fish population at South Georgia. British Antarctic Survey Bullettin 59: 63-74.

Calì F., Riginella E., La Mesa M., Mazzoldi C. Life history strategies of *Notothenia rossii* and *N. coriiceps* along the Southern Scotia Arc. Paper.

Calvo J., Morriconi E., Rae G.A. (1999). Reproductive biology of the icefish *Champsocephalus esox* (Günther, 1861) (Channichthyidae). Antarctic Science 11: 140-149.

Chavan V. (2012). GBIF Version 1.0, Copenhagen: Global Biodiversity Information Facility. GBIF Secretariat. Accessible at http://www.gbif.org.

Cheshire K.J.M., Ye Q., Fredberg J., Short D., Earl J. (2013). Aspects of reproductive biology of five key fish species in the Murray Mouth and Coorong. South Australian Research and Development

Institute (Aquatic Sciences), Adelaide. SARDI Publication F2009/000014-3. SARDI Research Report Series 699, 63pp.

Colombo M., Damerau M., Hanel R., Salzburger W., Matschiner M. (2015). Diversity and disparity through time in the adaptive radiation of Antarctic notothenioid fishes. Journal of Evolutionary Biology 28: 376-394.

Conover D.O., Schultz E.T. (1997). Natural selection and the evolution of growth rate in the early life history: what are the trade-offs? In: Chambers R.C., Trippel E.A. (eds) Early Life History and Recruitment in Fish Populations. Chapman and Hall, London, 305-332.

Dando P.R. (1984). Reproduction in estuarine fish. In: Potts G.W., Wooton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, Oxford, 155-170.

Daniels A. (1978). Nesting behaviour of *Harpagifer bispinis* in Arthur Harbour, Antarctic Peninsula. Journal of Fish Biology 12: 465-474.

Detrich H.W., Jones C.D., Kim S., North A.W., Thurber A., Vacchi M. (2005). Nesting behaviour of the icefish *Chaenocephalus aceratus* at Bouvetøya, Southern Ocean (CCAMLR subarea 48.6). Polar Biology 28: 828-832.

Dettai A., Berkani M., Lautredou A.C., Couloux A., Lecointre G., Ozouf-Costaz C., Gallut C. (2012). Tracking the elusive monophyly of nototheniid fishes (Teleostei) with multiple mitochondrial and nuclear markers. Marine Genomics 8: 49-58.

Duarte C.M., Alcaraz M. (1989). To produce many small or few large eggs: a size-independent reproductive tactic of fish. Oecologia 80: 401-404.

Duhamel G., Kock K.H., Balguerias E., Hureau J.C. (1993). Reproduction in fish of the Weddell Sea. Polar Biology 13: 193-200.

Eastman J.T. (2005). The nature of the diversity of Antarctic fishes. Polar Biology 28: 93-107.

Eastman J.T., Amsler M.O., Aronson R.B., Thatje S., McClintock J.B., Vos S.C., Kaeli J.W., Singh H., La Mesa M. (2013). Photographic survey of benthos provides insights into the Antarctic fish fauna from the Marguerite Bay slope and the Amundsen Sea. Antarctic Science 25(01): 31-43.

Eastman J., Eakin R.R. (2015). Notothenioid classification and list of species. http://www.oucom.ohiou.edu/dbms-eastman.

Ekau W. (1982). Biological investigations on *Notothenia ramsayi* Regan 1913 (Pices Notothenoidei, Nototheniidae). Archiv für Fischereiwissenschaft 33: 43-68.

Ekau W. (1989). Egg development of *Trematomus eulepidotus* Regan 1914 (Nototheniidae, Pisces) from the Weddell Sea. Cybium 13: 213–219.

Ekau W. (1991). Reproduction in high Antarctic fish. Meeresforschung 33: 159-167.

Evans C.W., Cziko P., Cheng C.H.C., DeVries A.L. (2005). Spawning behaviour and early development in the naked dragonfish *Gymnodraco acuticeps*. Antarctic Science 17: 319–327.

Everson I. (1994). Timescale of ovarian maturation in *Notothenia coriiceps*: Evidence for a prolonged adolescent phase. Journal of Fish Biology 44: 997-1004.

Everson I., Kock K.H., Parkes G. (1996). Ovarian development associated with first maturity in three Antarctic channichthyid species. Journal of Fish Biology 49: 1019-1026.

Felsenstein J. (1985). Phylogenies and the comparative method. The American Naturalist 125: 1-15.

Fernández D.A., Calvo J., Franklin C.E., Johnston I.A. (2000). Muscle fiber types and size distribution in sub-Antarctic notothenioid fishes. Journal of Fish Biology 56: 1295-1311.

Ferrando S., Castellano L., Gallus L., Ghigliotti L., Masini M.A., Pisano E., Vacchi M. (2014). A demonstration of nesting in two Antarctic icefish (genus *Chionodraco*) using a fin dimorphism analysis and ex situ videos. PLoS ONE 9(3): e90512. doi:10.1371/journal.pone.0090512.

Fischer W., Hureau J.C. (1985). FAO species identification sheets for fishery purposes. Southern Ocean (Fishing areas 48, 58 and 88) (CCAMLR Convention Area). Rome, FAO. Vol. 2.

Fisher J.A.D., Frank K.T., Leggett W.C. (2010). Global variation in marine fish body size and its role in biodiversity ecosystem functioning. Marine Ecology Progress Series 405: 1-13.

Froese R., Pauly D. (2014). FishBase. World Wide Web electronic publication. www.fishbase.org. (11/2014).

Garland J.R., Harvey P.H., Ives A.R. (1992). Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic Biology 41: 18-32.

Gon O., Heemstra P.C. (1990). Fishes of the Southern Ocean. J. L. B. Smith Institute of Ichthyology, Grahamstown, 462 pp.

Hanchet S.M., Rickard G.J., Fenaughty J.M., Dunn A., Williams M.J. (2008). A hypothetical life cycle for Antarctic toothfish *Dissostichus mawsoni* in Antarctic waters of CCAMLR Statistical Area 88. CCAMLR Science 15: 35–54.

Harvey P.H., Pagel M.D. (1991). The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford, 239 pp.

Harvey P.H., Purvis A. (1991). Comparative methods for explaining adaptations. Nature 351: 619-624.

Hislop J.R.G. (1984). A comparison of the reproductive tactics and strategies of cod, haddock, whiting and Norway pout in the North sea. In: Potts G.W., Wootton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, London, 311-329.

Hourigan T.F., Radtke R.L. (1989). Reproduction of the Antarctic fish *Nototheniops nudifrons*. Marine Biology 100: 277-283.

Hunter J.R., Lo N.C.H., Leong R.J.H. (1985). Batch fecundity in multiple spawning fishes. NOAA Technical Report, NMFS 36: 67-77.

Huston M.A., Wolverton S. (2011) Regulation of animal size by eNPP, Bergmann's rule, and related phenomena. Ecological Monographs 81(3): 349-405.

Kaschner K., Watson R., Trites A.W., Pauly, D. (2006). Mapping world-wide distributions of marine mammal species using a relative environmental suitability (RES) model. Marine Ecology Progress Series 316: 285-310.

Kaschner K., Ready J.S., Agbayani E., Rius J., Kesner-Reyes K., Eastwood P.D., South A.B., Kullander S.O., Rees T., Close C.H. (2007). AquaMaps: predicted range maps for aquatic species. Available online: www.aquamaps.org.

Kaschner K., Rius-Barile J., Kesner-Reyes K., Garilao C., Kullander S.O., Rees T., Froese R. (2013). AquaMaps: predicted range maps for aquatic species. World Wide Web electronic publication. www.aquamaps.org, version 08/2013.

Knust R., Gerdes D., Mintenbeck K. (2012). The expedition of the research vessel ‘’Polarstern’’ to the Antarctic in 2011 (ANTXXVII/ 3) (CAMBIO). Berichte zur Polar- und Meeresforschung - Reports on Polar and Marine Research, Bremerhaven, 644: 1-202.

Knust R., Schröder M. (2014). The Expedition PS82 of the Research Vessel POLARSTERN to the southern Weddell Sea in 2013/2014. Berichte zur Polar- und Meeresforschung - Reports on Polar and Marine Research, Bremerhaven, 680: 1-155.

Kock K.H. (1992). Antarctic Fish and Fisheries. Cambridge University Press, Cambridge, 359 pp.

Kock K.H. (2005). Antarctic icefishes (Channichthyidae): a unique family of fishes. A review, Part I. Polar Biology 28(11): 862-895.

Kock K.H., Kellermann A. (1991). Reproduction in Antarctic notothenioid fish. Antarctic Science 3: 125-150.

Kock K.H., Everson I. (1997). Biology and ecology of mackerel icefish, *Champsocephalus gunnari*: an Antarctic fish lacking haemoglobin. Comparative Biochemistry and Physiology 118(4): 1067-1077.

Kock K.H., Jones C.D., Wilhelms S. (2000). Biological characteristics of Antarctic fish stocks in the southern Scotia Arc region. CCAMLR Science 7: 1-41.

Kock K.H., Jones C.D. (2002). The biology of the icefish *Cryodraco antarcticus* Dollo, 1900 (Pisces, Channichthyidae) in the southern Scotia Arc (Antarctica). Polar Biology 25(6): 416-424.

Kock K.H., Pshenichnov L., Jones C.D., Skora K.E., Frolkina Z.A. (2004). Joinville–D'Urville Islands (Subarea 48.1) a former fishing ground for the spiny icefish (*Chaenodraco wilsoni*), at the tip of the Antarctic Peninsula revisited. CCAMLR Science 11: 1-20.

Kock K.H., Pshenichnov L.K., Devries A.L. (2006). Evidence for egg brooding and parental care in icefish and other notothenioids in the Southern Ocean. Antarct Science 18: 223–227.

Kock K.H., Reid K., Croxall J., Nicol S. (2007). Fisheries in the Southern Ocean: an ecosystem approach. Philosophical Transactions of the Royal Society 362: 2333-2349.

Kock K.H., Pshenichnov L., Jones C.D., Gröger J., Riehl R. (2008). The biology of the spiny icefish (Chaenodraco wilsoni Regan, 1914). Polar Biology 31: 381–393.

Kolm N., Ahnesjö N. (2005). Do egg size and parental care coevolve in fishes? Journal of Fish Biology 66: 1499-1515.

Kompowsky A. (1992). Studies on *Psilodraco breviceps* Norman, 1937 (Pisces, Notothenioidei, Bathydraconidae) from the region of South Georgia. Acta Ichthyologica et Piscatoria 22(1): 3-14.

Konecki T., Tarcett T.E. (1989). Eggs and larvae of *Nototheniops larseni* from the spongocoel of a hexactinellid sponge near Hugo Island, Antarctic Peninsula. Polar Biology 10: 197-198.

Kuhn K.L., Near T.J., Jones C.D., Eastman J.T. (2009). Aspects of the biology and population genetics of the Antarctic nototheniid fish *Trematomus nicolai*. Copeia 2: 320–327.

La Mesa M., Caputo V., Rampa R., Vacchi M., (2003). Macroscopic and histological analyses of gonads during the spawning season of *Chionodraco hamatus* (Pisces, Channichthyidae) off Terra Nova Bay, Ross Sea, Southern Ocean. Polar Biology 26: 621–628.

La Mesa M., Caputo V., Eastman J.T. (2006a). Gametogenesis and reproductive strategies in some species of the Antarctic fish genus *Trematomus* (Nototheniidae) from Terra Nova Bay, Ross Sea. Polar Biology 29: 963-970.

La Mesa M., Caputo V., Rampa R., Eastman J.T. (2006b). Gametogenesis in the Antarctic plunderfishes *Artedidraco lönnbergi* and *Artedidraco skottsbergi* (Pisces: Artedidraconidae) from the Ross Sea. Antarctic Science 18: 183-190.

La Mesa M., Caputo V., Eastman J.T. (2007). Gametogenesis in the dragonfishes *Akarotaxis nudiceps* and *Bathydraco marri* (Pisces, Notothenioidei: Bathydraconidae) from the Ross Sea. Antarctic Science 19: 64–70.

La Mesa M., Caputo V., Eastman J.T. (2008). The reproductive biology of two epibenthic species of Antarctic nototheniid fish of the genus *Trematomus*. Antarctic Science 20(4): 355-364.

La Mesa M., Caputo V., Eastman J.T. (2010). Some reproductive traits of the Tristan klipfish, *Bovichtus diacanthus* (Carmichael 1819) (Notothenioidei: Bovichtidae) from Tristan da Cunha (South Atlantic). Polar Biology 33: 337-346.

La Mesa M., Eastman J.T., (2011). Antarctic silverfish: life strategies of a key species in the high-Antarctic ecosystem. Fish and Fisheries. DOI: 10.1111/j.1467-2979.2011.00427.

La Mesa M., Riginella E., Mazzoldi C., Ashford J. (2014). Reproductive resilience of ice-dependent Antarctic silverfish in a rapidly changing system along the western Antarctic Peninsula. Marine Ecology 36: 235-245.

La Mesa M., Riginella E., Melli V., Bartolini F., Mazzoldi C. (2015). Biological traits of a sub-Antarctic nototheniid, *Patagonotothen ramsayi*, from the Burdwood Bank. Polar Biology. doi 10.1007/s00300-015-1663-6.

Laptikhovsky V. (2006). Latitudinal and bathymetric trends in egg size variation: a new look at Thorson's and Rass's rules. Marine Ecology 27: 7-14.

Levin L.A., Bridges T.S. (2001). Pattern and diversity in the reproduction and development. In: McEdward L. (eds) Ecology of Marine Invertebrate Larvae. CRC Press, London, 2-48.

Licandeo R.R., Barrientos C.A., González M.T. (2006). Age, growth rates, sex change and feeding habits of notothenioid fish *Eleginops maclovinus* from the central-southern Chilean coast. Environmental Biology of Fishes 77: 51-61.

Lisovenko L.A. (1987). Reproductive biology of Antarctic fish in relations to conditions of their habitat. In Skarlato O.A., Alekseev A.P., Liubimova T.G. (eds) Biological resources of the Arctic and Antarctic. Moscow: Nauka, 337–357 (in Russian).

Lisovenko L.A., Trunov I.A. (1988). New information on the reproduction of ionah glassfish, *Neopagetopsis ionah*, of the Lazarev Sea. English Translation in Journal of Ichthyology 29(3): 27–33.

Lucassen M. (2012). The expedition of the research vessel "Polarstern" to the Antarctic in 2012 (ANT-XXVIII/4). Berichte zur Polar- und Meeresforschung = Reports on polar and marine research. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, 652: 1-90.

Maddison W.P., Maddison D.R. (2011). Mesquite: a modular system for evolutionary analysis. Version 2.75, http://mesquiteproject.org.

Martins E.P. (2000). Adaptation and the comparative method. Trends in Ecology & Evolution 15(7): 296-299.

Meneghesso C., Riginella E., La Mesa M., Donato F., Mazzoldi C. Unveiling the biology of two Antarctic Artedidraconidae species: reproduction and age-length relationship of *Artedidraco skottsbergi* and *Dolloidraco longedorsalis* from the Weddell Sea. Paper.

Mileykovsky S.A. (1971). Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Marine Biology 10: 193-213.

Militelli M.I., Macchi G. J., Rodrigues K.A. (2015). Maturity and fecundity of *Champsocephalus gunnari*, *Chaenocephalus aceratus* and *Pseudochaenichthys georgianus* in South Georgia and Shag Rocks Islands. Polar Science 9: 258-266.

Miller P.J. (1984). The tokology of gobioid fishes. In: Potts G.W., Wooton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, Oxford, 119-154.

Morales-Nin B., Moranta J., Balguerias E. (2000). Growth and age validation in high-Antarctic fish. Polar Biology 23: 626–634.

Moreno C.A. (1980). Observations on food and reproduction in *Trematomus bernacchii* (Pisces: Nototheniidae) from the Palmer Archipelago. Copeia 1: 171-173.

Murua H., Kraus G., Saborido-Rey F., Witthames P.R., Thorsen A., Junquera S. (2003). Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal of Northwest Atlantic Fisheries Science 33: 33-54.

Near T.J., Cheng C.-H.C. (2008). Phylogenetics of notothenioid fishes (Teleostei: Acanthomorpha): Inferences from mitochondrial and nuclear gene sequenze. Molecular Phylogenetics and Evolution 47: 832-840.

Near T.J., Dornburg A., Kuhn K.L., Eastman J.T., Pennington J.N., Patarnello T., Zane L., Fernández D.A., Jones C.D. (2012). Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. Proceedings of the National Academy of Science 109: 3434-3439.

North A.W. (1998). Growth of young fish during winter and summer at South Georgia, Antarctica. Polar Biology 19(3): 198-205.

North A.W. (2002). Larval and juvenile distribution and growth of Patagonian toothfish around South Georgia. Antarctic Science 14(1): 25-31.

North A.W., White M.G. (1987). Reproductive strategies of Antarctic fish. In Kullander S.O., Fernholm B. (eds) Proceedings of the V Congress of the European Ichthyological Society, Stockholm 1985, 381-390.

North A.W., Ward P. (1989). Initial feeding of fish larvae during winter at South Georgia. Cybium 13: 357-364.

OBIS (2015). Data from the Ocean Biogeographic Information System. Intergovernmental Oceanographic Commission of UNESCO. http://www.iobis.org (consulted on 2015 May).

Permitin Y.Y. (1973). Fecundity and reproductive biology of icefish (Channichthyidae), fish of the family Muraenolepidae and dragonfish (Bathydraconidae) of the Scotia Sea (Antarctica). Journal of Ichthyology 13(2): 204-215.

Permitin Y.Y., Silyanova Z.S. (1971). New data on the reproductive biology and fecundity of fishes of the genus *Notothenia* (Richardson) in the Scotia Sea (Antarctica). Journal of Ichthyology 11: 693-705.

Potts G.W. (1984). Parental behaviour in temperate marine teleost with special reference to the development of nest structures. In: Potts G.W., Wootton R.J. (eds) Fish reproduction: strategies and tactics. Academic Press, London, 223-244.

Pshenichnov L.K. (1998). Unsteadiness of generative and sex structure of the spiny icefish population (*Chaenodraco wilson*). In: Proceedings of all-union conference problems of fishery forecasts, Murmansk (in Russian).

Pshenichnov L.V. (2004). Some peculiarities of *Chionobathyscus dewitti* biology in the Ross Sea. WG-FSA-04/90, CCAMLR, Hobart, Australia, 6pp.

Rae G.A., Calvo J. (1995). Fecundity and reproductive habits in *Patagonotothen tessellata* (Richardson, 1845) from the Beagle Channel, Argentina. Antarctic Science 7: 235-240.

Ready J., Kaschner K., South A.B., Eastwood P.D., Rees T., Rius J., Agbayani E., Kullander S., Froese R. (2010). Predicting the distributions of marine organisms at the global scale. Ecological Modelling 221: 467-478.

Regan C.T. (1916). Larval and postlarval fishes. British Antarctic ("Terra Nova") Expedition 1910. Natural History Report Zoology 4: 125-156.

Riginella E., Mazzoldi C., Ashford J., Jones C.D., Morgan C., La Mesa M. Life history strategies of the Scotia Sea icefish, *Chaenocephalus aceratus*, along the Southern Scotia Ridge. Paper.

Rochet M.J., Cornillon P.A., Sabatier R., Pontier D. (2000). Comparative analysis of phylogenetic and fishing effects in life history patterns of teleost fishes. Oikos 91: 255-270.

Roff D.A. (1992). Evolution of life histories: theory and analysis. Chapman and Hall, New York, 527 pp.

Rutschmann S., Matschiner M., Damerau M., Muschick M., Lehmann M.F., Hanel R., Salzburger W. (2011). Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Molecular Ecology 20: 4707-4721.

Sapota M.R. (1999). Gonad development and embryogenesis of *Notothenia coriiceps* from South Shetlands - Antarctica. Polar Biology 22: 164-168.

Shandikov G.A., Faleeva T.I. (1992). Features of gametogenesis and sexual cycles of six notothenioid fishes from East Antarctica. Polar Biology 11: 615-621.

Stearns S.C. (1977). The evolution of life history traits: a critique of the theory and review of the data. Annual Review of Ecology and Systematics 8: 145-171.

Stearns S.C. (1992). The Evolution of Life Histories. Oxford University Press, Oxford, 249 pp.

Vacchi M., Williams R., La Mesa M. (1996). Reproduction in three species of fish from the Ross Sea and Mawson Sea. Antarctic Science 8: 185-192.

Vacchi M., Bottaro M., Pisano E., Eastman J.T., Eakin R.R. (2007). Aspects of gonadal morphology in the South Georgian plunderfish *Artedidraco mirus* (Perciformes: Artedidraconidae). Polar Biology 30: 125–131.

Van der Molen S., Matallanas J. (2003). Oocyte development and maturity classification of *Gerlachea australis* from the Weddell Sea, Antarctica. Polar Biology 26: 653–658.

Vanella F.A., Calvo J., Morriconi E.R., Aureliano D.R. (2005). Somatic energy content and histological analysis of the gonads in Antarctic fish from the Scotia Arc. Scientia Marina 69: 305-316.

Verhoeven K.J.F., Simonsen K.L., McIntyre L.M. (2005). Implementing false discovery rate control: increasing your power. Oikos 108: 643-647.

Waite E.R. (1916). Fishes. Australasian Antarctic Expedition 1911–1914. Scientific Reports, Series B, Zoology 3: 1-92.

Ware D.M. (1975). Relation between egg size, growth, and natural mortality of larval fish. Journal of Fish Research Board of Canada 32: 2503-2512.

White M.G., Burren P.J. (1992). Reproduction and larval growth of *Harpagifer antarcficus* Nybelin (Pisces, Notothenioidei). Antarctic Science 4: 421-430.

White M.G., Veit R.R., North A.W., Robinson K. (1996). Egg-shell morphology of the Antarctic fish, *Notothenia rossii* Richardson, and the distribution and abundance of pelagic eggs at South Georgia. Antarctic Science 8(3): 267-271.

Annex 1. Notothenioid species list and relative environmental data and life history traits values included in the analyses. Lat= mean latitude of species distribution; Temp= mean water temperature; Δt= maximum temperature - minimum temperature; Prim Prod= mean primary production; Rel fec= mean relative fecundity; GSI= maximum female gonadosomatic index; Max size= maximum size; "?" is presents when egg type and/or parental care are only suggested; *present study (in the case of relative fecundity, when literature data were available, the mean value was calculated and reported).

Species	Lat (°S)	Temp (°C)	Δt (°C)	Prim Prod $\left(\frac{\mathrm{mgC}}{\mathrm{m^2*day}}\right)$	Sea ice cover	Egg size (mm) (1)	Rel fec (2)	GSI (3)	Max size (cm) (4)	Egg type (5)	Parental care (6)	References
Artedidraconidae												
Artedidraco mirus	54	1.67	0.6	467	0.01	3.0	16.0		13	Demersal?		(1) Regan 1916; (2) Vacchi *et al.* 2007; (4) Gon and Heemstra 1990; (5) Duhamel *et al.* 1993; Ekau 1991; Kock and Kellermann 1991
Artedidraco shackletoni	72	-1.685	0.25	296	0.66	3.2	12.4	16.0	15	Demersal?		(1, 2, 3) Ekau 1991; (4) Gon and Heemstra 1990; (5) Duhamel *et al.* 1993; Ekau 1991; Kock and Kellermann 1991
Artedidraco skottsbergi	69	-1.295	0.99	296	0.48	3.6*	17*	28*	11	Demersal?		(2) La Mesa *et al.* 2006b; (1, 2, 3) Meneghesso *et al.* unpubl.; (4) Gon and Heemstra 1990; (5) Duhamel *et al.* 1993; Ekau 1991; Kock and Kellermann 1991
Dolloidraco longedorsalis	71	-0.53	2.6	388	0.645	3*	15*		14	Demersal?		(1, 2, 3) Meneghesso *et al.* unpubl.; (4) Gon and Heemstra 1990; (5) Duhamel *et al.* 1993; Ekau 1991; Kock and Kellermann 1991
Bathydraconidae												
Akarotaxis nudiceps	72.5	-0.785	2.25	447	0.665		16.2		14*	Demersal?		(2, 5) Ekau 1991; (4) Present study
Bathydraco marri	69.5	-0.175	1.79	286	0.455		40.3		25*	Demersal	yes?	(2) Duhamel *et al.* 1993; (4) Present study; (5, 6) Waite 1916; La Mesa *et al.* 2007
Cygnodraco mawsoni	72	-1.64	0.3	277	0.64	3.6	10.2		47*			(1, 2)Ekau 1991; (4) Present study
Gerlachea australis	73	-1.5	0.58	380	0.56	3.0	15.5*		28*	Demersal	yes	(1) Lisovenko 1987; (2) Duhamel *et al.* 1993; (2-4) Present study; (4) Gon and Heemstra 1990; (5, 6) Van der Molen and Matallanas 2003
Gymnodraco acuticeps	69.5	-1.35	0.88	321.5	0.475	3.6			37*	Demersal	yes	(1, 5, 6) Evans *et al.* 2005; (4) Present study
Parachaenichthys charcoti	64.5	-0.94	1.1	315	0.215	4.0		25.0	53	Demersal	yes	(1, 3, 4, 5, 6) Barrera-Oro and Lagger 2010
Parachaenichthys georgianus	55	1.52	0.8	434	0.01	4.0	10.8	30.0	59	Demersal		(1,3) North and Ward 1989; (2) North and White 1987; (4) Gon and Heemstra 1990; (5) Permitin 1973
Prionodraco evansii	69	-1.61	0.36	380.5	0.61	3.7			15	Demersal		(1, 5) Kock and Kellermann 1991; (4) Gon and Heemstra 1990
Psilodraco breviceps	54.5	2.095	1.45	468	0.01		90.0		20			(2) Kompowsky 1992; (4) Gon and Heemstra 1990
Racovitzia glacialis	67.5	-0.61	2.44	365.5	0.52	4.3	22.6		27*			(1, 2) Kock and Kellermann 1991; (2) Duhamel *et al.* 1993; (2) Ekau 1991; (4) Present study
Bovichtidae												
Bovichtus diacanthus	38.5	14.38	3.58	497	0	1.5	106.0	9.2	19			(1, 2, 3, 4) La Mesa *et al.* 2010
Cottoperca gobio	48	9.42	5.02	1237	0	2.4			80	Demersal	no?	(1, 4, 5, 6) Arkhipkin *et al.* 2014
Channichthyidae												
Chaenocephalus aceratus	59	0.31	2.74	347.5	0.135	4.7*	5.8*	28*	75	Demersal	yes	(2) Kock and Kellermann 1991; (2, 3) Riginella *et al.* unpubl.; (5, 6) Detrich *et al.* 2005
Chaenodraco wilsoni	69	-1.295	0.99	342.5	0.435	4.9	5.8	35.7	43	Demersal	yes	(1, 2, 5, 6) Kock *et al.* 2008; (2) Kock and Kellermann 1991; (3) Pshenichnov 1988; (4) Gon and Heemstra 1990
Champsocephalus esox	53.5	4.92	6.56	948.5	0	2.7	32.5	22.0	35			(1, 2, 3, 4) Calvo *et al.* 1999
Champsocephalus gunnari	57	1.28	4.16	408.5	0.085	4.1	19.3	27.0	65			(1, 2, 3, 4) Kock *et al.* 2005
Channichthys rhinoceratus	50	2.925	1.97	420.5	0	4.5	12.0	12.0	52			(1, 2, 4) Kock *et al.* 2005; (3) Kock and Kellermann 1991
Chionobathyscus dewitti	69.5	0.295	1.73	289	0.415	4.2	7.6		60	Egg carrying	yes	(1, 2, 5, 6) Kock *et al.* 2006; (4) Gon and Heemstra 1990
Chionodraco hamatus	69	-1.425	0.73	339	0.495	5.0	5.9*	39.0	49	Demersal	yes	(1, 2) Vacchi *et al.* 1996; (3) La Mesa *et al.* 2003; (2) Present study; (5, 6) Kock 2005; Ferrando *et al.* 2014
Chionodraco myersi	69.5	-1.625	0.33	375	0.565	4.2	14.4		39	Demersal?	yes?	(1, 2) Ekau 1991; (2) Duhamel *et al.* 1993; (4) Morales Nin *et al.* 2000; (5, 6) Kock 2005; Ferrando *et al.* 2014
Chionodraco rastrospinosus	63.5	-0.945	0.95	319	0.25	5.3*	5.5*	29*	49	Demersal?	yes?	(1, 2, 3) Present study; (2, 3, 4, 5, 6) Kock 2005; (5, 6) Ferrando *et al.* 2014
Cryodraco antarcticus	69	-1.235	1.11	351	0.43	4.4	10.2	34*	65	Demersal?	yes?	(1, 2) Ekau 1991; (1, 2, 3) Kock and Jones 2002; (2) Duhamel *et al.* 1993; (3) Present study; (5, 6) Pshenichnov 2004
Neopagetopsis ionah	69	-1.16	1.26	387	0.375	6.5*	3.3*	49*	56	Demersal*	yes*	(1, 2, 3, 5, 6) Present study; (2) Lisovenko and Trunov 1989; (2) Shandikov and Faleeva 1987; (4) Gon and Heemstra 1990
Pagetopsis macropterus	69	-1.385	0.81	356.5	0.51	4.0	7.1		33	Demersal	yes	(1) Lisovenko 1987; (2) Kock *et al.* 2004; (4) Gon and Heemstra; (5, 6) Kock 2005

Species	Lat (°S)	Temp (°C)	Δt (°C)	Prim Prod $\left(\frac{\mathrm{mgC}}{\mathrm{m}^2 * \mathrm{day}}\right)$	Sea ice cover	Egg size (mm) (1)	Rel fec (2)	GSI (3)	Max size (cm) (4)	Egg type (5)	Parental care (6)	References
Pagetopsis maculatus	70	-1.665	0.25	365.5	0.68	4.8	21.4		25			(1) Kock and Kellermann 1991; (2) Duhamel *et al.* 1993; (4) Gon and Heemstra
Pseudochaenichthys georgianus	59.5	0.585	2.77	405	0.125	4.5	8.0	30.0	57			(1, 2, 3) Kock and Kellermann 1991; (2) Vanella *et al.* 2005; (2) Militelli *et al.* 2015; (4) Gon and Heemstra 1990
Harpagiferidae												
Harpagifer antarcticus	62	0.05	3.9	350	0.415	2.6	87.5	60.0	10	Demersal	yes	(1, 2, 3) White and Burren 1992; (4) Gon and Heemstra 1990; (5, 6) Daniels 1978
Nototheniidae												
Dissostichus eleginoides	49.5	8.795	13.55	927	0	4.7	23.5	15.0	215	Pelagic		(1, 2, 3) Kock and Kellerman 1991; (4) Fischer and Hureau 1985; (5) North 2002
Dissostichus mawsoni	61.5	-1.005	1.39	243.5	0.33	4.3	22.5	20.0	200	Pelagic?		(1, 2) Kock and Kellerman 1991; (3, 5) Hanchet *et al.* 2008; (4) Frose and Pauly 2014
Gobionotothen gibberifrons	60	2.185	7.03	415.5	0.17	2.5	82.0	27.0	55			(1, 2, 3) Kock and Kellermann 1991; (4) Gon and Heemstra 1990
Gobionotothen marionensis	53.5	2.3	5.82	450.5	0.135	1.6	300.0	14.0	20			(1, 2) Kock and Kellermann 1991; (3) Permitin and Silyanova 1971; (4) Gon and Heemstra 1990; North 1998
Gobionotothen acuta	50	3.32	2.76	460.5	0	1.6			35			(1) Kock and Kellermann 1991; (4) Gon and Heemstra 1990
Lepidonotothen larseni	57.5	0.395	3.49	356.5	0.185	2.0	82.3*	20.0	23	Demersal	yes	(1, 2, 3) Kock and kellermann 1991; (2) Present study; (5, 6) Konecki and Targett 1989
Lepidonotothen nudifrons	63.5	-0.86	1.26	329	0.23	2.5	53.3*	25.0	15	Demersal	yes	(1) Kock and Jones 2005; (2) Present study; (3) Kock and Kellermann 1991; (4) Gon and Heemstra 1990; (5, 6) Hourigan and Radtke 1989
Lepidonotothen squamifrons	50.5	3.25	3.92	435	0	1.9	160*	30.0	55	Demersal	yes	(1, 2, 3) Kock and Kellerman 1991; (2) Present study; (4) Gon and Heemstra 1990; (5, 6) Eastman *et al.* 2013
Notothenia coriiceps	62	2.375	8.03	394.5	0.285	4.7	13*	39.0	62	Pelagic	no	(1, 2) Kock and Kellerman 1991; (2) Calì *et al*. unpubl.; Sapota 1999; (4) Gon and Heemstra 1990; (5, 6) White *et al.* 1996
Notothenia rossii	56	4.1	9.56	469	0.055	5*	14.4*	50.0	92	Pelagic	no	(1, 2, 3) Kock and Kellerman 1991; (1, 2) Calì *et al*. unpubl.; (4) Gon and Heemstra 1990; (5, 6) White *et al.* 1996
Patagonotothen guntheri	52.5	5.275	6.07	936	0	1.4	300.0		27			(1, 2) Alekseyeva 1993; (4) Gon and Heemstra 1990
Patagonotothen ramsayi	46	9.91	7.28	1115.5	0	2.2	351.9*	11.0	47	Demersal	yes?	(1, 2, 5, 6) Arkhipkin *et al.* 2013; (2) La Mesa *et al*. 2015; (2, 3) Brickle *et al.* 2006; (4) Ekau 1982
Patagonotothen tessellata	46	10.18	7.38	1072	0	1.5	340.0	40.0	30	Demersal	yes	(1, 2, 3, 5, 6) Rae and Calvo 1995; (4) Fernández *et al*. 2000
Pleuragramma antarctica	69	-1.445	0.69	334.5	0.51	2.0	123.9*	46.0	25	Cryopelagic	no	(1, 2, 3) Kock and Kellermann 1991; (2, 5, 6) La Mesa and Eastman 2011; (2) La Mesa *et al.* 2014; (4) Gon and Heemstra 1990
Trematomus bernacchii	69.5	-1.4	0.78	288.5	0.485	4.5	9.5		34	Demersal	yes	(1) Kock *et al.* 2000; (1, 5, 6) Moreano 1980; (2) Vacchi *et al.* 1996; (4) Gon and Heemstra 1990
Trematomus borchgrevinki	69	-1.32	0.94	323	0.52	4.0	23.0		28			(1, 2) Kock and Kellermann 1991; (4) Gon and Heemstra 1990
Trematomus eulepidotus	69	-1.295	0.99	329	0.445	4.3	23.5	33*	35	Demersal	yes	(1, 2) La Mesa *et al.* 2008; (1) Kock and Kellermann 1991; (3) Present study; (5, 6) Ekau 1989
Trematomus hansoni	65.5	-0.09	3.4	341.5	0.415	3.8	20.9	36.0	41	Demersal?		(1, 3, 5) La Mesa *et al.* 2006a, 2008; (2) Vacchi *et al.* 1996; (2) Duhamel *et al.* 1993; (4) Gon and Heemstra 1990
Trematomus lepidorhinus	69	-1.64	0.3	341.5	0.655		42.3		31	Demersal?		(2) Kock and Kellermann 1991; (2) Duhamel *et al.* 1993; (4) Gon and Heemstra 1990; (5) La Mesa *et al.* 2008
Trematomus loennbergii	69.5	-1.565	0.45	385.5	0.62	3.5	40.0		30	Demersal?		(1, 2) Kock and Kellermann 1991; (2) Ekau 1991; (4) Gon and Heemstra 1990; (5) La Mesa *et al.* 2008
Trematomus nicolai	71.5	-1.625	0.33	274.5	0.63		16.7		40	Demersal?		(2) Kock and Kellermann 1991; (4) Kuhn *et al.* 2009; (5) La Mesa *et al.* 2008
Trematomus pennellii	69	-1.365	0.85	307.5	0.51	3.8	19.0		30	Demersal?		(1, 2) Kock and Kellermann 1991; (4) Gon and Heemstra1990; (5) La Mesa *et al.* 2008
Trematomus scotti	69	-1.465	0.65	397.5	0.485	2.5	116*	37*	19	Demersal?		(1, 2) Kock and Kellermann 1991; (2) Duhamel *et al.* 1993; (2, 3) Present study; (5) La Mesa *et al.* 2008
Trematomus tokarevi	65	-0.485	2.19	323.5	0.41	3.8			22	Demersal?		(1) Lisovenko 1987; (4) Gon and Heemstra 1990; (5) La Mesa *et al.* 2008
Trematomus vicarius	54	1.5	0.24	409	0.01	3.8			35	Demersal?		(1) Burchett *et al.* 1983; (4) Gon and Heemstra 1990; (5) La Mesa *et al.* 2008
Pseudaphritidae												
Pseudaphritis urvillii	40.5	12.5	15	471.5	0	0.5		16.0	36	Pelagic?		(1, 3, 5) Chesire *et al.* 2013; Bray and Thompson 2011
Eleginopsidae												
Eleginops maclovinus	44	10.76	6.22	1524	0	1.1	796.0	22.5	90	Pelagic		(1, 2, 3, 4, 5) Brickle *et al.* 2005