

Comparative population genetics, larval dispersal and evolutionary aspects of Antarctic fishes (Notothenioidei)

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SUMMARY

In this thesis, population genetic structures and evolutionary aspects of speciation in notothenioid fishes from the Southern Ocean have been investigated. Special emphasis was given to the role of pelagic larval dispersal on gene flow between geographically separated populations, since notothenioids are characterized by unusually long pelagic larval durations of up to one year, which is assumed to counteract genetic divergence of populations and ultimately allopatric speciation processes. By elucidating the population genetic structures of selected notothenioid species, inferences were made about the level of gene flow between populations. The major goal of this thesis was to compare the population genetic structures of sympatric species within the Atlantic sector of the Southern Ocean to determine factors responsible for regulating gene flow. Species-specific traits, such as larval durations, were expected to result in differences between the observed patterns, while environmental factors, such as oceanographic currents or frontal systems, were assumed to influence multiple species in a similar way. In addition, it has been tested whether the evolution of notothenioids fulfills the criteria of an adaptive radiation, of which only a few examples are known from the marine realm.

In **Chapter I**, the population genetic structure of seven notothenioid species from the southern Scotia Arc, collected at the area of the Antarctic Peninsula and South Orkney Islands, have been compared with mitochondrial DNA sequences and microsatellite markers. The investigated species comprise three crocodile icefishes (Channichthyidae: *Chaenocephalus aceratus*, *Champscephalus gunnari*, *Chionodraco rastrospinosus*) and four cod icefishes (Nototheniidae: *Gobionotothen gibberifrons*, *Lepidonotothen squamifrons*, *Trematomus eulepidotus*, *T. newnesi*). The results show that in the investigated area, populations of all species are genetically not significantly differentiated over distances of about 250 km, and that the direction of gene flow is in congruence with the prevailing current of the Antarctic Circumpolar Current (ACC). The results from this comparative approach highlight the role of passive larval dispersal with the currents for gene flow. The only exceptions are the channichthyids *C. aceratus* and *C. gunnari*, which show a small, but significant differentiation with microsatellite markers.

In order to investigate the role of larval dispersal on gene flow on a larger geographic scale, the population genetic structures of *C. aceratus* and *C. gunnari* have been investigated over large parts of their distribution range in the Atlantic sector of the Southern Ocean (**Chapter II**). More specifically, populations at the Antarctic Peninsula region, South Orkney Islands, South Georgia Island and Bouvet Island, which are connected by the ACC, but separated by long stretches of deep ocean basins, were investigated. The examined populations show significant differentiation except for the area of the southern Scotia Arc. Therefore, long distances of open ocean seem to restrict gene flow. At the same time, levels of self-recruitment are high indicating that larvae are effectively retained near their spawning locations. In addition, the levels of population divergence are likely influenced by demographic factors, such as effective population sizes and generation times. Population size reductions during the last glacial maximum inferred from Bayesian Skyline Plots (BSPs) indicate that sub-

Antarctic populations are largely influenced by glacial cycles, similar to populations in the high-Antarctic realm, further driving population divergences and speciation events.

In **Chapter III**, the population genetic structure of the nototheniid *Lepidonotothen larseni* in the Atlantic sector of the Southern Ocean has been examined, whose early pelagic developmental stages are among the longest known from notothenioids (about 18 months). The results indicate significant population structure among specimens from South Orkneys, South Georgia and Bouvet Island. In combination with the results of Chapter II, the study suggests that the pelagic larval time *per se* does not imply high levels of gene flow in notothenioids, although it allows for occasional long-distance dispersal. Consequently, it can be assumed that allopatric speciation (in its peripatric subform) plays a major role in the evolution of notothenioid fishes, similar to Antarctic invertebrates, which are characterized by short, if at all, pelagic larval stages.

In **Chapter IV**, the ecological diversification of notothenioids has been tested as evidence for an adaptive radiation. Based on stable carbon (C) and nitrogen (N) isotopes, 25 species were characterized for their feeding ecology and foraging habitats to test whether ecological diversification along the benthic-pelagic axis followed a single directional trend or evolved independently in several lineages. Phylogenetic and isotopic disparity-through-time analyses showed that the trophic diversification did not follow a single directional trend but evolved several times in parallel within different notothenioid lineages possibly as a result of interspecific competition, which is characteristic for adaptive radiations.

The results presented in this thesis show that gene flow between notothenioid populations is highly restricted in the Atlantic sector of the Southern Ocean, despite a high potential for gene flow by long pelagic larval phases and strong oceanographic currents. Moreover, the degrees of population divergences seem to be further related to demographic factors and specific life-history traits, such as generation-time-effects and spawning behavior (inshore vs. offshore). Allopatric speciation in the subform of peripatric speciation is a highly eligible mode of speciation in sub-Antarctic notothenioids and may have been driven by glacial periods, during which population sizes were substantially decreased, similar to high-Antarctic species and Antarctic invertebrates. In addition, the ecological diversification of notothenioids is in congruence with an adaptive radiation.

ZUSAMMENFASSUNG

In dieser Doktorarbeit wurden die populationsgenetischen Strukturen und weitere evolutionäre Aspekte von Artbildungsprozessen innerhalb von Antarktischen (Notothenioidei) aus dem Südpolarmeer untersucht. Ein besonderer Schwerpunkt war es, den Einfluss pelagischer Larvenverdriftung auf den Genfluss zwischen geografisch getrennten Populationen zu ermitteln, da notothenioide Fische durch ungewöhnlich lange, pelagische Larvenphasen von bis zu über einem Jahr charakterisiert sind, wodurch erhöhter Genfluss einer Auseinanderentwicklung von Populationen und damit auch allopatrischer Artbildung entgegen wirken kann. Die Untersuchung der populationsgenetischen Strukturen erlaubt es, Rückschlüsse auf das Ausmaß des genetischen Austauschs zwischen Populationen zu ziehen. Das Hauptziel dieser Arbeit war es, durch einen Vergleich der genetischen Populationsstrukturen sympatrischer Arten, Faktoren zu identifizieren, die maßgeblich den Genfluss beeinflussen. Dabei wurde angenommen, dass artspezifische Merkmale, wie z. B. die Dauer der Larvenphase, in Unterschieden zwischen den beobachteten Populationsstrukturen zum Ausdruck kommen, während Umweltfaktoren, wie ozeanografische Strömungen und Fronten, verschiedene Arten gleichermaßen beeinflussen. Des Weiteren wurde untersucht, ob die Evolution der Antarktischen den Kriterien einer Adaptativen Radiation entspricht, für welche es im marinen Lebensraum bisher nur wenige bekannte Beispiele gibt.

In **Kapitel I** wurden, basierend auf mitochondrialen Gensequenzen und Mikrosatelliten, die populationsgenetischen Strukturen von sieben notothenioiden Arten verglichen, die am südlichen Schottischen Bogen im Gebiet der Antarktischen Halbinsel und den Südlichen Orkneyinseln vorkommen. Zu den untersuchten Arten gehören drei Krokodileisfische (Channichthyidae: *Chaenocephalus aceratus*, *Champscephalus gunnari*, *Chionodraco rastrospinosus*) und vier Antarktisdorsche (Nototheniidae: *Gobionotothen gibberifrons*, *Lepidonotothen squamifrons*, *Trematomus eulepidotus*, *T. newnes*). Die Ergebnisse zeigen, dass im Untersuchungsgebiet die Populationen aller Arten über eine Distanz von ca. 250 km genetisch nicht oder nur marginal zu differenzieren sind und dass die Richtung des Genflusses mit der vorherrschenden Strömungsrichtung des Antarktischen Zirkumpolarstromes übereinstimmt. Die Ergebnisse dieser vergleichenden Untersuchung unterstreichen die Bedeutung passiver, pelagischer Larvenverdriftung mit der Strömung für den Genfluss. Die einzigen Ausnahmen waren die Krokodileisfische *C. aceratus* und *C. gunnari*, die anhand von Mikrosatelliten eine geringe, aber dennoch signifikante genetische Differenzierung zeigten.

Um den Einfluss der Verdriftung pelagischer Entwicklungsstadien auf den Genfluss eingehender zu untersuchen, wurden in **Kapitel II** die populationsgenetischen Strukturen von *C. aceratus* und *C. gunnari* über weite Teile ihres Verbreitungsgebietes im atlantischen Sektor des Südpolarmees analysiert. Hierbei wurden anhand von Mikrosatelliten und mitochondrialer DNS-Sequenzen (D-loop, Cyt *b*) Populationen in der Region der Antarktischen Halbinsel, Südlichen Orkneyinseln, Südgeorgien und Bouvetinsel verglichen, die zwar hydrografisch durch den Antarktischen Zirkumpolarstrom miteinander verbunden, jedoch über weite Distanzen durch tiefe

Ozeanbecken getrennt sind. Die Populationen zeigen signifikante genetische Unterschiede mit Ausnahme des bereits in Kapitel I untersuchten Gebiets am südlichen Schottischen Bogen. Daher kann angenommen werden, dass weite Distanzen offenen Ozeans dem Genfluss durch Larvenverdriftung effektiv entgegenwirken. Gleichzeitig konnte gezeigt werden, dass die Selbstrekrutierung der einzelnen Populationen hoch ist, weshalb effektive Mechanismen für die Retention von Larven vorhanden sein müssen. Zusätzlich wurde nachgewiesen, dass die genetische Divergenz der Populationen durch demografische Faktoren beeinflusst wird, wie z. B. Populationsgrößen und Generationszeiten. Basierend auf sog. *Bayesischen Skyline Plots* wurden Populationsgrößenreduzierungen während des letzten glazialen Maximums nachgewiesen, was darauf hinweist, dass sub-antarktische Populationen ähnlich stark wie hoch-antarktische Populationen durch Eiszeiten beeinflusst werden, wodurch wiederum die genetische Auseinanderentwicklung von Populationen begünstigt und Speziation gefördert werden.

In **Kapitel III** wurde die genetische Populationsstruktur des nototheniiden *Lepidonotothen larseni* im atlantischen Sektor des Südpolarmeeres analysiert, der durch eine der längsten bekannten pelagischen Entwicklungsphasen innerhalb der Notothenioidei charakterisiert ist, welche ca. 18 Monate dauert. Die Ergebnisse zeigen auch hier eine signifikante Differenzierung der Populationen von den Südlichen Orkneyinseln, Südgeorgien und der Bouvetinsel. In Kombination mit den Ergebnissen aus Kapitel II kann daher angenommen werden, dass die Dauer der pelagischen Entwicklung kein Indikator für die Höhe des realen Genflusses ist, obwohl durch sie zeitweilig eine Verdriftung über lange Distanzen stattfindet. Folglich kann angenommen werden, dass allopatrische Speziation eine ebenso wesentliche Rolle in der Evolution notothenioider Fische spielt, wie bei antarktischen Evertebraten, die in der Regel durch eine höchstens kurze Larvalphase gekennzeichnet sind.

In **Kapitel IV** wurde die ökologische Diversifikation während der Evolution notothenioider Fische, als Nachweis für eine Adaptative Radiation, untersucht. Basierend auf stabilen Isotopen von Kohlenstoff und Stickstoff wurden 25 Arten bezüglich ihrer Nahrungsökologie und Habitatspezialisierung charakterisiert, um zu überprüfen, ob ihre ökologische Diversifikation einem gerichteten Trend entlang der benthisch-pelagischen Achse folgte oder mehrfach unabhängig in unterschiedlichen Stammlinien entstanden ist. Phylogenetische und sog. *Disparity-through-time* Analysen zeigten, dass die trophische Diversifikation nicht einem einzelnen gerichteten Trend folgte, sondern mehrfach und parallel innerhalb verschiedener notothenioider Stammlinien evolvierte, was wahrscheinlich eine Folge zwischenartlicher Konkurrenz war und charakteristisch für eine Adaptative Radiation ist.

Die Ergebnisse dieser Dissertation zeigen, dass der Genfluss zwischen notothenioiden Populationen innerhalb des atlantischen Sektors des Südpolarmeeres niedrig ist, obwohl ein hohes Potential hierfür durch lange pelagische Entwicklungsstadien und starke ozeanografische Strömungen gegeben ist. Das Ausmaß der genetischen Divergenz zwischen Populationen ist neben geografischer Isolation zusätzlich durch demografische Faktoren und artspezifische Lebensstrategien beeinflusst, wie z. B. Generationszeit-Effekten und Laichverhalten (küstennah – schelfkantennah). Aus den Ergebnissen lässt sich folgern, dass allopatrische Speziation, insbesondere in ihrer peripatrischen Form, ein legitimer Artbildungsprozess bei sub-antarktischen Notothenioidei ist. Darüberhinaus wird die Artbildung durch Eiszeiten begünstigt,

während derer auch die Populationen in sub-antarktischen Gebieten substantiell in ihrer Größe reduziert werden, ähnlich wie es für hoch-antarktische Arten und Evertebraten bekannt ist. Zudem konnte gezeigt werden, dass die ökologische Diversifikation der Notothenioidei wahrscheinlich durch eine Adaptive Radiation entstanden ist, für die es bisher nur wenige Nachweise im marinen Lebensraum gibt.

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GENERAL INTRODUCTION

Speciation in the sea

Speciation, the evolutionary process of reproductive isolation between populations by which new biological species arise, is central for our understanding of evolution and biodiversity (Coyne & Orr 2004). According to the biological species concept (BSC), a species can be defined as a “group of interbreeding natural populations that are reproductively isolated from other such groups” (Mayr 1963; 1995; but see Coyne & Orr 2004 for a selected variety of other definitions). The process of speciation therefore involves the emergence of reproductive isolating barriers that allow groups of the same ancestral species to diverge, until they are unable to reproduce ‘fit’ offspring. The most common form of reproductive isolation arises by *geographic* separation of populations (allopatric speciation), whereas speciation based on *biological* barriers that can arise between groups of the same species with overlapping spatial distributions (sympatric speciation), is notably rare (Coyne & Orr 2004). Allopatric speciation can further be subdivided into vicariant speciation and peripatric speciation (Mayr 1954; Coyne & Orr 2004). In the former, reproductive isolation occurs after the split of a population into two or more populations by arising geographic barriers, whereas in the latter a new population is founded by a few individuals without any continuation of genetic exchange with the source population. Regardless of the underlying bases for allopatric speciation, the geographic isolation of populations causes gene flow (the transfer of genes or alleles from one population to another) to cease, while natural selection and genetic drift lead to genetic divergence of the populations and ultimately to reproductive isolation. Therefore, high levels of gene flow counteract speciation events.

In contrast to terrestrial and lacustrine ecosystems, barriers to gene flow are less common (or at least less obvious) in the marine realm. The more or less continuous habitat and high dispersal potential of many marine species, especially during their early developmental stages as larvae, promote gene flow over large geographic distances and may prevent the genetic divergence of populations (Palumbi 1994). Indeed, the level of gene flow and, hence, population structure, is correlated with dispersal ability in a wide variety of taxa (Bohonak 1999). Nonetheless, dispersal ability is not a perfect indicator of gene flow (Bohonak 1999) and an increasing number of studies revealed restricted gene flow in marine species with high dispersal abilities (e.g. Siegel *et al.* 2003; Shanks 2009). Therefore, identifying the ecological biotic and abiotic factors, which additionally influence gene flow is crucial for our understanding of speciation processes.

One of the probably most fascinating subjects in evolutionary studies of marine organisms are Antarctic fishes (Notothenioidei). Notothenioidei dominate the Antarctic ichthyological species assemblages on the shelves and slopes of the Southern Ocean in terms of diversity and abundance. Their evolutionary success and ecological diversity in combination with a relatively well known phylogeny make them highly interesting for evolutionary research. Yet, one of the great mysteries in the evolution of notothenioids is still unsolved: how could notothenioids evolve into the great species diversity we see today, while they are characterized by high dispersal abilities due to

unusually prolonged pelagic larval stages often exceeding one year, which theoretically counteract speciation events by increased levels of gene flow?

To answer this question population genetic methods can be employed, as they allow examining the structure of populations and, hence, the magnitudes of gene flow. Although the population genetic structures of many notothenioid species have already been examined (see below), much debate exists about the general validity of the observed patterns, as a direct comparison between studies and species is hindered by different sampling designs, methods, genetic markers and species biogeography. Therefore, the goal of this thesis is to elucidate the population genetic structures of sympatric species in a comparative approach, to assess the role of larval dispersal for gene flow in notothenioids. Moreover, a comparative approach allows identifying putative biotic and abiotic factors additionally involved in gene flow regulation. The results of this thesis will not only contribute to our knowledge of speciation processes in notothenioids, but also expand our understanding of speciation processes of marine organisms in general.

Evolutionary history of Antarctic Notothenioidei

The Southern Ocean around Antarctica is a unique evolutionary site, as its marine fauna is thermally, geographically and oceanographically isolated since approximately 25-22 million years ago (mya; Dayton *et al.* 1994; Eastman & McCune 2000; Eastman 2005). After the separation of the Antarctic continent from the rest of Gondwana, the Antarctic Circumpolar Current (ACC), the worlds largest current, started to flow continuously around Antarctica (Kennett 1982). The subsequent cooling of Antarctic waters to temperatures as low as -1.86°C and the formation of the Antarctic Polar Front (PF) lead to an effective circumpolar hydrographic barrier

for many marine organisms. From a biological perspective, these changing environmental conditions resulted in major changes of the Antarctic species composition (Balushkin 1992; Eastman 1993). Without adaptations to the cold or range shifts into milder waters of the north or deep-sea, many species became extinct.

The Antarctic ichthyofauna, as known today, is characterized by low species diversity and high endemism. Although encompassing about 10% of the worlds oceans (Laws 1985), the Southern Ocean harbors only about 1% (322 species from 50 families) of the estimated global fish diversity (32,500 species), with 88% of the Antarctic species being endemic (Eastman 2005; Nelson 2007). The three most speciose taxa on the shelves and slopes of the Southern Ocean belong to the two perciform groups Notothenioidei and Zoarcidae as well as the scorpaeniform family Liparidae (Eastman

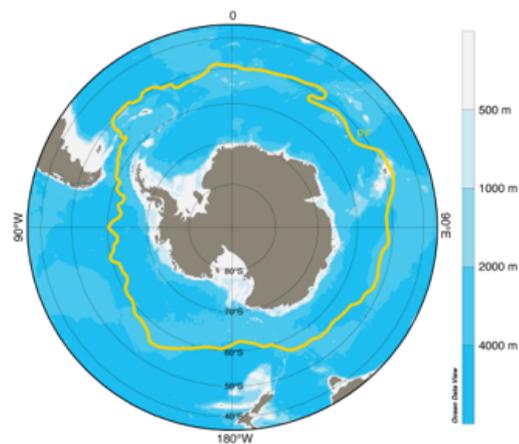


Fig. 1 The Southern Ocean around the Antarctic continent and the location of the Antarctic Polar Front (PF; after Orsi *et al.* 1995)

2005). Although they together account for nearly 88% of species diversity (Eastman 2005), notothenioid fishes clearly dominate the species assemblages not only in terms of diversity (Eastman & Clarke 1998), but also abundance and biomass (>90%; e.g. DeWitt 1971; Ekau 1990; Jones *et al.* 2008).

The causes for the ecological and evolutionary success of notothenioids in the Southern Ocean are tightly linked to their unique evolutionary history. In contrast to liparids and zoarcids, which probably invaded the Southern Ocean from the North Pacific in a secondary radiation after the polar climatic conditions developed (Eastman 1993), notothenioids are indigenous (Andriashev 1965). Their survival and diversification in the ice-laden Antarctic waters were facilitated by their acquired key innovation to express antifreeze glycoproteins (AFGPs), which prevent body fluids from freezing (DeVries & Wohlschlag 1969; DeVries 1988). In the large absence of direct competitors for ecological resources this ability gave ancestral notothenioids the opportunity to diversify by adapting to open ecological niches (Eastman & Grande 1989).

Today, notothenioids consist of 131 recognized species from 8 families, of which 103 species from 5 families are considered endemic to the Southern Ocean ('Antarctic clade'; Eastman 1993; 2013). The most speciose families are the Nototheniidae (50 species), followed by Artedidraconidae (29), Bathydraconidae (16), Channichthyidae (16) and Harpagiferidae (11). The least speciose families are Bovichthidae (7), Eleginopidae (1) as well as Pseudaphritidae (1), and these have, with one exception within the Bovichthidae, a non-Antarctic distribution along the coasts of South America, Australia or New Zealand (Gon & Heemstra 1990; Eastman 2013). Regardless of their distribution range, all notothenioids are characterized by the lack of a swim bladder and it is assumed that this trait is derived from a common ancestor with a benthic life strategy (e.g. Andersen 1984; Eastman 1993). Although more than half of the modern notothenioid species are also benthic, they now occupy all habitats from the ocean floor to the water column, thereby showing a variety of benthic, epibenthic, semi-pelagic, cryopelagic and truly pelagic life strategies (Eastman 1993). The evolutionary trend of notothenioids therefore follows a benthic-pelagic axis, a trend called 'pelagization', related to adaptations for feeding in the water column (e.g. Andriashev 1987; Eastman 1993; Klingenberg & Ekau 1996). In compensation for a lacking swim bladder, buoyancy in pelagic species was gained by morphological and physiological adaptations, as for example reduced skeletal mineralizations and increased lipid depositions (reviewed in Eastman 1993). In addition, morphological changes for swimming in the pelagic realm resulted in a transition from wide-headed fish with short posterior bodies and pectoral fins in benthic species towards more slender and elongated bodies in pelagic ones (Klingenberg & Ekau 1996). But in contrast to the AFGPs, these adaptations were not derived only once by a common ancestor, but independently in several lineages of the Notothenioidei (Klingenberg & Ekau 1996) and bursts of lineage diversification post-date the acquisition of AFGPs by about 10 million years (Near *et al.* 2012). In summary, the rapid evolution of Notothenioidei from a common ancestor and the diversification into various ecological niches paralleled with respective adaptations makes notothenioid fishes a rare example of a marine adaptive radiation.

Adaptive radiation of Notothenioidei

Adaptive radiation is “the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage” (Schluter 2000). According to Schluter (2000), an adaptive radiation can be detected by four criteria: (i) common ancestry, (ii) phenotype-environment correlation, (iii) trait utility (in the sense that physiological and morphological traits enhance the performance in a certain environment), and (iv) rapid speciation. The most popular examples of adaptive radiations are from terrestrial organisms, such as the Darwin finches on Galápagos, which show a remarkable diversity in beak morphology (Schluter 2000). Adaptive radiations in teleost fishes are primarily known from freshwater systems, such as the colourful cichlids from the Great Lakes of East Africa, which diversified into more than 1500 species, or the three-spined sticklebacks, which repeatedly radiated into benthic and limnetic ecomorphs in post-glacial lakes from a marine ancestor (see Matschiner *et al.* 2010 and references therein). By contrast, examples of marine adaptive radiations are scarce. The two most recognized groups of marine fishes fulfilling the above mentioned criteria of an adaptive radiation are probably those of *Sebastes* rockfishes from the northeastern Pacific Ocean and Antarctic notothenioids (Eastman 1993; Clarke & Johnston 1996; Eastman & Clarke 1998; Johns & Avise 1998).

Glacial cycles as drivers of notothenioid speciation

The evolution of polar organisms, including notothenioid fishes, is hypothesized to have been driven by climatic ‘diversity pumps’ (Clarke & Crame 1992). Antarctic species were repeatedly subject to major glaciation and de-glaciation cycles associated with severe implications for species’ survival and distribution (Kennett 1982; Eastman 1993; Petit *et al.* 1999). During major glacial periods the Antarctic icesheet extended as far as the edge of the continental shelf (Ingólfsson 2004; Gersonde *et al.* 2005), sometimes eradicating Antarctic marine bottom communities on the large scale (Thatje *et al.* 2005). The periodical fragmentation of populations by ice, isolation in refugia during glacial maxima and re-colonization events are supposed to have been key mechanisms for allopatric speciation (Hewitt 1996; Rogers 2007). This is especially true for polar marine bottom invertebrates, which are broadly characterized by high endemism and scarcity of pelagic larval stages (Thorson 1950; Mileikovsky 1971; Thatje 2012). However, notothenioid fishes always develop through unusually prolonged pelagic developmental stages often exceeding one year, regardless of the adult life strategy (Kellermann 1986; 1989; North 2001). During this time, larvae and/or juveniles may disperse with the currents over large geographic scales and facilitate gene flow even between distant populations, thereby counteracting allopatric speciation. On the other hand, long pelagic larval stages may promote colonization of remote habitats or re-colonization of destructed habitats. Therefore, colonization events and peripatric speciation may have been a primary mechanism of allopatric speciation in notothenioids, especially among the relatively isolated sub-Antarctic islands. Although population genetic methods are helpful in elucidating the recent and historical patterns of connectivity between populations and their implications for speciation, the population genetic structures of notothenioids and the role of larval dispersal for gene flow are still insufficiently known. Therefore, the comparative approach used in this

thesis will also be used to examine how species and populations were influenced during past climate changes.

Population genetics of notothenioids

Knowledge about the connectivity of populations is not only central for our understanding of evolutionary processes, but also ecology, biogeography, phylogeography and conservation management. In consideration of the large size of the oceans and small size of most marine propagules, direct observations of migration between populations are often impossible (Hellberg *et al.* 2002). Population genetic tools, which measure the distribution of genetic variation within species and populations, are therefore widely applied to make indirect inferences about population connectivity (Hedgecock *et al.* 2007; Lowe & Allendorf 2010). In addition, the genetic trail of migrations and imprints of other past demographic events, such as population expansions and bottlenecks, can be deduced.

From 1991 until February 2009, the beginning of my PhD-project, 21 population genetic studies on notothenioids had been published (Table 1). Although 17 species were examined in total, more than half of these studies focused on the 2 species most interesting to fisheries, namely the Mackerel icefish *Champsocephalus gunnari* and the Patagonian toothfish *Dissostichus eleginoides*. Since 2009, 5 additional population genetic studies on 7 notothenioid species have been published, resulting in a total of 26 studies on 21 species, excluding my publications in the presented thesis.

Table 1 Population genetic studies on Antarctic notothenioids, including the presence and scale of population differentiation. Geographical scale is categorized into between sectors (between Atlantic, Indian and Pacific ocean sectors of the Southern Ocean), within sectors (within ocean sectors) and regional (significant differentiation between populations separated by less than 250 km). If not indicated, the respective geographic scale was not assessed. RFLP=random fragment length polymorphism, STR=short tandem repeats (e.g. microsatellites), mtDNA=mitochondrial DNA sequences, RAPD=random amplified polymorphism DNA, nDNA=nuclear DNA sequences

Study	Family	Species	Marker type	geographical scale of genetic differentiation		
				Between sectors	Within sector	Regional <250 km
(Carvalho & Warren 1991)	Channichthyidae	<i>Champsocephalus gunnari</i>	Allozymes	yes	yes	yes
(Schneppenheim <i>et al.</i> 1994)	Nototheniidae	<i>Lepidonotothen squamifrons</i>	Allozymes	yes	no	no
(Williams <i>et al.</i> 1994)	Channichthyidae	<i>Champsocephalus gunnari</i>	RFLP		no	no
(Duhamel <i>et al.</i> 1995)	Channichthyidae	<i>Champsocephalus gunnari</i>	Allozymes	no	no	no
	Nototheniidae	<i>Notothenia rossii</i>	Allozymes		no	no
(Clément <i>et al.</i> 1998)	Channichthyidae	<i>Chaenodraco wilsoni</i>	Allozymes	no		
	Channichthyidae	<i>Chionodraco hamatus</i>	Allozymes	no		
	Channichthyidae	<i>Chionodraco myersi</i>	Allozymes	yes		
	Channichthyidae	<i>Cryodraco antarcticus</i>	Allozymes	no		
	Channichthyidae	<i>Neopagetopsis ionah</i>	Allozymes	yes		
	Channichthyidae	<i>Pagetopsis macropterus</i>	Allozymes	no		

Table 1 continued

Study	Family	Species	Marker type	geographical scale of genetic differentiation		
				Between sectors	Within sector	Regional <250 km
(Reilly & Ward 1999)	Nototheniidae	<i>Dissostichus eleginoides</i>	STR			yes
(Smith & McVeagh 2000) ¹	Nototheniidae	<i>Dissostichus eleginoides</i>	Allozymes, STR	no, yes	no, partly	
(Smith & Gaffney 2000) ²	Nototheniidae	<i>Dissostichus eleginoides</i>	Allozymes, STR, mtDNA	no, yes, yes	no, partly, yes	
(Appleyard <i>et al.</i> 2002)	Nototheniidae	<i>Dissostichus eleginoides</i>	RFLP, STR	yes, no	no, no	no, no
(Parker <i>et al.</i> 2002)	Nototheniidae	<i>Dissostichus mawsoni</i>	RAPD	yes		
(Patarnello <i>et al.</i> 2003)	Channichthyidae	<i>Chionodraco hamatus</i>	mtDNA	yes	no	no
(Appleyard <i>et al.</i> 2004)	Nototheniidae	<i>Dissostichus eleginoides</i>	RFLP, STR		no, no	
(Shaw <i>et al.</i> 2004)	Nototheniidae	<i>Dissostichus eleginoides</i>	RFLP, STR		yes, yes	no, no
(Smith & Gaffney 2005)	Nototheniidae	<i>Dissostichus mawsoni</i>	RFLP, mtDNA	no, no		
(Kuhn & Gaffney 2006)	Channichthyidae	<i>Champsocephalus gunnari</i>	mtDNA, nDNA	yes, no	yes, yes	
(Rogers <i>et al.</i> 2006) ³	Nototheniidae	<i>Dissostichus eleginoides</i>	STR, mtDNA	yes, yes	yes, yes	
(Zane <i>et al.</i> 2006) ⁴	Nototheniidae	<i>Pleuragramma antarctica</i>	mtDNA	yes	no	no
(Janko <i>et al.</i> 2007)	Nototheniidae	<i>Trematomus bernacchii</i>	mtDNA, nDNA	yes, no	no, no	no, no
	Nototheniidae	<i>Trematomus newnesi</i>	mtDNA, nDNA	no, no	no, no	
(Papetti <i>et al.</i> 2007) ⁵	Channichthyidae	<i>Chaenocephalus aceratus</i>	STR			no
(Jones <i>et al.</i> 2008)	Channichthyidae	<i>Chaenocephalus aceratus</i>	mtDNA		no	no
	Nototheniidae	<i>Lepidonotothen larseni</i>	mtDNA		no	no
	Nototheniidae	<i>Lepidonotothen squamifrons</i>	mtDNA		yes	no
	Nototheniidae	<i>Notothenia coriiceps</i>	mtDNA		no	no
(Kuhn & Gaffney 2008) ⁶	Nototheniidae	<i>Dissostichus mawsoni</i>	mtDNA, nDNA	yes, yes	yes, yes	no, no
(Kuhn <i>et al.</i> 2009)	Nototheniidae	<i>Trematomus nicolai</i>	mtDNA	no		
(Matschiner <i>et al.</i> 2009)	Nototheniidae	<i>Gobionotothen gibberifrons</i>	STR, mtDNA		no, no	no, no
(Papetti <i>et al.</i> 2009) ⁷	Channichthyidae	<i>Chaenocephalus aceratus</i>	STR		yes	yes
(Papetti <i>et al.</i> 2012)	Channichthyidae	<i>Chionodraco rastrispinosus</i>	STR		no	no
(Van de Putte <i>et al.</i> 2012)	Nototheniidae	<i>Trematomus bernacchii</i>	STR, mtDNA	yes, yes	yes, yes	no, yes
	Nototheniidae	<i>Trematomus hansonii</i>	STR, mtDNA	yes, yes	yes, yes	
	Nototheniidae	<i>Trematomus newnesi</i>	STR, mtDNA	yes, no	no, no	no, no

¹STR results depend on locus. Either only pairwise population comparisons for single loci or overall differentiation was tested. The Polar Front appears as barrier to gene flow.

²No significance tests available. The Polar Front appears as barrier to gene flow.

³Populations from Falkland Islands are significantly differentiated from any other populations south of the Polar Front. Without the Falkland population, intra-sector differentiation is not significant anymore when measured by mtDNA.

⁴In contrast to the other studies the significances are based on $p < 0.01$. If $p < 0.05$ is used, differentiations occur within ocean sectors. Regardless of significance level, population differentiations exist between sampling years at the same location.

⁵Significant differentiations only between year classes.

⁶Results depend on SNP selection.

⁷Significant differentiations also between sampling years.

Although the results of these studies are as manifold as the employed genetic markers, sampling designs and species, a general trend is the decrease of genetic homogeneity among populations with distance, indicating an isolation-by-distance relationship. On a regional scale (less than 250 km) the vast majority of species showed genetic homogeneity. Even within sector population differentiations are predominantly insignificant, sometimes over several thousand kilometers. Although a marginal majority of studies revealed significant population differentiations between ocean sectors, many populations of species with circum-Antarctic distributions were repeatedly found to be not significantly differentiated, as, for example, in the benthopelagic Antarctic toothfish *Dissostichus mawsoni* (Smith & Gaffney 2005) or the truly pelagic Antarctic silverfish *Pleuragramma antarctica* (Zane *et al.* 2006). Moreover, populations of strictly benthic species, such as the Blackfin icefish *Chaenocephalus aceratus* or the Humped rockcod *Gobionotothen gibberifrons*, which are confined to shelf areas as adults, were not significantly differentiated over their distribution range on sub-Antarctic islands (Papetti *et al.* 2007; Jones *et al.* 2008; Matschiner *et al.* 2009). The seemingly high levels of gene flow among populations separated by deep ocean over large geographic scales, but connected by currents like the ACC, regardless of the adult life strategy, suggest that gene flow is mediated via dispersal of pelagic developmental stages such as eggs, larvae or juveniles. This finding is corroborated by genetic breaks that have been found over much shorter geographic distances, where oceanographic barriers exist. For example, populations of *D. eleginoides* are not significantly differentiated over large parts of its circumpolar distribution range, but populations in close proximity and separated by the Antarctic Polar Front (PF) show little connectivity (Shaw *et al.* 2004; Rogers *et al.* 2006). Hence, oceanographic features are an important factor regulating population connectivity of notothenioids by either enhancing or attenuating larval dispersal, as has also been shown in species from warmer waters with distinctly shorter pelagic larval stages (e.g. Taylor & Hellberg 2003; Bay *et al.* 2006; Cowen & Sponaugle 2009). However, the general validity of the observed patterns is limited by varying sampling designs, genetic marker types and species' biogeography, what makes general inferences about gene flow by larval dispersal a challenging task.

With the development of new genetic markers and techniques, a transition from allozyme based studies towards microsatellite and DNA sequence based studies on notothenioids took place from 1999 until 2006 (see Material and Methods section for a short introduction to genetic markers used in population genetic studies). At the same time, this transition led to increasing numbers of studies, which detected significant population differentiations (e.g. ~27% of allozyme vs. ~61% of microsatellite based data). The higher resolution of microsatellites and DNA sequences as compared to allozymes allow discriminating population structures on finer geographic scales. For example, populations of *D. eleginoides* from the South American shelf, sub-Antarctic islands and Antarctic shelf could be differentiated by microsatellites and DNA sequences, but not allozymes (Smith & Gaffney 2000; Smith & McVeagh 2000). Marker-dependent results apply also for populations of *C. gunnari* (Duhamel *et al.* 1995; Kuhn & Gaffney 2006), *Chionodraco hamatus* (Clément *et al.* 1998; Patarnello *et al.* 2003), *D. mawsoni* (Smith & Gaffney 2005) and others.

Similar to the type of genetic marker, the inferred level of population connectivity is influenced by the sampling design. In general, the distribution range of a species determines the maximum distance over which levels of gene flow can be assessed. As a consequence, species with circum-Antarctic distributions may by default show genetic homogeneity on larger geographic scales than those endemic to specific locations, thereby prohibiting a direct comparison among species. Moreover, the collection of specimen in the Southern Ocean remains challenging with regard to its remoteness and climatic peculiarities, often leading to only small sample sizes used in population genetic studies accompanied by statistical shortcomings.

Overall, the variety of genetic marker types, sampling locations, sample sizes and analyses utilized in population genetic studies of notothenioids resulted in equally variable conclusions about the connectivity between populations. Therefore, one major goal of this thesis was to assess the population genetic structure of notothenioids in a comparative approach based on sympatric species, multiple genetic markers and reasonable sample sizes. In this way, species-specific traits and environmental factors shaping the population genetic structures of notothenioids can be identified and the generality of the observed patterns validated.

Factors regulating population connectivity by larval dispersal

The connectivity of marine populations is greatly enhanced by dispersal of pelagic larval and juvenile stages, especially in demersal species, whose populations are confined to island shelves. The pelagic larval duration (PD) is often correlated to dispersal distance and considered a main determinant of population structure (Riginos & Victor 2001; Shanks *et al.* 2003; Siegel *et al.* 2003; Shanks 2009). Nonetheless, populations are repeatedly found to be highly structured despite long PDs (e.g. Barber *et al.* 2002; Levin 2006; Marko *et al.* 2007; White *et al.* 2010), owing to interactions between biological and physical factors. A suitable way to better understand the responsible factors regulating larval dispersal and gene flow is to compare multiple species at the same locations, since biological differences between species are likely to cause differences in population structures, while environmental barriers will cause genetic breaks in many species in a similar way (Avice 1996; Wares *et al.* 2001; Pelc *et al.* 2009; Sivasundar & Palumbi 2010; Selkoe *et al.* 2010).

Physical barriers change over ecological as well as evolutionary time scales and comprise land barriers and oceanography, including environmental gradients. While topographic barriers (incl. geographic isolation) are relatively static over long periods of time, oceanographic variables have more dynamic impacts on dispersal. Identified oceanographic barriers are currents, eddies, up/downwelling and fronts (Gaylord & Gaines 2000; Wares *et al.* 2001; Sponaugle *et al.* 2002; Largier 2003; McCulloch & Shanks 2003; Sotka *et al.* 2004; Shanks & Brink 2005; Longhurst 2007; Cowen & Sponaugle 2009; Galarza *et al.* 2009; Schunter *et al.* 2011), often coupled with environmental barriers between adjacent water masses, such as temperature or salinity gradients (Reiss *et al.* 2000; Banks *et al.* 2007; Teske *et al.* 2008). Putative oceanographic retention mechanisms for larvae at their spawning sites are gyres and eddies forming on the lee side of islands or shelf-break frontal systems (but see Cowen

2002). But once larvae get entrained in oceanic currents, they may be transported over long distances before they find suitable habitat for settlement.

Biological factors influence where, when and how larvae are dispersed. Adult traits (potential fecundity, spawning behavior, parental care), egg traits (buoyancy) and larval traits (size at hatch, pelagic larval stage duration, rate of development, behavior, swimming and sensory capabilities) are likely to determine the extent to which larvae are successfully retained at their natal site or dispersed with the currents (Kingsford *et al.* 2002; Sponaugle *et al.* 2002). Therefore, a list of species-specific traits is compiled for the study species in the Material and Methods section.

MATERIAL AND METHODS

Study Area: Scotia Arc and Atlantic Sector of the Southern Ocean

The islands of the Scotia Arc and Bouvet (BO) in the Atlantic sector of the Southern Ocean were chosen as study sites (Fig. 2) and are ideal to examine the influence of pelagic larval dispersal and oceanography on the population genetic structure of notothenioids. The Scotia Arc consists of a series of islands and ridges that stretch in an eastward bow from South America to the Antarctic Peninsula and encompass the Scotia Sea. From north to south the islands comprise Shag Rocks (SR), South Georgia (SG), South Sandwichs (SS), South Orkneys (SO) and South Shetlands (SSh), including Elephant Island (EI). These islands are each separated by approximately 120-1800 km of deep ocean, whereas BO is located about 1800-3400 km further east. Hence, the notothenioids living on the island shelves are (ecologically speaking) forming discrete populations.

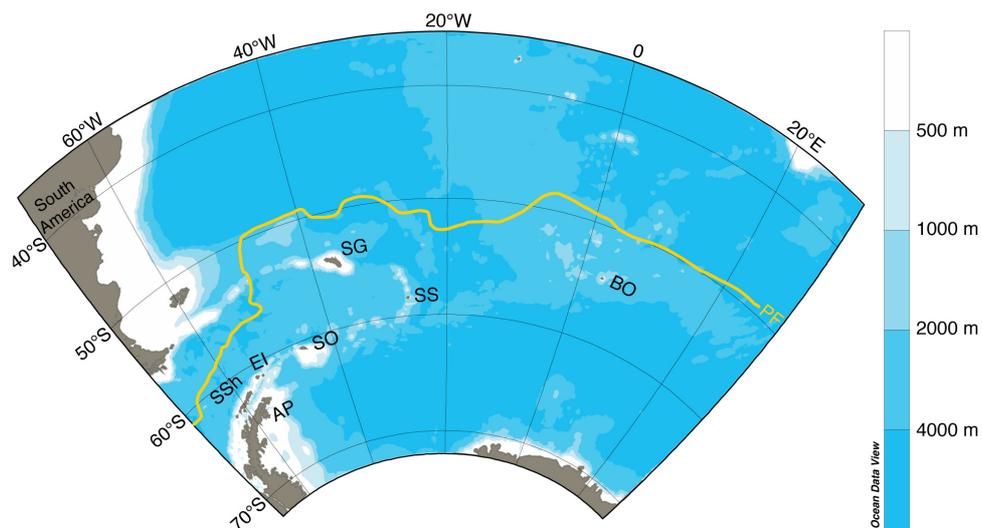


Fig. 2 The study area in the Atlantic sector of the Southern Ocean. Notothenioid specimens were collected at South Shetland Islands (SSh), Elephant Island (EI), Antarctic Peninsula region (AP), South Orkney Islands (SO), South Georgia Island (SG) and Bouvet Island (BO). No specimens collected at South Sandwich Islands (SS). PF=Polar Front (after Orsi *et al.* 1995)

Although separated geographically, the islands in the study area are all connected by the world's largest current, the Antarctic Circumpolar Current (ACC). The ACC flows clockwise without interruption around Antarctica thereby crossing the Scotia Sea and is well studied owing to its importance in driving the global ocean circulation (Orsi *et al.* 1995; Schmitz 1995; Rintoul 2000). The high velocity and net volume of the ACC are supposed to have a homogenizing effect on populations around Antarctica even when thousands of kilometres apart, as larvae dispersed with the ACC promote gene flow between distant populations (Eastman 1993). However, the ACC also acts as a barrier to migration and dispersal in latitudinal direction as three full frontal systems within the ACC, which appear as variable, meandering latitudinal bands, bear characteristic changes in water mass properties (Nowlin & Klinck 1986; Orsi *et al.* 1995; Moore *et al.* 1999; Rintoul 2000). From north to south these are the Subantarctic Front (SAF), Antarctic Polar Front (PF) and southern ACC front (SACCF). The SAF and PF are marked by sharp poleward declines of sea surface temperatures and salinities, associated with discontinuities between plankton (Ward *et al.* 2012), invertebrate (Hedgpeth 1969) and fish (Everson 1977) species assemblages and abundances on either side of the front (Deacon 1982). Moreover, the PF constitutes the northerly distribution boundary for many Antarctic species (Knox 1994). In contrast to the SAF and PF, the SACCF cannot be located by changes in surface water properties, but is instead characterized by a temperature maximum of 1.8-2.0°C of the underlying Upper Circumpolar Deep Water (UCDW) in approximately 500 m depth. It is the only oceanic front, which separates islands of the Scotia Arc and runs south of SG.

The oceanography in the southern Scotia Sea, in the area of AP, SSh, EI and SO, is influenced not only by the ACC, but also by water originating from the Weddell Sea to its south (Whitworth *et al.* 1994). Both water masses mix up in the so called Weddell-Scotia Confluence (WSC), which is characterized by a weak vertical stratification, with lower temperatures and salinities, but higher oxygen concentrations than in the adjacent waters (Deacon & Moorey 1975). In this region of the pack-ice zone that is ice-free during the austral summer, some water from the Weddell Sea flows northwards over the South Scotia Ridge filling the bottom layer of the southern Scotia Sea (Orsi *et al.* 1993). The WSC causes turbulences of water masses where the ACC and Weddell Sea water meet, thereby leading to potential retention mechanisms of larvae in the form of gyres.

Given the geographic and bathymetric separation of the Scotia Arc Islands and BO, in combination with the strong flow of the ACC, the sub-Antarctic islands in the Atlantic sector of the Southern Ocean are an ideal area to study the role of dispersal of long pelagic larval stages and oceanography in population structuring.

Study species

To assess the role of larval dispersal and other life-history characteristics on gene flow between populations, notothenioid species with differing life strategies were chosen. The study species comprise 3 members of crocodile icefishes (Channichthyidae) and 5 members of cod icefishes (Nototheniidae; Table 2). Despite differences in the adult life strategies, the early developmental stages may vary markedly between species, although generally characterized by unusually prolonged pelagic developmental stages

MATERIAL AND METHODS

(Fig. 3). Additional biological features that may influence the dispersal during early life history stages are summarized in Table 2 and comprise adult spawning characteristics (place of spawning: inshore-offshore, time of spawning, fecundity, parental care), egg characteristics (size, buoyancy), larval characteristics (hatching time, size at hatch, growth rates, feeding mode, migration behaviour, pelagic larval phase duration) and juvenile characteristics (behaviour, pelagic phase duration). Despite the wealth of publications on notothenioid species, many biological characteristics of notothenioids are still insufficiently known. For example, descriptions of notothenioid larvae are available for only 52 recognised species (Koubbi *et al.* 1990; North & Kellermann 1990) and the most comprehensive information exists for species of commercial interest, such as *C. gunnari*.

Table 2 List of study species and selected biological characteristics

Family Species	Distribution	Depths range [m]	Adult habit	Generation time [years]	Spawning location	Abs. fecundity [No. of eggs]
Channichthyidae						
<i>C. aceratus</i>	Scotia Arc region, Bouvet Is ¹	5-770 ²	Benthic ³	6-8 ⁴	Nearshore ⁵	3,082-22,626 ^{3,6}
<i>C. gunnari</i>	Scotia Arc region, Bouvet Is, Kerguelen Is, Heard Is ¹	0-700 ¹	Benthopelagic ³	3 ⁴	Nearshore ^{5,12}	1,294-31,045 ^{14,15}
<i>C. rastrospinosus</i>	South Orkney Is, South Shetland Is, Antarctic Peninsula ¹	0-1,000 ²	Benthopelagic ²	4-8 ^{20,21}	Deeper shelf ^{2,22}	1,464-5,136 ⁸
Nototheniidae						
<i>G. gibberifrons</i>	Scotia Arc region ²⁷	5-750 ²⁷	Benthic ^{1,9}	6-8 ⁴	Deeper shelf ²⁸	21,699-143,620 ⁹
<i>L. larseni</i>	Sub-Antarctic Islands, Antarctic Peninsula ²⁷	30-550 ²⁷	Benthic ²⁷ , semi-pelagic ³¹	4-5 ³²	?	1,815-9,745 ^{4,8,29}
<i>L. squamifrons</i>	Sub-Antarctic islands, intervening seamounts of the Indian Ocean sector, Scotia Arc region, Bouvet Is ^{27,33}	5-670 ^{36,37}	Benthic ^{1,9}	7-9 ³⁸	Deeper shelf? ^{4,24,38}	38,000-280,000 ^{39,40}
<i>T. eulepidotus</i>	Circum-Antarctic: nearshore and continental shelf and nearby islands ²⁷	70-550 ²⁷	Benthopelagic ^{1,2}	7 ⁴¹	Shallow shelf ⁴¹	1,400-12,854 ⁴²
<i>T. newnesi</i>	Circum-Antarctic: shallow shelf waters of the continent and adjacent islands ²⁷	0-400 ⁴³	Benthocryopelagic ⁴⁴	?	Shallow shelf? ⁴⁵	2,300-12,200 ⁴⁶

Continued

Table 2 continued

Family Species	Eggs	Spawning time ^a	Hatching time ^a	Egg size [mm] ^b	Size at hatch [mm] ^c	Larval growth rate [mm/day] ^d	Larval vertical migrations ^e
Channichthyidae							
<i>C. aceratus</i>	Bottom, guarded ⁷	May-Jun ⁸	Aug-Nov ^{8,9}	3.9-4.7 ^{10,11}	15 ⁹	0.11-0.22 ^{9,12}	Diurnal ¹³
<i>C. gunnari</i>	Benthopelagic or pelagic ¹ , scattered	Jun-Jul ⁸	Jan-Mar ^{8,16}	2.0-4.0 ^{17,18}	11 ¹²	0.08-0.26 ^{12,19}	Diurnal ¹³
<i>C. rastrospinosus</i>	Demersal ³ , scattered	Mar-May ^{8,23}	Sep-Oct ^{16,24}	4.8-5.3 ^{22,25}	17 ¹⁶	0.21-0.37 ²⁶	?
Nototheniidae							
<i>G. gibberifrons</i>	Demersal ²⁹ , scattered	Aug-Sep ⁸	Nov-? ⁸	1.5-2.5 ⁵	7 ¹⁶	0.01-0.37 ^{12,30}	Partly diurnal ¹²
<i>L. larseni</i>	Demersal ^{33,34}	Jul-Sep ^{8,23}	Sep-Nov ^{16,26}	1.6-2.0 ³³	7 ²⁶	0.08 ²⁶	Diurnal ¹³
<i>L. squamifrons</i>	Demersal ²⁹ , scattered	Feb-Mar ^{23,26}	Apr-Jun ³⁸	1.3-1.7 ^{29,38}	7 ¹⁶	~0.08-0.14 ³⁸	?
<i>T. eulepidotus</i>	Substrate ⁴¹ , non-guarded	Apr ²³	Sep ⁴¹	2.8-4.3 ^{24,41}	11 ⁴¹	~0.08-0.16 ⁴¹	?
<i>T. newnesi</i>	?	Mar-May ^{46,47}	Sep-Nov ⁴⁵	?	9 ²⁶	0.37 ^{12,45}	?

^aSpawning and hatching times as reported for the southern Scotia Arc region (South Shetlands, Antarctic Peninsula). Times at other locations may differ.

^bMaximum values as reported at time of hatching.

^cLowest values reported in the literature.

^dDepending on larval size, increases over time (age). Values rounded.

^eVertical migration behaviour may vary with season and intensities differ between species.

¹Iwami & Kock (1990), ²Hureau (1985a), ³Permitin (1973), ⁴Kock & Kellermann (1991), ⁵Burchett *et al.* (1983), ⁶Lisovenko & Sil'yanova (1980), ⁷Detrich *et al.* (2005), ⁸Kock (1989), ⁹Ślósarczyk (1987), ¹⁰Kock (1979), ¹¹Everson (1968), ¹²North (1990), ¹³North (1988), ¹⁴Kock (1981), ¹⁵Lisovenko & Zakharov (1988), ¹⁶Kellermann (1989), ¹⁷Lönnerberg (1905), ¹⁸Olsen (1955), ¹⁹Duhamel (1987), ²⁰Kock (2005a), ²¹LaMesa & Ashford (2008a), ²²Kock (2005b), ²³Kock *et al.* (2000), ²⁴Kock & Jones (2005), ²⁵Gubsch (1982), ²⁶Kellermann (1986), ²⁷DeWitt *et al.* (1990), ²⁸Casaux *et al.* (1990), ²⁹Permitin & Sil'yanova (1971), ³⁰North (1991), ³¹Eastman (1993), ³²North & White (1987), ³³Andriashev (1965), ³⁴Konecki & Targett (1989), ³⁵Schneppenheim *et al.* (1994), ³⁶Duhamel (1981), ³⁷Ekau (1990), ³⁸Duhamel & Ozouf-Costaz (1985), ³⁹Lisovenko & Sil'yanova (1979), ⁴⁰Kock (1992), ⁴¹Ekau (1989), ⁴²Ekau (1991), ⁴³Tiedtke & Kock (1989), ⁴⁴Andriashev (1987), ⁴⁵Radtko *et al.* (1989), ⁴⁶Shust (1987), ⁴⁷Jones & Kock (2006)

The pelagic development of young notothenioid fishes, from hatching until settlement of juveniles, may take between 3 (*Artedidraco skottsbergi*) and 18 months (*Lepidonotothen larseni*) as reported from the Weddell Sea (Kellermann 1989). However, the actual larval stage, from hatching until all fin rays are formed, is shorter and takes between 2 (*D. eleginoides*, *Notothenia rossii*) and 6 months (*Notothenia*

coriiceps) at SG (North 2001) or 3 (*N. rossii*) and 15 months (*Notothenia cyanobranca*) at Kerguelen (Indian sector; Koubbi *et al.* 2009). For the study species, the total pelagic durations are between 8 (*G. gibberifrons*) and 18 months (*L. larseni*), whereas the larval stages last between 3 (*C. aceratus*, *C. gunnari*, *T. newnesi*) and 8 months (*L. larseni*), depending on latitudinal location. Figure 3 summarizes the pelagic phase of the investigated species, based on previous reviews and publications describing the occurrence of the respective developmental stages. The soft transition between stages is mostly caused by prolonged spawning periods of several months.

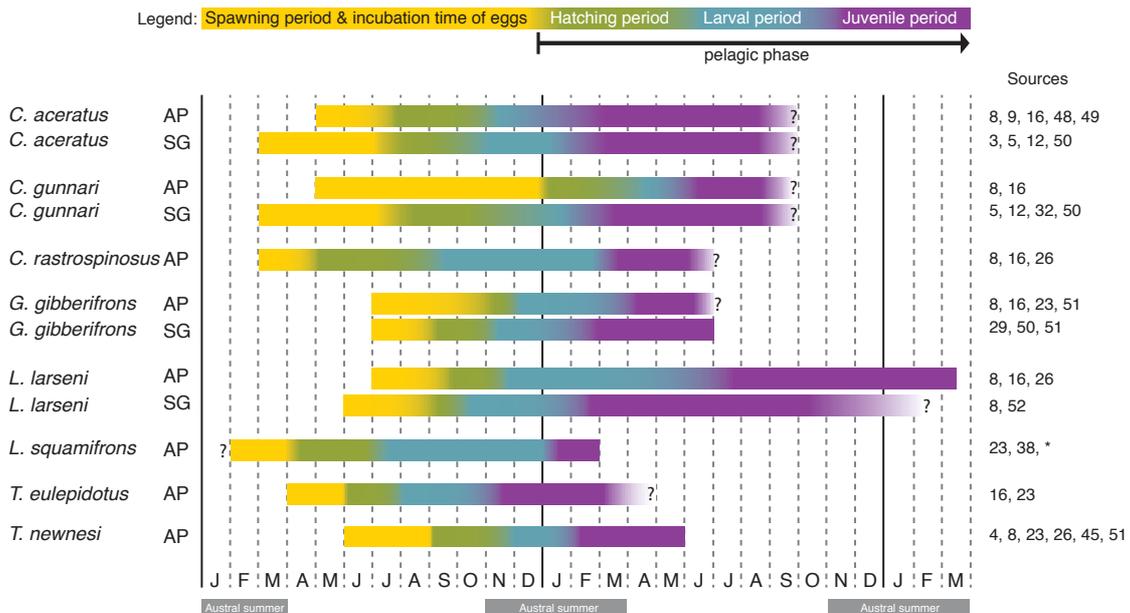


Fig. 3 Occurrence and duration of early developmental stages of the study species. AP=Antarctic Peninsula region, SG=South Georgia Island. Sources as in Table 2, with the following additions: ⁴⁸Kellermann & Ślósarczyk (1984), ⁴⁹Ślósarczyk (1986), ⁵⁰North (2001), ⁵¹Kellermann (1990), ⁵²Efremenko (1983). *see ²⁶ for deviating reports

Population genetic methods

Population genetics is concerned with the distribution of genetic variation among species, populations and individuals. It makes implicit use of the genetic signatures left by past demographic events, such as migrations, colonizations, population size changes, inbreeding, isolation or adaptation, to infer levels of gene flow on ecological and evolutionary time scales. Putting it the other way around, the population genetic signatures we find today in any given organism can be used to infer the ecological or evolutionary factors that caused them.

Molecular markers for population genetics

Different genetic markers change at different rates, allowing inferences about populations at varying time scales (Sunnucks 2000). The most widely applied molecular markers in population genetic studies are allozymes and DNA based markers (Avisé 2004). Since allozymes are enzymes likely to be affected by selection, inferences about demographic processes may be challenging, as the assumptions of the neutral theory of evolution would be violated. DNA based markers superseded

allozymes in population genetic studies when the polymerase chain reaction (PCR) was developed. Molecular markers based on DNA include amplified fragment-length polymorphisms (AFLP), restriction fragment-length polymorphisms (RFLP) and randomly amplified polymorphic DNAs (RAPD), but owing to their drawbacks like dominance and difficulties in reproducibility, their utilization has ceased (Schlötterer 2004). Instead, microsatellites, single nucleotide polymorphisms (SNP) and sequences of mitochondrial DNA (mtDNA) as well as nuclear DNA (nDNA) are now the markers of choice in population genetic studies (Avice 2004; Schlötterer 2004). However, with the increasing availability of high-throughput sequencing technologies, genomic approaches will revolutionize our understanding of population genetic structures in the future (Tautz *et al.* 2010; e.g. Pool *et al.* 2010). For examination of the population genetic structures of notothenioids in this thesis, I used microsatellites and mtDNA as molecular markers, as their specific properties allow to make highly resolved inferences about recent and historical levels of gene flow.

Microsatellites

Microsatellites are tandemly repeated, non-coding, nuclear DNA sequences of 1-6 base pairs (bp) in length (Goldstein & Schlötterer 1999). They are single-locus, co-dominant and highly polymorphic markers that are widespread in both eukaryotic and prokaryotic genomes (Oliveira *et al.* 2006). Their mutation rates range from 10^{-2} to 10^{-6} per generation and therefore exceed the base substitution rates of coding sequences (Schlötterer 2000). Their high levels of polymorphism can be used to identify single individuals (genetic fingerprinting; Jeffreys *et al.* 1985) and give high statistical power to assess the recent genetic relationship among individuals and populations on fine spatial and temporal scales (Jarne & Lagoda 1996), even when they are only weakly differentiated, as often found in marine organisms (Waples 1998). Hence, microsatellites are the current “marker of choice” for population genetic studies (Jarne & Lagoda 1996), especially in high gene flow species such as marine fishes (Hauser & Carvalho 2008).

Nonetheless, microsatellites have their own drawbacks. Reliable estimates of genetic differentiation require an adequate set of multiple loci (≥ 6 ; Koskinen *et al.* 2004) to overcome stochastic biases, which may result from evolutionary processes acting on each locus independently (Nei *et al.* 1977; Waples 1998). Unfortunately, the development of suitable loci is usually species-specific and takes considerable time and expenses (Zane *et al.* 2002). However, cross amplification among taxa is possible and may facilitate their use in closely related species. In the presented PhD thesis, I made use of previously published microsatellites isolated from channichthyid and nototheniid species (Papetti *et al.* 2006; Van Houdt *et al.* 2006; Susana *et al.* 2007) and cross amplified them, if possible, in all study species. Another drawback of microsatellites results from their mutation model and fast mutation rate. Changes in allele size of microsatellites are thought to occur under the step-wise mutation model (SMM; Kimura & Ohta 1973; Ohta & Kimura 1973) through slipped-strand mispairing during replication (Levinson & Gutman 1987), in which the repeat motif can either be added or lost. In combination with the fast mutation rate, the SMM can lead to homoplasmy and saturation of a locus, potentially underestimating genetic divergence (Nauta & Weissing 1996). In addition, technical problems with microsatellites may arise

during amplification in the form of null alleles, stuttering and large allele dropouts, but these can be accounted for (Van Oosterhout *et al.* 2004).

Mitochondrial DNA

The animal mitochondrial genome is a valuable tool for studying population genetics, phylogeography and phylogenetics (Awise *et al.* 1987; Awise 2004; Ballard & Whitlock 2004). The mtDNA is highly conserved, without introns and only short intergenic regions, thereby facilitating applications of markers in closely related species (Awise *et al.* 1987; Kocher *et al.* 1989; Gissi *et al.* 2008). It is much smaller than the nuclear genome and is usually a circular molecule of approximately 14-18 kilobases (kb) length. Since mtDNA is haploid and, with few exceptions, maternally inherited, it is assumed to lack recombination during replication (but see Piganeau *et al.* 2004) and its effective population size (N_e) is only $\frac{1}{4}$ than that of nDNA, thus, making it more prone to impacts of genetic drift (Wright 1931; Ohta 1972; Brown *et al.* 1979). As a consequence, as a single locus mtDNA provides a more sensitive tool to measure population differentiation than a single locus of nDNA (Birky *et al.* 1989). In addition, the average mutation rates of mtDNA genes are higher than those of nDNA genes (Brown *et al.* 1979), thereby allowing differentiations on lower taxonomic levels (Hwang & Kim 1999; but see Piganeau *et al.* 2004). Nonetheless, the mutation rates of mtDNA markers may vary considerably between species and genes (see e.g. Galtier *et al.* 2009). As mtDNA is only a small proportion of the whole genome and is effectively one locus, mtDNA genes might not necessarily depict species' history (Pamilo & Nei 1988). Thus, similar to microsatellites, caution must be exercised when inferences about gene flow and evolutionary histories are based on single mtDNA loci.

In the presented PhD thesis, I used previously published primers to amplify partial mtDNA sequences of cytochrome *b* and/or the displacement loop (D-loop) of the hypervariable control-region, to examine the demographic histories and population differentiations of notothenioids. Although the non-coding control region has the highest mutation rate of the mitochondrial genome (Brown 1985) and, thus, a high taxonomic resolution, both loci are highly suitable for inferences on the population level (Lee *et al.* 1995; Hwang & Kim 1999) and widely applied in population genetic studies of teleost fishes.

Testing the genetic population structures and demographic histories

Inferences about the population genetic structures and demographic histories of the study species were derived from a range of analyses based on microsatellite and mtDNA data. Fast evolving microsatellites were primarily employed to infer information about recent demographic events, whereas the slower substitution rate of mtDNA allowed testing for historical demographic events. These demographic events comprise population differentiations, levels and directionality of gene flow, population expansions or declines and the geographic distribution of genetic diversity. The applied methods partly vary between markers due to their specific attributes and are described in the following chapters in more detail (see e.g. Excoffier & Heckel 2006 for a review about the population genetic analyses and softwares available).

AIM AND OUTLINE OF THIS THESIS

The goal of this thesis was to investigate aspects of the evolutionary processes, which drove the diversification of notothenioid fishes in the Southern Ocean. In particular, the population genetic structures of sympatric species in the Atlantic sector of the Southern Ocean were compared to evaluate the role of pelagic larval dispersal on gene flow between populations. Gene flow by larval dispersal should result in genetic similarities among populations and counteract population divergences as well as allopatric speciation. By comparing sympatric species, species-specific traits that might lead to differences in the observed patterns can be assessed and inferences about the generality of the observed patterns can be made (chapters I, II and III). Moreover, it has been tested whether pelagization in notothenioids evolved independently within different lineages or whether it followed a single directional trend. The results of this study are helpful to characterize the evolution of notothenioids as a rare example of a marine adaptive radiation (chapter IV).

This thesis is divided into the following chapters:

Chapter I

Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica

This study compares the population genetic structure of seven notothenioid species inhabiting the southern Scotia Arc. The species comprise the three channichthyids *Chaenocephalus aceratus*, *Champscephalus gunnari*, *Chionodraco rastrospinosus* and the four nototheniids *Gobionotothen gibberifrons*, *Lepidonotothen squamifrons*, *Trematomus eulepidotus* and *T. newnesi*. Samples were collected from the shelves of South Orkney Islands as well as Elephant Island and the Antarctic Peninsula. The objective of this study is to assess the generality of population connectivity in the Southern Scotia Arc, an area influenced by two water regimes.

Chapter II

Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae)

This chapter extends the population genetic study of the channichthyids *C. aceratus* and *C. gunnari* (chapter I) by comparing specimens from four locations in the Atlantic sector of the Southern Ocean (Elephant Island, South Orkney Islands, South Georgia Island and Bouvet Island). The larger sampling area allows for estimating the gene flow by pelagic propagule dispersal over long geographic distances of deep-ocean areas. Again, the comparative approach is used to identify and validate species-specific and environmental factors involved in gene flow regulation and population divergence.

Chapter III

Population genetic structure of *Lepidonotothen larseni* revisited: *cyt b* and microsatellites suggest limited connectivity in the Atlantic sector of the Southern Ocean

In this study the population genetic structure of the nototheniid *Lepidonotothen larseni* has been examined. This species is characterized by the longest pelagic developmental phase known from notothenioids. Therefore, the population genetic structure of *L. larseni* can be used to validate previous findings of population connectivity in the Atlantic Sector of the Southern Ocean as well as to evaluate the role of pelagic larval dispersal and other factors for regulating gene flow.

Chapter IV

Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation

Notothenioids represent a species flock in the marine realm. To examine their evolution for characteristics of an adaptive radiation, 25 notothenioid species have been ecologically described with respect to their habitat and trophic level by stable isotopic analyses. Subsequent phylogenetic and disparity-through-time analyses have been applied to test whether ecological niches in the water column were colonized within a single directional trend or independently within several lineages, of which the latter would be characteristic for an adaptive radiation.

CHAPTER I

Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica

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Abstract

The Antarctic fish fauna is characterized by high endemism and low species diversity with one perciform suborder, the Notothenioidei, dominating the whole species assemblage on the shelves and slopes. Notothenioids diversified *in situ* through adaptive radiation and show a variety of life history strategies as adults ranging from benthic to pelagic modes. Their larval development is unusually long, lasting from a few months to more than a year, and generally includes a pelagic larval stage. Therefore, the advection of eggs and larvae with ocean currents is a key factor modulating population connectivity. Here, we compare the genetic population structures and gene flow of seven ecologically distinct notothenioid species of the southern Scotia Arc based on nuclear microsatellites and mitochondrial DNA sequences (D-loop/cytochrome *b*). The seven species belong to the families Nototheniidae (*Gobionotothen gibberifrons*, *Lepidonotothen squamifrons*, *Trematomus eulepidotus*, *T. newnesi*) and Channichthyidae (*Chaenocephalus aceratus*, *Champsocephalus gunnari*, *Chionodraco rastrospinosus*). Our results show low population differentiation and high gene flow for all investigated species independent of their adult life history strategies. In addition, gene flow is primarily in congruence with the prevailing ocean current system, highlighting the role of larval dispersal in population structuring of notothenioids.

Introduction

The Southern Ocean surrounding the Antarctic continent is a unique marine environment and its fish fauna is characterized by a high degree of endemism at low species diversity (Andriashev 1987; Eastman 1993; 2005). The northern boundary of the Southern Ocean is delimited by the Antarctic Convergence at about 50-60°S, which is marked by a sharp decrease of surface temperature from north to south and constitutes a thermal barrier for many marine organisms existing since approximately 22-25 My (Dayton *et al.* 1994; Eastman & McCune 2000). Beside its thermal isolation, the formation of deep circum-polar currents like the Antarctic Circumpolar Current (ACC) as well as large distances and deep ocean basins between the Antarctic continental shelf and those of adjacent continents form additional oceanographic, geographic and bathymetric barriers to migration and dispersal. The Antarctic

ichthyofauna as known today consists of 322 species from 50 families, with about 88% being endemic to the waters south of the Antarctic Convergence. A single group of fish, the perciform suborder Notothenioidei, dominates the species assemblage on the shelves and slopes (Andriashev 1987; Eastman 2005).

Notothenioids consist of 131 species in 8 families, with 104 species out of 5 of these families being endemic to the Antarctic region, where they constitute up to 77% of species diversity and 91% of biomass on the shelves and slopes of the continent and nearby islands (Eastman 2005). Together with members of the families Zoarcidae (24 species) and Liparidae (70 species), they comprise 88% of the Antarctic fish fauna (Eastman & McCune 2000). But unlike the latter two families that probably invaded the area from North Pacific waters, notothenioids diversified *in situ* in the course of an adaptive radiation (Eastman 1993; Clarke & Johnston 1996; Eastman & McCune 2000; Matschiner *et al.* 2011). This radiation is thought to have been triggered by the acquisition of antifreeze glycoproteins (AFGPs) that keep body fluids from freezing in the ice-laden waters of Antarctica (Cheng 1998; Matschiner *et al.* 2011). While the cooling of Antarctic waters as well as repeated expansions and retreats of the Antarctic ice sheet forced most Antarctic species of the Oligocene to either shift their distribution northwards or into deeper waters, or otherwise led to their extinction (Briggs 2003; Barnes & Conlan 2007), notothenioids radiated in the absence of competitors and filled vacant ecological niches (Eastman 1991). Although about one-half of today's species show a demersal life-style (as is also presumed for their ancestors), the notothenioid radiation is largely based on diversification related to niches in the water column (Eastman 1993). Since notothenioids lack a swim bladder, buoyancy for pelagization is gained through extended lipid depositions and reduced ossification (Eastman 1993), resulting in a variety of epibenthic, semipelagic, cryopelagic and pelagic life strategies. Adaptive radiations in the marine realm are rare compared to those known from freshwater systems like e.g. cichlid fishes in the Great Lakes of East Africa (Seehausen 2006; Salzburger 2009; Matschiner *et al.* 2010) or are camouflaged by subsequent dispersal in the course of evolution. The notothenioids therefore constitute a prime example for a marine adaptive radiation, making their ecological and morphological diversification a highly interesting target for evolutionary studies (Eastman 2000).

In contrast to the variety of adult life history strategies, the early larval development in notothenioids is always pelagic. Depending on the species and locality, the larval stage may be completed within two months after hatching or last more than one year (Kellermann 1986; 1989; North 2001). During this stage, strong currents like the clockwise ACC (Westwind Drift) or the counterclockwise Eastwind Drift along the Antarctic continent are likely to modulate larval dispersal away from the shelves into the open ocean. This may cause substantial losses to spawning populations and can lead to source-sink relationships by transporting larvae downstream towards distant shelf habitats. As White (1998) pointed out, the prolonged pelagic early life history strategy in notothenioids is at odds with a successful larval survival strategy. However, ichthyoplankton studies have shown that larval abundances for demersal species are surprisingly high on the shelf areas and decrease with increasing distance to the coast, despite the fact that their distributions are generally influenced by bathymetry, hydrography and seasonal events (Loeb *et al.* 1993; White 1998). Local retention mechanisms, such as gyres formed behind islands or shelf-break frontal systems limit

offshore transport of larvae (White 1998) and should increase genetic heterogeneity between populations of different shelves, thereby fostering speciation.

Population genetic studies in notothenioids provide evidence that the oceanography of the Southern Ocean indeed has large influence on the genetic structure of populations. Populations of species with circumpolar distributions as for example the pelagic Antarctic toothfish *Dissostichus mawsoni* and the more sedentary benthopelagic Patagonian toothfish *D. eleginoides* are not significantly differentiated over large parts of their distribution range (Smith & Gaffney 2005; Rogers *et al.* 2006). However, these results do not imply complete absence of genetic heterogeneity, as other genetic markers were able to resolve differentiations on varying geographic scales (Parker *et al.* 2002; Shaw *et al.* 2004; Kuhn & Gaffney 2008). Populations connected along currents like the ACC are often found to be more closely related than those located in proximity but separated across frontal systems like e.g. the Polar Front (Shaw *et al.* 2004; Rogers *et al.* 2006). Even strictly benthic species like the humped rockcod *Gobionotothen gibberifrons* show no signs of differentiation among populations separated geographically by nearly 2000 km and bathymetrically by deep basins (Matschiner *et al.* 2009). By combining oceanographic data with population genetic signatures, Matschiner *et al.* (2009) showed that dispersal of pelagic larvae in *G. gibberifrons* is most probably the major means of gene flow in this otherwise benthic species. The contribution of larval dispersal to population structure and in the long term on species' biogeography is still lively debated not only in notothenioids, but also in fish from warmer waters with distinctly shorter pelagic early life stages (e.g. Taylor & Hellberg 2003; Bay *et al.* 2006; Cowen & Sponaugle 2009). In particular, it is unclear what influence a prolonged pelagic early life stage and the existence of strong currents (which together should result in high levels of gene flow among populations) had on the adaptive radiation in notothenioids.

In this study, we compare the genetic signatures derived from microsatellites and mitochondrial (mt) DNA sequences of seven notothenioid species with different life history strategies and larval durations inhabiting the southern Scotia Arc (Table I-1). It is the first time that the genetic population structures based on two types of genetic markers are compared between multiple notothenioid species. We also included data obtained from drifting buoys to infer the influence of larval dispersal with oceanic currents on gene flow.

Table I-1 Distribution area and selected life history characteristics for all seven study species. Spawning and hatching times are for the region of South Shetland Islands and the Antarctic Peninsula

Family - Species	Distribution range	Depths range [m]	Adult habit	Eggs	Spawning time	Hatching time	Abs. fecund.	Gen. time [years]
Channichthyidae								
<i>C. aceratus</i>	Scotia Arc region, Bouvet Is ^a	5-770 ^b	benthic ^c	bottom, guarded ^d	May-Jun ^e	Aug-Nov ^{e,f}	3082-22626 ^{c,g}	6-8 ^h
<i>C. gunnari</i>	Scotia Arc region, Bouvet Is, Kerguelen Is, Heard Is ^a	0-700 ^a	benthopelagic ^c	benthopelagic or pelagic ^c , scattered	Jun-Jul ^e	Jan-Mar ^{e,i}	1294-31045 ^{i,k}	3 ^h
<i>C. rastrospinosus</i>	South Orkney Is, South Shetland Is, Antarctic Peninsula ^a	0-1000 ^b	benthopelagic ^b	demersal ^c , scattered	Mar-May ^{e,l}	Sep-Oct ^{l,m}	1464-5136 ^e	4-8 ^{n,o}
Nototheniidae								
<i>G. gibberifrons</i>	Scotia Arc region ^p	5-750 ^p	benthic ^{a,f}	demersal ^q , scattered	Aug-Sep ^e	Nov-? ^e	21699-143620 ^e	6-8 ^h
<i>L. squamifrons</i>	Sub-Antarctic Islands, intervening seamounts of the Indian Ocean sector, Scotia Arc region, Bouvet Is ^{b,f}	5-670 ^{s,t}	benthic ^{a,f}	demersal ^q , scattered	Feb-Mar ^{l,u}	Apr-Jun ^v	38000-280000 ^{w,x}	7-9 ^v
<i>T. eulepidotus</i>	Circum-Antarctic: nearshore and continental shelf and nearby islands ^p	70-550 ^p	benthopelagic ^{a,b}	substrate ^y , non-guarded	Apr ^l	Sep ^y	1400-12854 ^{h,z}	7 ^y
<i>T. newnesi</i>	Circum-Antarctic: shallow shelf waters of the continent and adjacent islands ^p	0-400 ^{aa}	benthocryopelagic ^{bb,cc}	?	Mar-May ^{dd,ee}	Sep-Nov ^{h,cc}	2300-12200 ^{dd}	?

^aIwami & Kock (1990), ^bHureau (1985a), ^cPermitin (1973), ^dDetrich *et al.* (2005), ^eKock (1989), ^fŚlósarczyk (1987), ^gLisovenko & Sil'yanova (1980), ^hKock & Kellermann (1991), ⁱKellermann (1989), ^jKock (1981), ^kLisovenko & Zakharov (1988), ^lKock *et al.* (2000), ^mKock & Jones (2005), ⁿKock (2005a), ^oLa Mesa & Ashford (2008a), ^pDeWitt *et al.* (1990), ^qPermitin & Sil'yanova (1971), ^rSchneppenheim *et al.* (1994), ^sDuhamel (1981), ^tEkau (1990), ^uKellermann (1986), ^vDuhamel & Ozouf-Costaz (1985), ^wLisovenko & Sil'yanova (1979), ^xKock (1992), ^yEkau (1989), ^zEkau (1991), ^{aa}Tiedtke & Kock (1989), ^{bb}Andriashev (1987), ^{cc}Radtke *et al.* (1989), ^{dd}Shust (1987), ^{ee}Jones & Kock (2006)

Our study area is the southern Scotia Arc, consisting of the tip of the Antarctic Peninsula (AP), South Shetland Islands (SSh) including Elephant Island (EI) located about 200 km north of the Peninsula and the South Orkney Islands (SO) approximately 420 km further east (Fig. I-1). The shelves of the AP and SSh/EI are separated by trenches of more than 500 m depth, whereas the SO shelf is separated by depths of 2000-3000 m. This region of the Seasonal Pack-Ice Zone, which is ice-free during the austral summer, is largely influenced by two water regimes: the ACC flowing eastward through the Scotia Sea in the north, and water originating from the Weddell Sea in the south (Whitworth *et al.* 1994).

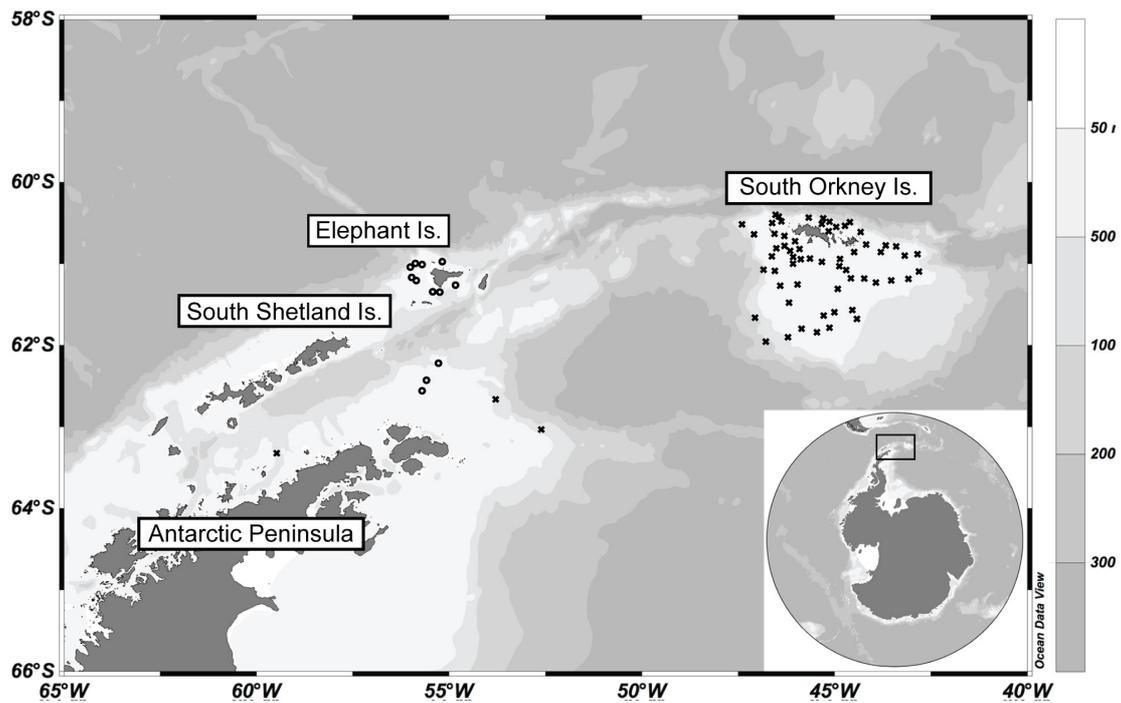


Fig. I-1 Study area and sampling localities in the southern Scotia Arc, Antarctica. Open circles = stations sampled during ANT-XXIII/8 2006, crosses = stations sampled during US AMLR 2009 finfish survey

The species investigated in this study comprise the three channichthyids *Chaenocephalus aceratus*, *Champscephalus gunnari* and *Chionodraco rastrospinosus* as well as the four nototheniids *Gobionotothen gibberifrons*, *Lepidonotothen squamifrons*, *Trematomus eulepidotus* and *T. newnesi*, which are all among the most abundant species in the southern Scotia Arc. Their life histories differ in a variety of traits (Table I-1): *C. aceratus*, *G. gibberifrons* and *L. squamifrons* are benthic species of which the two former ones spend most of their time resting on the bottom (Fanta *et al.* 1994; Kock & Jones 2005). *C. gunnari*, *C. rastrospinosus* and *T. eulepidotus* show a benthopelagic life style preying for food in the water column (Rutschmann *et al.* 2011). Vertical migrations between near bottom layers during the day and sub-surface waters during the night are known from several notothenioids including *C. gunnari* (Kock & Everson 1997). *T. newnesi* shows a remarkable feeding plasticity and is considered a benthocryo-pelagic species (La Mesa *et al.* 2000). It is generally benthivorous, but carries out vertical migrations during summer feeding on pelagic organisms. In winter, when there is sea ice cover, *T. newnesi* switches to a cryo-pelagic mode and feeds on organisms under the ice (Daniels 1982; Casaux *et al.* 1990; La Mesa *et al.* 2000).

The aim of this study is to investigate the role of prolonged larval stages on the genetic population structures of notothenioids. Dispersing larvae should result in high similarities between geographically separated populations even in those species that show a more sedentary habit as adults. We hypothesize that a high connectivity between populations is a general characteristic of notothenioids when hydrographic currents connect geographically separated populations.

Methods

Sampling

The specimens analysed in this study were collected during expedition ANT-XXIII/8 aboard RV Polarstern in December 2005-January 2006 and U.S. AMLR (United States Antarctic Marine Living Resources) survey in February-March 2009 aboard RV Yuzhmorgeologiya. Sampling sites were located on the shelves at the tip of the AP, EI (the most easterly island of the SSh), and SO to their east (Fig. I-1). Muscle tissue of the specimens was stored in 95% ethanol. DNA was extracted using two different protocols depending on the cruise. All 2005-2006 samples were extracted with the BioSprint 96 workstation (QIAGEN) following the manufacturer's protocol, whereas DNA from the AMLR 2009 samples was extracted by incubating muscle tissue in 300 μ l 5%-Chelex solution containing 12 μ l Proteinase K (20 mg/ml) for 3 hours at 55 °C, followed by a denaturation step of 25 minutes at 98 °C in a thermomixer.

MtDNA sequencing and data analysis

Depending on the amplification success, mitochondrial gene sequences were either generated from the control region/D-loop (*C. aceratus*, *C. gunnari*, *C. rastrospinosus* and *G. gibberifrons*) or cytochrome *b* (*cyt b*; *L. squamifrons*, *T. eulepidotus* and *T. newnes*). Partial D-loop or *cyt b* were amplified with the primers LPR-02 and HDL2 (Derome *et al.* 2002) or NotCytbF and H15915n (Matschiner *et al.* 2011), respectively. For amplification of the D-loop region 2 μ l template DNA were mixed with 7.5 μ l Taq PCR Master Mix (QIAGEN), 0.5 μ l of each 10 μ M primer, 1 μ l bovine serum albumin and 14.5 μ l sterile water. A simplified hot start at 94°C for 2 min initiated the PCR profile followed by 35 cycles of 94°C for 30 s, 52°C for 30 s and 72 °C for 90 s. Thermocycling finished with a final elongation step at 72°C for 7 min. Cytochrome *b* sequences were amplified using Phusion polymerase (Finnzymes) following the manufacturer's manual at 57°C annealing temperature. PCR products were purified by adding 2 μ l ExoSAP-IT (USB Corporation) to 5 μ l PCR product following the manufacturer's instructions.

Sequencing PCR with forward primers was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). After purification with BigDye XTerminator (Applied Biosystems), sequencing products were run on an AB3130x/ Genetic Analyzer (Applied Biosystems). Sequences were automatically aligned with CodonCode Aligner (CodonCode Corp.), inspected by eye and corrected manually if necessary.

Basic sequence properties as well as intraspecific sequence polymorphisms measured as nucleotide diversity (π) and haplotype diversity (h) were examined with DNASP 5.10 (Librado & Rozas 2009). Population structure among sampling localities was assessed by analysis of molecular variance (AMOVA) based on traditional *F*-statistics and calculated with 16000 permutations as implemented in ARELQUIN 3.5 (Excoffier & Lischer 2010).

Phylogenetic trees were inferred with the Maximum Likelihood (ML) method implemented in PAUP* 4.0a112 (Swofford 2003), whereby models of sequence evolution were selected according to BIC (Posada 2008). On the basis of these phylogenies, haplotype genealogies were constructed following the method described in Salzburger *et al.* (2011).

The statistical power of both mtDNA and microsatellites to detect significant genetic differentiation between populations was tested with POWSIM 4.0 (Ryman & Palm 2006) using both the χ^2 test and Fishers exact test. Various levels of differentiation (measured as F_{ST} in the range from 0.001 to 0.08) were tested by combining different effective population sizes (N_e) and times since divergence (t). In addition, POWSIM allows calculating the α error (type I error), which is the probability of rejecting the null hypothesis of genetic homogeneity although it was true by drawing the alleles directly from the base population ($t=0$).

Microsatellite genotyping and data analysis

In addition to mtDNA sequences we included data of twelve previously published microsatellites in our analyses. Microsatellites Cr15, Cr38, Cr127, Cr236, Cr259 were originally isolated from *Chionodraco rastrispinosus* (Papetti *et al.* 2006), Trne20, Trne35, Trne37, Trne53, Trne55, Trne66 from *Trematomus newnesi* (Van Houdt *et al.* 2006) and Ca26 from *Chaenocephalus aceratus* (Susana *et al.* 2007). Marker sets for each species were composed of 8 to 10 microsatellites, depending on the amplification success (Online Resource 1).

All amplification reactions contained 5 μ l Multiplex Master Mix (QIAGEN), 0.2 μ l of each 10 μ M primer, 0.8 μ l template DNA and water added to a final volume of 10 μ l. All reactions contained primers for up to 3 microsatellites of which the forward primers were fluorescently labelled. The PCR profile was 95°C for 15 min followed by 35 cycles of 94°C for 30 s, 59°C for 90 s, 72°C for 90 s and final elongation at 72°C for 10 min. Fragment lengths were determined with GeneScan LIZ500 size standard (Applied Biosystems) on an AB3130xl Genetic Analyzer (Applied Biosystems) and scored with GENEMAPPER 4.0 (Applied Biosystems).

Alleles were automatically binned with TANDEM (Matschiner & Salzburger 2009) and subsequently converted with CONVERT (Glaubitz 2004). We used a 3D factorial correspondence analysis as implemented in GENETIX (Belkhir *et al.* 2001) to visualize outliers in the data. Suspicious individuals with potential errors in the data were either corrected, re-genotyped or otherwise completely removed from the dataset.

Microsatellites were tested for the presence of null alleles, stuttering and large allele dropout with MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004). Allele size ranges, Hardy-Weinberg-Equilibrium (HWE) per population and species as well as genotypic linkage-disequilibrium between loci were examined in GENEPOP 4.0.10 (Raymond & Rousset 1995).

The number of alleles per sample and locus were calculated with FSTAT (Goudet 1995; 2001) using the implemented rarefaction method to account for differences in sample sizes.

Population structure was assessed performing an AMOVA based on traditional F -statistics as implemented in ARLEQUIN 3.5 (Excoffier & Lischer 2010). To account for biases attributed to null alleles present in the data, F_{ST} values were also calculated excluding null alleles with FREENA (Chapuis & Estoup 2007). ARLEQUIN was further used to test loci for HWE in each population. Molecular diversities were measured as mean number of pairwise differences and average gene diversity. The Garza-Williamson index was calculated as indicator of recent demographic history. This statistic is sensitive to population size reductions since a recent bottleneck usually reduces the number of alleles more than the allele size range hence leaving "vacant"

positions in between. The index is supposed to be very small in populations having experienced a recent bottleneck and close to one in stationary populations (Garza & Williamson 2001). In a similar approach populations were tested for a recent reduction in effective population size with BOTTLENECK 1.2.02 (Piry *et al.* 1999) using the two-phase model (TPM) of mutation with 10% infinite allele model (IAM) and 90% single step mutation model (SMM) with a variance of 15% and 1000 iterations. Significance was tested with the Wilcoxon signed-rank test. In addition, we used the mode-shift indicator as qualitative descriptor of allele frequency distribution. A normal L-shaped distribution indicates populations in mutation-drift equilibrium whereas a shifted mode is a sign for recent bottlenecks. We considered that a population truly underwent a bottleneck if this was indicated by all three measurements (low Garza-Williamson index, significant Wilcoxon signed-rank test and mode shift).

To further analyse the structure of populations and to identify clusters of individuals, we used a Bayesian approach based on the genotypes of microsatellites as implemented in STRUCTURE 2.3.1 (Pritchard *et al.* 2000). For every data set we ran simulations for up to 6 clusters (k) with 20 iterations each. The parameters were set to 10000 steps of burn-in period and 100000 MCMC replications thereafter. The admixture model was used as we expected a weak population structure as often encountered in marine fishes with long larval phases (and indicated by our calculated F_{ST} values). Alpha was inferred from an initial value of 1.0 and correlated allele frequencies with lambda set to 1.0. In a second approach we ran the program with the same settings but incorporated *a priori* information about the sampling sites to help with clustering. We followed the method of Evanno *et al.* (2005) to calculate Δk as indicator of the most likely number of clusters. The power of microsatellites to detect significant population structure was tested in the same way as the mtDNA sequences using POWSIM 4.0 (Ryman & Palm 2006), but in the F_{ST} range of 0.001-0.01.

Isolation-with-migration analyses

The directionality and extent of gene flow between AP/EI and SO populations was examined for the combined data set of mtDNA sequences and nuclear microsatellites with the isolation-with-migration (IM) model as implemented in IMA2 (Hey & Nielsen 2007). The Hasegawa-Kishino-Yano (HKY) model of sequence evolution was applied to mitochondrial sequences, and a stepwise mutation model (SMM) was assumed for all microsatellite loci. Inheritance scalars of 0.25 and 1 were assigned to mtDNA and microsatellite loci, respectively. Appropriate prior parameter ranges were determined in a series of initial runs. We chose wide population size parameter ranges $\Theta_1, \Theta_2, \Theta_A \in (0,500]$, a divergence time prior $t \in (0,10]$, and exponential migration rate priors m_1, m_2 with distribution means of 5.0. Each run included 80 Metropolis-coupled Markov chains. Geometric heating scheme parameters were chosen to optimize chain swap rates, and set to $h_a = 0.97$ and $h_b = 0.86$. Per population comparison, ten replicate runs were conducted for 4.5 million generations, discarding the first 500 000 generations as burn-in. The consistent genealogies of run replicates were jointly analysed in IMA2's 'Load Trees'-mode. The migration rates per year (M) were calculated from the resulting parameters $M = \Theta \times m/2$ under consideration of the species' generation times.

Drifter analysis

In order to compare directionality of gene flow and ocean currents, we also analysed trajectories of satellite-tracked drifting buoys (hereafter called drifters) of the Global Drifter Program (Lumpkin & Speer 2007), following established protocols (Matschiner *et al.* 2009). Interpolated drifter data was downloaded from <http://www.aoml.noaa.gov/phod/dac/gdp.html> for all drifters passing the AP/SO region (55-65°S, 40-60°W) between 15 February 1979 and 31 December 2009. Three polygons were mathematically defined to encompass the AP, EI, and SO shelf areas at 500m depth. Polygon vertices were 65.0°S, 60.0°W; 65.0°S, 54.5°W; 63.9°S, 54.1°W; 63.3°S, 52.3°W; 62.2°S, 54.3°W; 62.1°S, 55.4°W; and 63.3°S, 60°W for the AP, 62.7°S, 60°W; 61.4°S, 54.1°W; 61.1°S, 53.9°W; 60.8°S, 55.7°W; and 61.9°S, 60.0°W for EI, and 62.3°S, 44.8°W; 61.5°S, 44.0°W; 61.2°S, 42.4°W; 60.8°S, 42.8°W; 60.3°S, 46.6°W; 60.5°S, 47.3°W; and 61.6°S, 46.9°W for SO. Trajectories of drifters passing these polygons were plotted for 90 days, starting with the day of departure from one of the polygons (Fig. I-2).

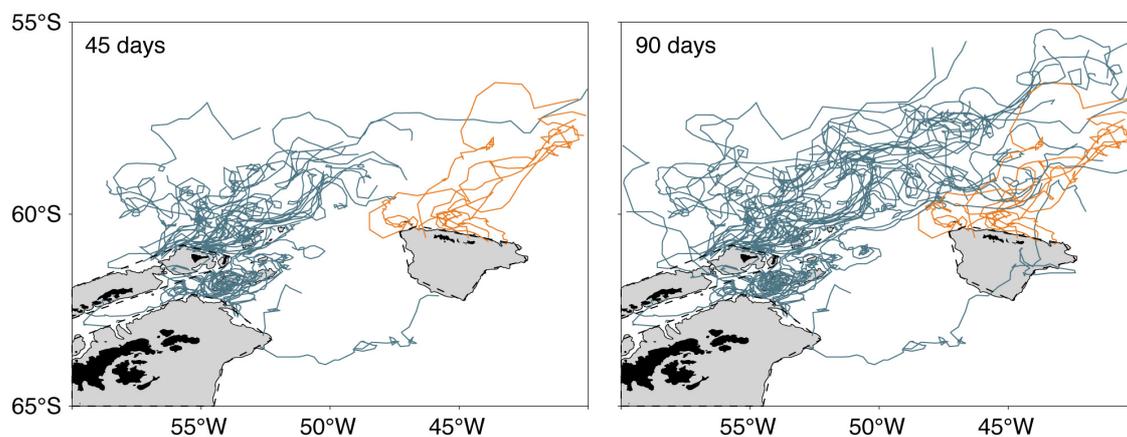


Fig. I-2 Trajectories of surface drifters 45 and 90 days after leaving the shelves (500 m isobaths contour line) of South Shetland/Antarctic Peninsula (blue) and South Orkney Islands (yellow)

Results*mtDNA – genetic diversity and demographic history*

The number of individuals successfully sequenced varied between species and ranged from 49 (*L. squamifrons*) to 194 (*G. gibberifrons*; Online Resource 2). The D-loop region could be amplified in 4 out of 7 species (*C. aceratus*, *C. gunnari*, *C. rastrispinosus* and *G. gibberifrons*). For the remaining three species *L. squamifrons*, *T. eulepidotus* and *T. newnesi* amplification of the D-loop region consistently failed. Therefore, we amplified a part of the mt cyt *b* gene as an alternative population genetic marker. Although we are aware that the use of two mtDNA markers is not optimal and hinders a direct comparison between datasets without restrictions, we found both markers to resolve the population genetic structures in a similar fashion. All sequences generated were submitted to GenBank (Accession nos JN241690-JN241831) and a list of haplotypes per shelf area can be found in Online Resource 3. Although a clear relationship between sample size or sequence length and the number of haplotypes could generally be expected, no such trend was found ($R^2=0.06$ and $R^2=0.18$, respectively). Similarly, the genetic diversities varied between species, but irrespective of the locus or phylogenetic relationship. Remarkably, the three high-Antarctic species

C. rastrispinosus, *T. eulepidotus* and *T. newnesi* had the highest nucleotide diversities (each >0.004) and a similar pattern arose from the haplotype diversities. On the population level, most samples from the AP region had higher diversities than SO samples. Unique to *C. rastrispinosus* the diversity for SO was higher than for AP.

mtDNA – genetic population structure

Power analyses revealed poor capabilities of the mtDNA sequences to detect subtle differentiations among populations for both D-loop and *cyt b*. High probability to detect true differentiation as low as $F_{ST} = 0.01$ was only evident for *G. gibberifrons* (Online Resource 4). Finescale genetic differentiations measured as pairwise F_{ST} between sampling localities (AP, EI and SO) were neither high nor significant for any species (data not shown). We therefore combined the AP and EI samples to test differentiation along the prevailing current from the AP/EI region towards SO. After pooling, differentiation between sample localities remained non significant and ranged between -0.003 ($p = 0.48$) for *C. gunnari* and 0.04 ($p = 0.06$) for *C. rastrispinosus* (Table I-2). The constructed haplotype genealogies support these findings and reflect genetic diversity rather than differentiations between localities (Fig. I-3).

Table I-2 Population differentiation (F -statistics) between Antarctic Peninsula and South Orkney Islands samples based on mtDNA and microsatellites

	Species						
	<i>C. aceratus</i>	<i>C. gunnari</i>	<i>C. rastrispinosus</i>	<i>G. gibberifrons</i>	<i>L. squamifrons</i>	<i>T. eulepidotus</i>	<i>T. newnesi</i>
mtDNA							
F_{ST}	-0.0056	-0.0027	0.0440	0.0027	-0.0087	-0.0053	-0.0146
Microsatellites							
F_{ST}	0.0088*	0.0226**	-0.0004	0.0002	-0.0015	-0.0035	0.0067
F_{ST} ENA	0.0088	0.0219	0.0021	0.0002	-0.0006	-0.0018	0.0027
F_{IS}	0.022*	0.034*	0.182**	0.062**	0.074**	0.161**	0.006
F_{IT}	0.03*	0.06*	0.18*	0.06*	0.07*	0.16*	0.01

ENA = excluding null alleles.

ENA results without significances, AMOVA significances: * $p \leq 0.05$, ** $p \leq 0.01$

Microsatellites – genetic diversity and demographic history

Microsatellites were successfully genotyped for 56 (*C. rastrispinosus*) to 125 individuals (*C. aceratus*) per species (Online Resource 1). Individuals with missing data at one or more loci were excluded from the analyses. Significant genotypic linkage disequilibrium between loci was limited to the pair of Cr236 and Trne20 in *C. gunnari* ($p = 0.03$) (data not shown). On the species level, for all but *T. newnesi* the hypothesis of HWE could be rejected (data not shown) and null alleles might be present at least at one locus in every pertained species (Online Resource 5). On the population level, HWE is only evident for both *T. newnesi* populations and the EI samples of *C. aceratus* (Online Resource 6). The null alleles found in this study are probably a result from the cross-species amplifications with microsatellite primers that were originally isolated from other notothenioid species. Among all species, *G. gibberifrons* had the highest (24.0 ± 14.0) and *C. gunnari* the lowest (10.7 ± 7.7) allelic richness (data not shown).

Reductions in population size were examined for species and populations. On the species level, none showed signs of a bottleneck concurrently in all three indicators examined (Online Resource 7). The probability of heterozygosity excess was significant only in *C. gunnari* and *C. rastrispinosus* (both $p < 0.01$), while the Garza-Williamson index was low (0.48 ± 0.21) only in *T. newnesi*. For every species, the

frequency distributions of alleles were normal L shaped. On the population level, the only sample, which showed evidence for a recent bottleneck in all tests, was *T. newnesi* from the SO shelf. In *C. gunnari* from SO heterozygosity excess and mode shift were evident, but the Garza-Williamson index was relatively high (0.63 ± 0.20).

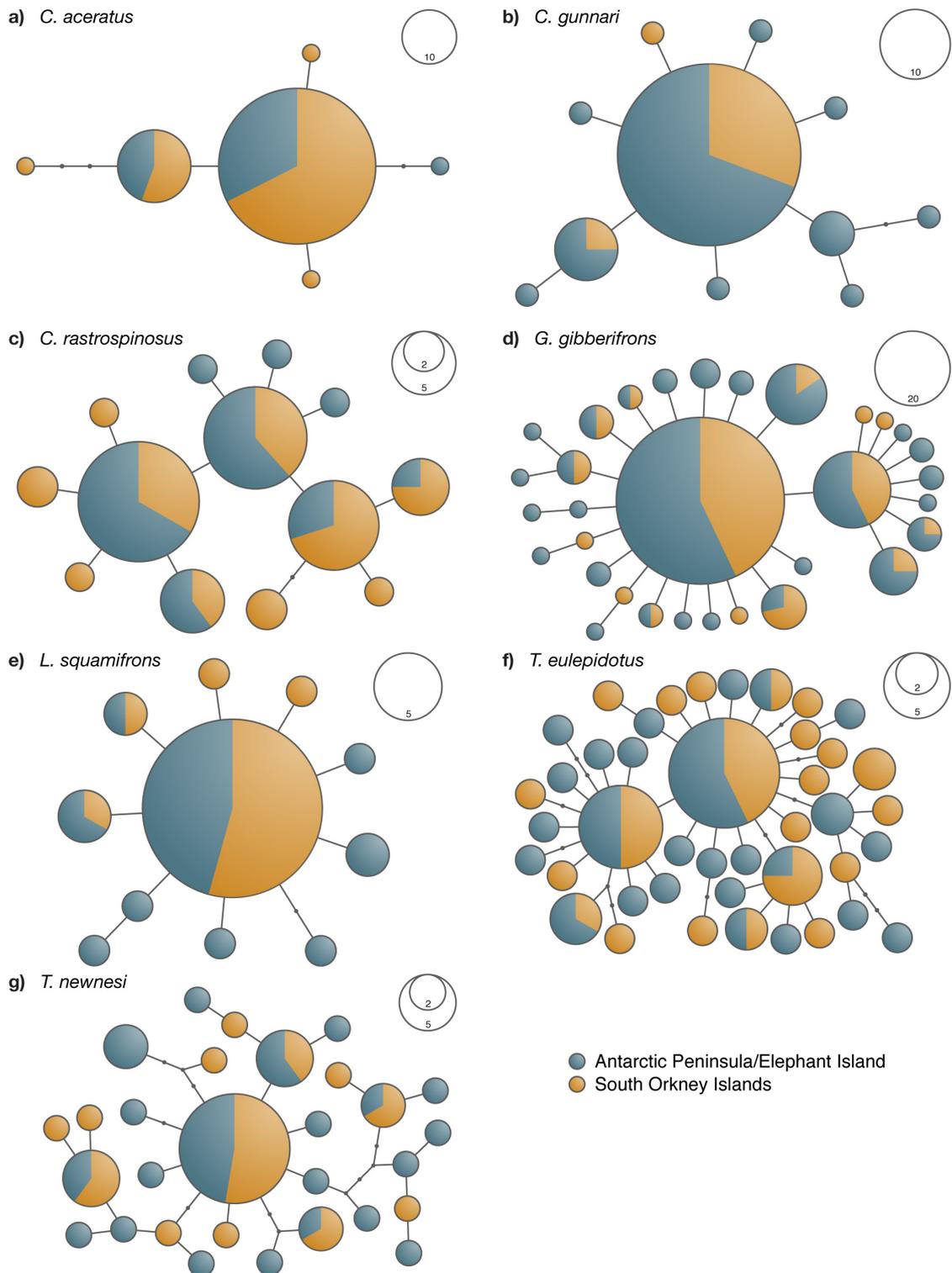


Fig. I-3 Unrooted haplotype genealogies based on mitochondrial D-loop (a-d) and *cyt b* sequences (e-g). Radii reflect number of individuals

Microsatellites – genetic population structure

The power of the microsatellites to detect significant population differentiation was generally much higher than for mtDNA sequences. Simulations suggest an average probability of 97% to detect a true differentiation of $F_{ST} = 0.01$ resulting from both the χ^2 (SD = 0.03) and Fisher exact (SD = 0.02) tests (Online Resource 4).

Population structure based on microsatellites was assessed with AMOVA (Table I-2) and in a Bayesian approach (Table I-3). Similar to mtDNA sequences, we found no significant differentiation between sampling localities of the AP and EI region (data not shown) and therefore combined these samples to test the genetic structure along the current system. In congruence with mtDNA data, differentiations between AP/EI and SO populations were minor in every species. Indeed, F_{ST} values were mostly one order of magnitude lower than for mtDNA except for *C. aceratus* and *C. gunnari*. These two species were also the only ones showing significant differentiation. Excluding null alleles from analyses did not alter the previous findings of low differentiation in any species, although it also changed F_{ST} values in some cases by one order of magnitude. The overall genetic variation can rather be explained by larger differences between individuals than between populations.

Table I-3 Number of clusters with highest mean posterior probability inferred from Bayesian analyses as indicated by ΔK (maximum ΔK in parenthesis)

	Species						
	<i>C. aceratus</i>	<i>C. gunnari</i>	<i>C. rastrospinosus</i>	<i>G. gibberifrons</i>	<i>L. squamifrons</i>	<i>T. eulepidotus</i>	<i>T. newnesi</i>
INA	1 (30.6)	1 (48.8)	2 (147.3)	1 (130.9)	1 (4.5)	2 (31.1)	1 (20.0)
INA locprior	1 (8.1)	1 (63.2)	1 (93.6)	1 (5.7)	1 (7.2)	1 (23.1)	1 (44.1)
ENA	1 (12.9)	1 (7.3)	2 (52.4)	1 (157.8)	1 (9.0)	1 (11.1)	no NA
ENA locprior	1 (88.7)	1 (69.3)	1 (63.0)	1 (9.8)	2 (1.0)	1 (12.9)	no NA

INA = including null alleles, ENA = excluding null alleles (according to MicroChecker 2.2.3), locprior a priori information of sampling sites incorporated in analysis

Table I-4 Isolation-with-migration results reflecting parameter bins with highest posterior probabilities (High Points)

Species	Parameter							
	t0	Θ_1	Θ_2	Θ_A	m1	m2	M1	M2
<i>C. aceratus</i>	0.129	7.25	14.74	141.20	0.05	4.55	0.18	33.56
<i>C. gunnari</i>	0.443	4.75	2.75	52.25	0.05	2.65	0.12	3.64
<i>C. rastrospinosus</i>	0.001	74.75	23.75	47.25	0.05	0.05	1.87	0.59
<i>G. gibberifrons I</i>	0.001	71.25	17.25	21.25	0.05	0.05	1.78	0.43
<i>G. gibberifrons II</i>	0.001	125.80	21.25	46.75	0.05	0.05	3.15	0.53
<i>L. squamifrons</i>	0.001	35.00	15.00	55.00	0.05	0.05	0.875	0.375
<i>T. eulepidotus</i>	0.193	25.00	375.00	35.00	0.05	0.05	0.625	9.375
<i>T. newnesi</i>	0.002	37.50	16.50	360.50	3.85	0.05	72.188	0.413

t0 = time since divergence, Θ_1 = effective AP/EI populations size, Θ_2 = effective SO populations size, Θ_A = effective ancestral populations size, m1 = migration rate from SO to AP/EI, m2 = migration rate from AP/EI to SO, M1 and M2 = accordant population migration rates

Drifter analyses

Between 15 February 1979 and 31 December 2009, a total of 73 drifters crossed the AP, EI, and SO shelf areas (Fig. I-2). Out of 64 drifters leaving the AP and EI shelf areas, one reached the SO shelf after 46 days, which is shorter than most notothenioid larval stages. This drifter had left the AP shelf at its easternmost end on 30 January 2008 and crossed the Philip Passage perpendicular to Weddell Sea Deep Water outflow (Heywood *et al.* 2002). This may have been facilitated by wind-driven surface

currents, and indicates that dispersal of passive particles from AP/EI to SO shelf areas is possible albeit comparatively rare. Most other drifters leaving the AP/EI shelf areas took a more northerly route in the ACC's main current and missed the SO shelf. Drifters leaving the SO shelf area dispersed in a north-eastern direction, and none of them reached the AP/EI region.

Microsatellites and mtDNA – isolation-with-migration model

The IM model was used to test whether gene flow is unidirectional with the prevailing current as expected by gene flow through passive larval dispersal or bidirectional through gene flow by adult migration. In congruence with our *F*-statistics, IMA2 did only resolve gene flow in *C. aceratus* and *C. gunnari*, for which significant population differentiation could be found. The remaining species either showed no migration or their time since divergence was about zero (Table I-4). Hence, IMA2 was not able to calculate migration rates when gene flow is too high and there is no clear separation between populations. In the following, we therefore present and discuss the isolation-with-migration results only for *C. aceratus* and *C. gunnari*. The migration parameters m_1 (from SO to AP/EI) and m_2 (from AP/EI to SO) derived from the models indicate asymmetric gene flow with the current in both species (Table I-4; Online Resource 8). Calculated population migration rates (effective rates at which genes come into populations per generation; M_1 , M_2) show a distinct pattern of gene flow with the current from AP/EI to SO, but are negligible from SO to AP/EI. With regard to private alleles in microsatellites, which may indicate a source-sink relationship by holding more unshared alleles in populations that act as sinks (and are usually found downstream), there is no uniform coherence between the direction of gene flow according to IMA2 estimates and the number of private alleles per population.

Discussion

Genetic structure and diversity

The results obtained in the present study show low or non-existing genetic population differentiation for seven of the most abundant notothenioid species in the southern Scotia Arc. These results seem to be independent of the adult life history strategies but are accordant to expectations for marine species with long pelagic larval stages. Both types of genetic markers used in this study show either no or only weak genetic structure among populations for all studied species. None of the obtained haplotype genealogies based on mtDNA shows a clear separation between localities (Table I-2) and *F*-statistics for both markers revealed, if at all, only minor differentiations. The only significant differentiations were found within the channichthyids *C. aceratus* and *C. gunnari*, while *C. rastrospinosus* and the nototheniid populations of *G. gibberifrons*, *L. squamifrons*, *T. eulepidotus* and *T. newnesi* generally lack a clear genetic structure. However, the channichthyid differentiations are not congruent among marker types and can therefore be used to discriminate between short and long term population dynamics. Microsatellites evolve faster than mtDNA and their higher diversities allow to infer present connectivity patterns better than mtDNA, which carries a longer-persisting signature of past events (Selkoe & Toonen 2006). Historical or long existing barriers to gene flow are hence more likely to be detected with mtDNA sequences. In our data, significant differentiations with microsatellite markers between samples from the AP/EI region and SO were only detected in the benthic *C. aceratus* and the benthopelagic *C.*

gunnari. However, the differentiation observed in both species is only minor. Our cluster analyses with the software STRUCTURE suggest in both cases the existence of only one population in the study area. As indicated by the low genetic differentiations measured by F_{ST} , STRUCTURE may not have been able to resolve the populations as separate entities. The small differentiations detected with mtDNA indicate homogenizing gene flow between AP/EI and SO. The highest differentiation of mtDNA sequences was found in the benthopelagic *C. rastrispinosus* ($F_{ST} = 0.04$), but even for this species, the differentiation was not significant. Although the differentiation measured by microsatellite data was close to zero in *C. rastrispinosus*, which indicates that the true differentiation might not be as high as measured with mtDNA, their power to detect subtle population differences was rather low in this case. Differences in the cluster analyses between runs including and excluding information about sampling localities, which result in one and two clusters respectively, suggest that a possible differentiation in *C. rastrispinosus* is more likely based on unrecognized factors (as e.g. sampling of cohorts) than geographically separated populations. In the four nototheniid species *G. gibberifrons*, *L. squamifrons*, *T. eulepidotus* and *T. newnesi*, the genetic differentiations between localities are generally low and not significant giving little evidence for the existence of barriers to gene flow between shelf areas in the southern Scotia Arc.

Our results add new information on the connectivity of nototheniid populations in the area. Previous studies on nototheniid population structures along the Scotia Arc were based on parasite infestation rates (Kock & Möller 1977; Siegel 1980a), morphometric characters (Kock 1981) and more recently on a variety of genetic markers (e.g. Papetti *et al.* 2009). Studies based on parasite infestation rates revealed differences between populations north and south of the ACC in *C. gunnari* and *C. aceratus*, but not among populations of *C. aceratus* and *C. rastrispinosus* along the southern Scotia Arc (Kock & Möller 1977; Siegel 1980a; b). For *C. gunnari*, four different populations had been identified based on morphometric characters from South Georgia, SO, SSh and EI (Kock 1981). Significant differentiation was also confirmed with genetic marker sets for *C. gunnari* populations north and south of the ACC (Kuhn & Gaffney 2006), but the island shelves along the southern Scotia Arc had not yet been compared. A recent publication on the genetic population structure of *C. aceratus* from the southern Scotia Arc based on microsatellites is in agreement with our results and shows that the populations on both sides of the Philip Passage are weakly, but significantly differentiated, while migration is still evident (Papetti *et al.* 2009). In our study, significant genetic differentiation in *C. aceratus* was detected with microsatellites, but not with mtDNA sequences. Hence, in the long run the migration rates in this sedentary species seem to be high enough (e.g. >1 individual per generation; Mills & Allendorf 1996) to counteract genetic drift and population differentiation. Microsatellite fragment lengths are susceptible to changes in frequency with every generation and F -statistics rather show a captured moment of population structure than history. Overall, the generally low genetic differentiation suggests high connectivity between populations in the study area.

With regard to the mutation rates of microsatellites and mtDNA, it seems to be counterintuitive that genetic differentiations between populations measured with fast evolving microsatellites are smaller than for slower evolving mtDNA as observed in *C. rastrispinosus* and to a lesser extent in *G. gibberifrons* and *T. newnesi*. A similar

pattern was already observed in populations of *D. eleginoides* from Heard and McDonald Island, Macquarie Island and South Georgia (Appleyard *et al.* 2002). Different genetic patterns between maternally inherited markers such as mtDNA and bi-parentally inherited markers such as nuclear microsatellites can arise from sexual differences in spawning behaviour or simply by genetic drift and population bottlenecks. Maternally inherited mtDNA is more affected by the latter two than nuclear DNA, since its effective population size is only one quarter that of nuclear DNA, which may result in higher divergences for mtDNA. Of the three species showing this discrepancy between markers in our study, spawning migrations are only known for *C. rastrorpinosus*, which migrates to shelf waters of 200-300 m depth to spawn (Kock 1989). However, it is currently unknown whether *C. rastrorpinosus* prefers specific spawning grounds and whether sexual differences in migration behaviour exist (Kock 2005b), leaving this explanation to speculation. By contrast, for all three species, at least one bottleneck indicator suggests that populations might have undergone a reduction in population size. We therefore cannot exclude bottlenecks and genetic drift as possible reasons for this pattern. However, these unusual differences between markers do not affect our general finding of low or absent population structure in the southern Scotia Arc.

According to genetic studies from the last decade, the levels of population differentiation in notothenioids vary widely and do not show a universal pattern. These studies primarily focused on single species targeted by the fisheries industry in the Southern Ocean like *C. gunnari*, *D. eleginoides* or *D. mawsoni* (Smith & McVeagh 2000; Appleyard *et al.* 2002; Parker *et al.* 2002; Shaw *et al.* 2004; Smith & Gaffney 2005; Kuhn & Gaffney 2006; Rogers *et al.* 2006; Kuhn & Gaffney 2008) and their results depend on the types of genetic markers used (allozymes, RAPDs, microsatellites, mt and nuclear DNA sequences). Although most species show genetic differences between single populations, a lack of differentiation over large parts of even circum-Antarctic distribution ranges is evident. In this regard, the adult life history strategy and habit seems to play only a minor role for the genetic structuring of populations, since this pattern can be found not only in active pelagic swimmers as the Antarctic silverfish *Pleuragramma antarcticum* (Zane *et al.* 2006), but also in strictly benthic species as *G. gibberifrons* (Matschiner *et al.* 2009). It seems plausible that gene flow between populations of notothenioids is primarily based on larval dispersal. Hence, oceanography is a key factor influencing the structure of notothenioid populations.

Migration and gene flow

The genetic population structures found in this study revealed ongoing gene flow in the southern Scotia Arc for all study species, regardless of their adult habit or larval stage duration. For the channichthyids *C. aceratus* and *C. gunnari* unidirectional gene flow from AP/EI to SO coincides with the current pattern of the ACC in the study area. Advection of eggs and pelagic larvae with the current is therefore a likely key feature connecting populations of notothenioid species that are bound to shelf and slope habitats during their adult stage. The pelagic phase of larvae takes about 400-500 days in *C. aceratus* (La Mesa & Ashford 2008b), but exact data for *C. gunnari* is still lacking. At South Shetlands *C. gunnari* spawns its eggs freely into the water in June-July and hatching occurs in January-March, leaving a time frame of about 8 months in which eggs may be dispersed. Young juveniles caught with a bottom trawl at South Georgia

were estimated to be 3.5-6.5 months old (North 2005). If growth rates are comparable between regions the pelagic phase of *C. gunnari* may hence be completed within 11.5 months (or about 350 days). Since both species have an absolute fecundity in the same order of magnitude the amount of larvae reaching the other shelf should be comparable or slightly less in *C. gunnari*. But as our results show, the migration rate of *C. aceratus* is about twice as high as in *C. gunnari*. This may indicate that the eggs of *C. gunnari* stay near the ground and are less advected by the current than the larvae, reducing the time of possible dispersal and raising genetic heterogeneity between populations. To this point, the role of putative differences between egg and larval dispersal on population structuring remains an open question and needs further investigations.

Overall, we conclude that the general pattern of weak or absent genetic population structure found for notothenioids with differing adult life history strategies is primarily based on the characteristic they all have in common, the pelagic larval phase. With regard to the adaptive radiation of notothenioids it remains unclear how species evolve rapidly while differentiation is counteracted by high gene flow through larval dispersal. It seems likely that notothenioid speciation events are restricted to periods when larval dispersal is hindered as e.g. during extended ice-coverages in the course of glacial maxima. To further examine the role of larval dispersal on population structure and adaptive radiation in notothenioids it is necessary to conduct further comparative population genetic studies over wider geographic scales including hydrographic features like the ACC.

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Electronic supplementary material

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CHAPTER II

Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae)

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Abstract

Dispersal via pelagic larval stages plays a key role in population connectivity of many marine species. The degree of connectivity is often correlated with the time that larvae spend in the water column. The Antarctic notothenioid fishes develop through an unusually long pelagic larval phase often exceeding one year. Notothenioids thus represent a prime model system for studying the influence of prolonged larval phases on the population structure in otherwise demersal species. Here, we compare the population genetic structure and demographic history of two sub-Antarctic crocodile icefish species (*Chaenocephalus aceratus* and *Champscephalus gunnari*) from the Scotia Arc and Bouvet Island in the Atlantic sector of the Southern Ocean to delineate the relative importance of species-specific, oceanographic and paleo-climatic factors to gene flow. Based on 7-8 microsatellites and 2 mitochondrial DNA markers (cytochrome *b*, D-loop) we detect pronounced population genetic structures in both species (AMOVA FSTs range from 0.04-0.53). High genetic similarities were found concordantly in the populations sampled at the Southern Scotia Arc between Elephant Island and South Orkney Islands, whereas the populations from Bouvet Island, which is located far to the east of the Scotia Arc, are substantially differentiated from those of the Scotia Arc region. Nonetheless, haplotype genealogies and Bayesian cluster analyses suggest occasional gene flow over thousands of kilometres. Higher divergences between populations of *C.gunnari* as compared to *C. aceratus* are likely caused by lower dispersal capabilities and demographic effects. Bayesian Skyline Plots reveal population size reductions during past glacial events in both species with an estimated onset of population expansions about 25,000 years ago.

Introduction

Larval dispersal plays a key role in population connectivity of marine organisms and is central to our understanding of marine ecology (Cowen & Sponaugle 2009). Drivers influencing dispersal can either be physical or biological, and their combination results in a diverse range of connectivity patterns between species (Cowen & Sponaugle 2009; Hellberg 2009). The extent of connectivity, and hence gene flow, between populations has great implications for evolution and the ability of species to cope with changing environmental conditions or impacts from human exploitation (Palumbi 1994; Botsford *et al.* 2001).

Gene flow through larval dispersal is especially important for benthic and demersal species, which are strictly confined to suitable habitats, such as shelf areas, during their adult stage. For these sedentary species, the degree of population connectivity may be greatly enhanced through a pelagic larval phase in the water column, where they are subject to passive drift by ocean currents. Therefore, the pelagic larval duration (PLD) is often thought to be a main factor determining the structure of marine populations (Shanks 2009) and the time spent adrift during larval stages was repeatedly found to be positively related to dispersal capability (e.g. Riginos & Victor 2001; Shanks *et al.* 2003). However, a growing number of studies indicate that populations can be highly structured even in species with long PLDs (e.g. Levin 2006; Marko *et al.* 2007; White *et al.* 2010).

Antarctic fishes of the perciform suborder Notothenioidei are ideal model organisms to study the influence of long PLDs on the connectivity of geographically distant populations. More than 100 notothenioid species from six families inhabit the shelves and slopes of the Antarctic continent and sub-Antarctic islands, where they are the dominant taxon in terms of diversity, abundance and biomass (Eastman 2005). Besides their ability to express antifreeze glycoproteins that prevent their body fluids from freezing in the cold Antarctic waters, they are also characterized by lacking a swim bladder (Eastman 1993). Although predominantly demersal, some notothenioids gained buoyancy by morphological adaptations, such as increased lipid depositions and reduced skeletal mineralizations, leading to benthic, epibenthic, semi-pelagic and pelagic life-history strategies (Eastman 1993). Despite differing in adult life-history strategies, the larval development of notothenioids is always pelagic and unusually long. The early developmental stages, from hatching of larvae until juveniles return to a demersal life, may take about 9 months, as for example in the benthic *Gobionotothen gibberifrons*, or last about one year, as in the benthopelagic *Champscephalus gunnari* and benthic *Chaenocephalus aceratus* (Kellermann 1989; Kock & Kellermann 1991; North 2001). During this time, larvae may be dispersed with the currents and gene flow by larval dispersal may counteract population divergence even between distantly located islands. The presumably great potential for dispersal of notothenioid larvae is further enhanced through the world's largest current system, the Antarctic Circumpolar Current (ACC). The ACC flows in easterly direction without interruption around Antarctica and is relatively well studied owing to its importance in driving the global ocean circulation (Efremenko 1983; Ślósarczyk 1986; Eastman 1993; Loeb *et al.* 1993; Orsi *et al.* 1995; Schmitz 1995; Rintoul 2000). The high velocity and net volume of the ACC are supposed to have a homogenizing effect on populations around Antarctica (Eastman 1993), and larvae dispersed with the ACC can significantly enhance the connectivity of populations, as indicated by similar species assemblages on islands connected by the ACC (e.g. Permitin 1973).

Previous population genetic studies revealed a diverse pattern of connectivity between notothenioid populations. In the Atlantic sector of the Southern Ocean (Fig. II-1), genetic homogeneity was found in populations of the benthic nototheniid *G. gibberifrons* along the Scotia Arc islands (Matschiner *et al.* 2009). A similar pattern arose previously from allozymes in the benthopelagic *C. gunnari* (Carvalho & Warren 1991 in Kock & Jones 2005) and from enzyme polymorphism data in the benthic

Lepidonotothen squamifrons (Schneppenheim *et al.* 1994), but later studies based on SNPs and mtDNA revealed differences between northern (South Georgia (SG), Shag Rocks) and southern (South Shetlands, SSh) populations in both cases (Kuhn & Gaffney 2006; Jones *et al.* 2008). Significant population differentiation between SG and SSh was also inferred for the nototheniid *Trematomus hansonii* (Van de Putte *et al.* 2012). More strikingly, preliminary studies found no significant differentiation between populations of the northern Scotia Arc islands and Bouvet Island (BO), which are separated by ~2300 km of deep-ocean, in 5 nototheniid species, including *C. gunnari* and *L. squamifrons* (Kuhn & Gaffney 2006; Jones *et al.* 2008). However, a significant population differentiation between these areas in the large *Dissostichus eleginoides* suggests that genetic homogeneity is not a general rule (Rogers *et al.* 2006). On a more regional scale, genetic homogeneity is the predominant pattern found in 8 nototheniid species in the southern Scotia Arc area (SSh including Elephant Island (EI), northern Antarctic Peninsula (AP) and South Orkney Islands (SO)) (Papetti *et al.* 2007; Van de Putte *et al.* 2012; Papetti *et al.* 2012; Damerou *et al.* 2012). Notable exceptions are the two icefishes *C. aceratus* and *C. gunnari*, which showed a slight, but significant differentiation between EI and SO populations based on microsatellites (Papetti *et al.* 2009; 2012; Damerou *et al.* 2012).

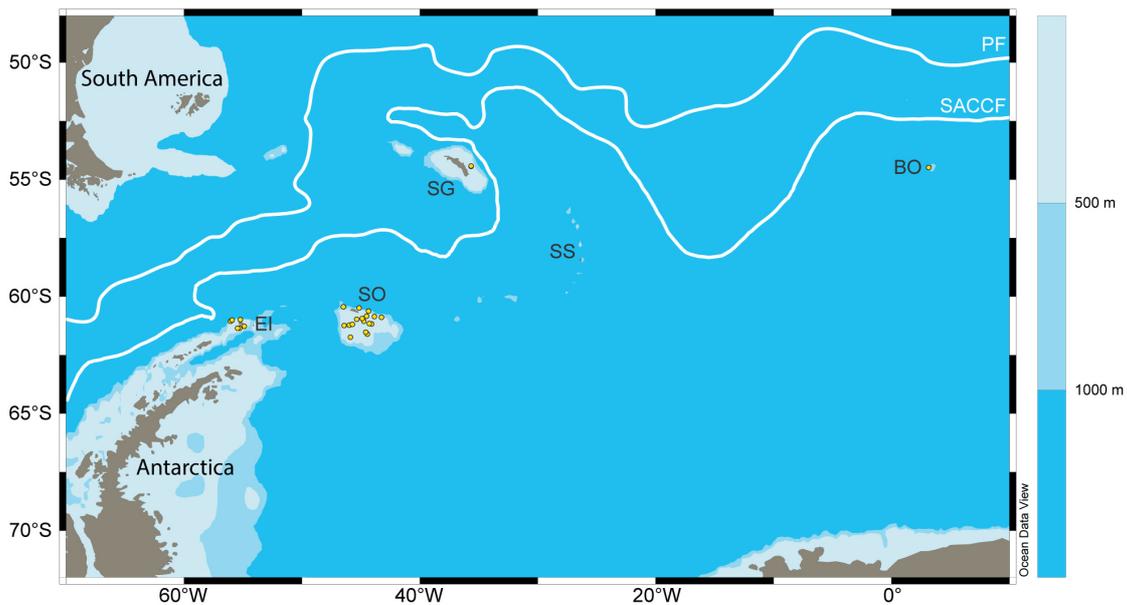


Fig. II-1 Map of the study area including sampling locations (yellow dots) and fronts (white lines). EI=Elephant Island, SO=South Orkney Islands, SG=South Georgia, SSh=South Sandwich Islands, BO=Bouvet Island, PF=Polar Front, SACCF=Southern Antarctic Circumpolar Current Front

In this study, we investigate the role of pelagic larval dispersal in two sub-Antarctic species of crocodile icefishes (Channichthyidae) in a comparative approach based on multiple markers to assess the effect of long PDs and strong currents on the genetic structures of populations which are geographically separated by large distances of deep ocean. We sampled Blackfin icefish (*Chaenocephalus aceratus*) and Mackerel icefish (*Champscephalus gunnari*) at four locations in the Southern Ocean (SG, SO, EI and BO) to encompass areas connected by the ACC in a west-east direction (Fig. II-1). Although connected by the ACC, SG is separated from the other islands by the Southern ACC Front (SACCF), which runs south of the island and might act as oceanographic barrier for gene flow.

Both study species inhabit the shelves and slopes down to about 700-770 m depth (Iwami & Kock 1990; Kock & Stransky 2000). Adult movement between islands is constrained by ocean basins of 3000 m depths and deeper. *C. aceratus* is a sedentary benthic species with a generation time of 6-8 years, while *C. gunnari* is an active benthopelagic species with a generation time of only 3 years (Kock & Kellermann 1991). Both species spawn inshore between austral autumn and winter (Kock 1989; 1992), and the pelagic development until juveniles return to a demersal life lasts throughout the winter of the following year (North 2001). Hence, the early development of both species is similar and takes more than one year. Nonetheless, the early developments differ in the following traits: *C. aceratus* is a nest-guarding species and larvae hatch after about 3-4 months of incubation, while *C. gunnari* spawns non-guarded demersal eggs and hatching occurs after about 5-6 months (Kock & Kellermann 1991; Everson *et al.* 2001; Detrich *et al.* 2005 and references therein). Consequently, eggs of *C. gunnari* are more likely to be dispersed with the currents, resulting in a higher potential for gene flow. Data on the exact larval duration is scarce, but the occurrence of transforming larvae and juveniles in summer and late summer suggests that the larval phase of both species is completed within 2-6 months, depending on location (e.g. Kellermann & Ślósarczyk 1984; North 1990; 2001). However, regional differences of spawning times and rates of development leave the aforementioned times as approximations (Everson 1984; North 2005). For example, latitudinal shifts in spawning times were reported for both species with a 1-2 months spawning delay in the southerly populations of SO and SSh as compared to SG (Kock & Kellermann 1991). Regardless of regional differences, the PDs are unusually long in both species.

We used population genetic tools to examine the connectivity between islands based on the hypothesis of genetically similar populations when larvae are dispersed successfully along the prevailing current of the ACC. Alternatively, larval behaviour and oceanographic retention mechanisms or barriers may substantially limit gene flow between localities (Loeb *et al.* 1993; Young *et al.* 2012). Given the equally long PDs of both species, observed differences between population genetic structures are likely caused by species-specific traits including demographic effects. We additionally assessed the demographic histories of both species to identify how species responded to major glacial cycles with Bayesian Skyline Plots.

Material and Methods

Sample collection

Samples of *C. aceratus* and *C. gunnari* were collected by bottom trawls between 131 and 398 m depth at four island shelves in the Atlantic Sector of the Southern Ocean: 1. Elephant Island (EI), 2. South Orkney Islands (SO), 3. South Georgia (SG) and 4. Bouvet Island (BO; Fig. II-1). Muscle tissues for DNA extraction were collected from adult specimen during three expeditions: From December 2005-January 2006 during ANT-XXIII/8 aboard RV Polarstern, in February-March 2009 during the U.S. AMLR (United States Antarctic Marine Living Resources) finfish survey aboard RV Yuzhmorgeologiya and from February-April 2011 during ANT-XXVII/3 again aboard RV Polarstern. All tissue samples were stored in 95% ethanol prior to DNA extraction.

Mitochondrial DNA sequencing

DNA extraction, amplification of partial cytochrome *b* (cyt *b*) and D-loop mitochondrial DNA (mtDNA) as well as sequencing followed previously established protocols (Damerau *et al.* 2012). D-loop sequences of samples from EI and SO generated as part of a former study (Accession Nos JN241690-JN241705) were complemented with new data from all four study locations. Cyt *b* sequences were newly amplified for all individuals. The additional sequencing for this study was done on a 3500 Genetic Analyzer (Applied Biosystems) following the manufacturers protocol. All sequences were automatically aligned with CODONCODE ALIGNER (CodonCode Corp.) and alignments were manually optimized.

Microsatellite genotyping

Nine microsatellite markers were genotyped in both species using previously published primers: Ca26 was originally isolated from *C. aceratus* (Susana *et al.* 2007), Cr15, Cr38, Cr127, Cr236, Cr259 from *Chionodraco rastrospinosus* (Papetti *et al.* 2006) and Trne20, Trne53, Trne66 from *Trematomus newnesi* (Van Houdt *et al.* 2006). Microsatellites Cr38 and Cr236 failed to amplify in *C. aceratus* whereas Ca26 was omitted in *C. gunnari*, resulting in 7 and 8 markers in the final dataset, respectively. PCR setups were used as in Damerau *et al.* (2012) and genotypes were subsequently assessed on a 3500 Genetic Analyzer (Applied Biosystems). We used GENEMAPPER v4.0 (Applied Biosystems) to score allele sizes before binning them automatically with TANDEM (Matschiner & Salzburger 2009).

mtDNA data analyses

DNA polymorphism was examined as haplotype (H_D) and nucleotide diversities (π) for every species and sampling site using DNASP v5.10 (Librado & Rozas 2009). Haplotype genealogies were constructed from phylogenetic trees as suggested by Salzburger *et al.* (2011). We selected the model of sequence evolution according to the Bayesian Information Criterion (BIC) in JMODELTEST (Posada 2008) and applied it to the inferred Maximum Likelihood (ML) tree, as implemented in PAUP* v4.0a112 (Swofford 2003).

Genetic differentiations within and among populations were measured by Analysis of Molecular Variance (AMOVA) based on traditional F -statistics with 16,000 permutations in ARLEQUIN v3.5 (Excoffier & Lischer 2010). The statistical power of the mtDNA sequences to detect significant genetic differentiations between populations was examined with POWSIM v4.0 (Ryman & Palm 2006) as described in Damerau *et al.* (2012) for F_{ST} values between 0.01-0.8.

The demographic histories were examined with Tajima's D and Fu's F neutrality tests (Tajima 1989; Fu 1997) using 1,000 simulation steps as implemented in ARLEQUIN v3.5. In these tests, negative significant deviations from neutrality might indicate either balancing selection or population expansion. In addition, population size changes were reconstructed from Bayesian Skyline Plots (BSPs) using BEAST v1.74 (Drummond *et al.* 2005; 2012) and visualized in TRACER v1.5 (Rambaut & Drummond 2007). Substitution models of sequence evolution and their parameters were chosen as described above. Substitution rates for each species and marker were inferred by constructing phylogenetic trees with publicly available sequences from Genbank (for accession numbers see Table II-S1, Supporting information) that were time-calibrated

as described in Matschiner *et al.* (2011), using their channichthyid and bathydraconid divergence date estimates as temporal constraints. The derived branch rates per site and million years (myr) for *cyt b* and D-loop were 0.0108 and 0.0149 in *C. aceratus* and 0.0107 and 0.0184 in *C. gunnari*, respectively. The substitution rates were set to the strict molecular clock model and estimated from a normal distribution with means based on the derived branching rates, variation of 0.01 and upper boundary of 0.08. To account for uncertainties with the exact substitution rate we further explored substitution rates of 0.02, 0.04 and 0.08 substitutions/site/myr. The time to the most recent common ancestor (tMRCA) was directly inferred from the tree prior. Each analysis was first run at species level (including sequences of all specimens of a species) for 50 million Markov Chain Monte Carlo (MCMC) generations with a burn-in of 10%. Parameters were checked for convergence and effective sampling sizes (ESS) of at least 100 in TRACER v1.5. We then applied the same analysis at population level (including sequences of all specimens of a population) and ran two replicate analyses to validate the consistency of the results. If parameters did not converge or ESS were less than 100, we extended the MCMC until these criteria were fulfilled.

Microsatellite data analyses

Microsatellite loci were tested for the presence of null-alleles with MICROCHECKER v2.2.3 (Van Oosterhout *et al.* 2004). The number of alleles per locus and sample site was calculated with FSTAT (Goudet 2001) using the implemented rarefaction method to allow for differences in sample sizes. Allele size ranges, genotypic linkage disequilibrium between loci and Hardy-Weinberg-Equilibrium (HWE) per species and population were analysed with GENEPOP v4.0.10 (Raymond & Rousset 1995).

The genetic population differentiation was assessed with AMOVA in ARLEQUIN v3.5 analogous to the mtDNA data, but including within individual variation. Where applicable, we repeated the AMOVA excluding null allele loci (ENA). Moreover, we calculated Jost's D as alternative measure of population differentiation with GENEALEx 6.5 (Peakall & Smouse 2012 and references therein). In contrast to F_{ST} measures, which are based on the variation of allele frequencies, Jost's D measures the proportion of allele variation among populations and may deliver more accurate results of population differentiations when heterozygosities are high (Jost 2008). In addition, we used a Bayesian approach to identify clusters of individuals with the software STRUCTURE v2.3.1 (Pritchard *et al.* 2000). For each species, 20 runs with 10^3 steps of burn-in and 10^5 MCMC replications were conducted for up to 6 admixing clusters (K). In a second pass, we incorporated information of individual sampling locations to help STRUCTURE with clustering.

Drifter trajectories

Trajectories of satellite-tracked drifting buoys (drifters) of the Global Drifter Program (Lumpkin & Pazos 2007) as means to compare the directionality of ocean currents with gene flow/larval dispersal were plotted following the protocols of Matschiner *et al.* (2009). For all drifters passing the regions of BO, EI, SG and SO between 15 February 1979 and 31 December 2009, interpolated data was downloaded from <http://www.aoml.noaa.gov/phod/dac/gdp.html>. Four polygons were defined to encompass the respective shelf areas at 500 m depth. Polygon vertices were 54.100°S 2.563°E, 54.100°S 4.025°E, 54.750°S 4.025°E, 54.750°S 2.563°E for BO, 61.668°S

54.642°W, 61.668°S 56.511°W, 60.890°S 56.511°W, 60.890°S 54.642°W for EI,

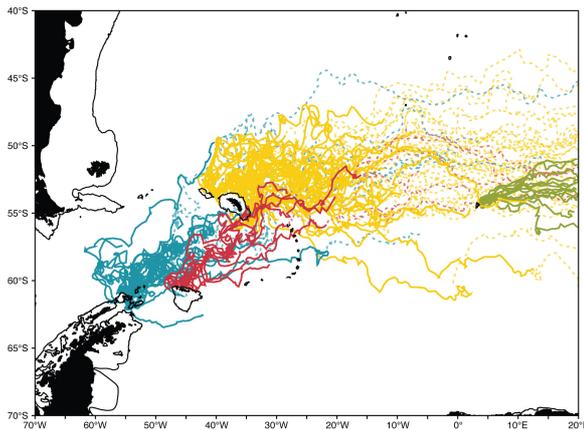


Fig. II-2 Trajectories of drifters leaving the 500m-isobath of the study areas Elephant Island (blue), South Orkney Islands (red), South Georgia (yellow) and Bouvet Island (green) for the duration of 105 (solid lines) and 400 (dashed lines) days

54.517°S 39.440°W, 53.735°S

39.440°W, 53.415°S 37.584°W,

53.735°S 35.779°W,

55.065°S 34.355°W, 55.513°S

35.779°W for SG and 62.249°S

44.839°W, 61.451°S 43.951°W,

61.207°S 42.374°W, 60.767°S

42.848°W, 60.308°S 46.621°W,

60.466°S

47.308°W, 61.634°S 46.861°W for

SO. The trajectories of drifters

leaving these polygons were plotted

for 105 and 400 days – the span of

potential pelagic larval dispersal in

our study species (Fig. II-2).

Results

mtDNA data

Overall, 247 *C. aceratus* and 192 *C. gunnari* specimens were analysed for this study.

Sequence alignments of *cyt b* generated from 241 *C. aceratus* individuals covered 697 base pairs (bp) while D-loop sequences from 179 individuals covered 333 bp. Less individuals could be sequenced of *C. gunnari*, resulting in alignments of 634 bp from 114 specimens for *cyt b* and 337 bp from 148 specimens for D-loop (Genbank accession numbers KC907761-KC907839). The genetic diversity for *cyt b* was about twice as high as in D-loop in both species (Table II-S2, Supporting information). Overall, *C. aceratus* showed slightly higher genetic diversity indices than *C. gunnari*. Populations at SG were genetically more diverse than at BO, EI and SO. These trends are also well reflected in the constructed haplotype genealogies (Fig. II-3). For *cyt b* no genetic variation was found at BO in *C. gunnari*. In general, POWSIM suggested higher power of *cyt b* than D-loop sequences to detect population differentiations, and this difference is more pronounced in *C. aceratus* (Fig. II-S1, Supporting information).

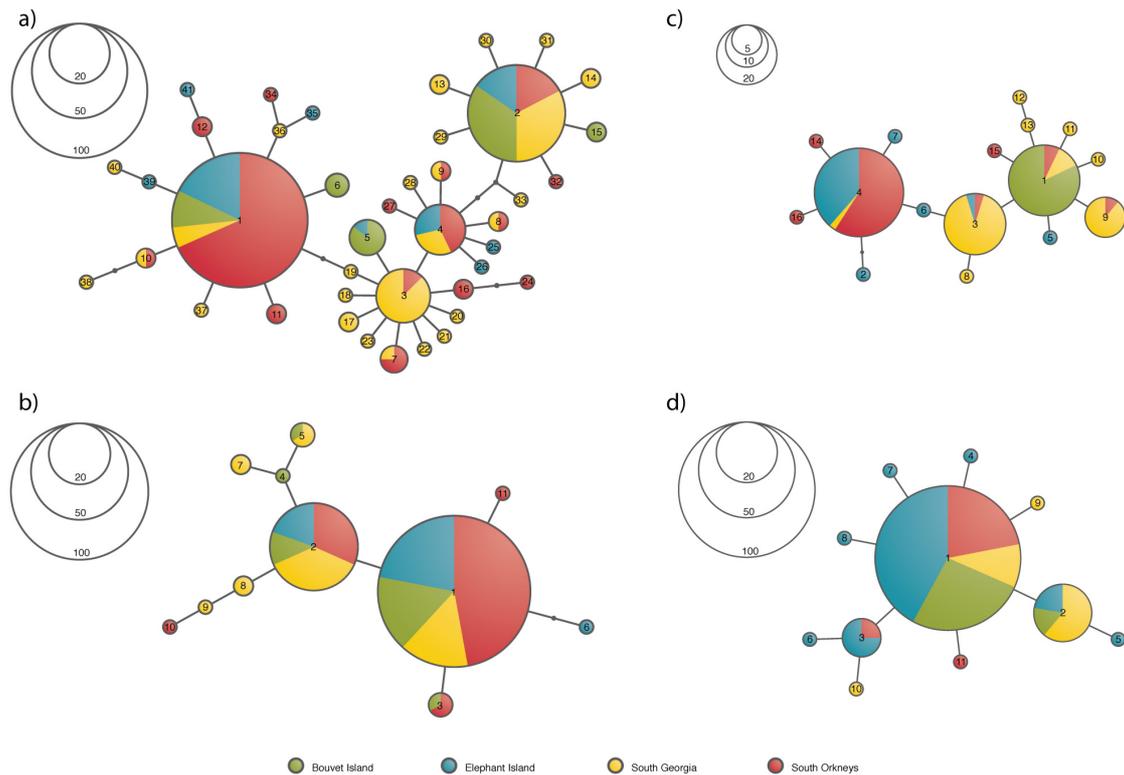


Fig. II-3 Haplotype genealogies for *C. aceratus* (a, b) and *C. gunnari* (c, d). Upper row cyt *b*, lower row D-loop

Microsatellite data

Microsatellites could be genotyped for 230 and 177 specimens of *C. aceratus* and *C. gunnari*, respectively (Table II-S3, Supporting information). All loci were polymorphic and showed no linkage-disequilibrium, but null-alleles were present at two loci (Cr38, Trne20) in all populations of *C. gunnari*. At BO, this species showed homozygote excess at most loci (Cr38, Cr127, Cr236, Trne20, Trne53), but given the low genetic diversity of mtDNA sequences, this finding is probably not a technical artefact. In general, genetic diversities were highest at SG and lowest at BO (Table II-S4, Supporting information). In accordance with mtDNA data, allelic diversity in *C. aceratus* was higher than in *C. gunnari*. The power of microsatellites to distinguish between slightly differentiated populations was higher than for mtDNA sequences as inferred from POWSIM and the proportion of significances reached 1 even for F_{ST} values as small as 0.005 (Fig. II-S1, Supporting information).

Genetic population structure

The genetic population differentiations estimated with F_{ST} were not congruent between markers, but showed some striking parallels between species. Global F_{ST} s for *C. aceratus* and *C. gunnari* were highly significant, with lowest F_{ST} values for microsatellites (0.021 and 0.022) and highest for cyt *b* (0.192 and 0.443, respectively, Table II-1). Microsatellite data show significant pairwise population differentiations with the highest degree of divergence for the populations at BO (Table II-2). Excluding null-allele loci from the analysis of *C. gunnari* did not alter the results. A comparison between values of Jost's *D* and F_{ST} suggests that the actual differentiation between populations is higher than indicated by F_{ST} .

CHAPTER II

Table II-1 Results of analysis of molecular variance (AMOVA) for the two study species *C. aceratus* and *C. gunnari* from 4 locations (Bouvet Island, Elephant Island, South Georgia, South Orkneys) measured by microsatellites, *cyt b* and D-loop mtDNA. ENA=excluding loci with null-alleles

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fst	P
<i>C. aceratus</i>						
Microsatellites						
Among pops	3	48.101	0.121	4.15	0.042	0
Within pops	226	660.271	0.116	3.97		
Within individuals	230	618.500	2.689	91.88		
Total	459	1326.872	2.926	100		
Cyt b						
Among pops	3	53.004	0.290	17.67	0.177	0
Within pops	237	320.168	1.351	82.33		
Total	240	373.212	1.641	100		
D-loop						
Among pops	3	2.247	0.012	5.07	0.051	0.008
Within pops	175	40.211	0.230	94.93		
Total	178	42.458	0.242	100		
<i>C. gunnari</i>						
Microsatellites ENA						
Among pops	3	61.433	0.207	8.18	0.082	0
Within pops	173	414.222	0.070	2.77		
Within individuals	177	399.000	2.254	89.06		
Total	353	874.655	2.531	100		
Cyt b						
Among pops	3	20.400	0.235	53.66	0.537	0
Within pops	110	22.328	0.203	46.34		
Total	113	42.728	0.438	100		
D-loop						
Among pops	3	3.601	0.029	14.41	0.144	0
Within pops	144	25.156	0.175	85.59		
Total	147	28.757	0.204	100		

Table II-2a-d Pairwise population differentiations

a) Genetic differentiation (F_{ST} |Jost's D) of *C. aceratus* populations based on microsatellites. BO=Bouvet Island, EI=Elephant Island, SG=South Georgia, SO=South Orkneys, *=significant differentiation at 5% level

	EI	SO	SG	BO
EI	-			
SO	0.03*10.12*	-		
SG	0.04*10.17*	0.01*10.06*	-	
BO	0.08*10.28*	0.07*10.25*	0.07*10.29*	-

b) Genetic differentiation (F_{ST}) of *C. aceratus* populations based on mtDNA. Below diagonal cytochrome *b*, above diagonal D-loop. BO=Bouvet Island, EI=Elephant Island, SG=South Georgia, SO=South Orkneys, *=significant differentiation at 5% level

	EI	SO	SG	BO
EI	-	-0.01	0.08*	-0.02
SO	0.03	-	0.13*	-0.02
SG	0.12*	0.28*	-	0.06*
BO	0.09*	0.26*	0.05*	-

c) Genetic differentiation (F_{ST} |Jost's D) of *C. gunnari* populations based on microsatellites; below diagonal $F_{ST}|D_{est}$ values including loci with null alleles (INA), above diagonal excluding loci with null alleles (ENA). BO=Bouvet Island, EI=Elephant Island, SG=South Georgia, SO=South Orkneys, *=significant differentiation at 5% level

	EI	SO	SG	BO
EI	-	0.02*10.10*	0.03*10.15*	0.17*10.50*
SO	0.03*10.08*	-	0.02*10.08*	0.13*10.31*
SG	0.04*10.12*	0.04*10.10*	-	0.16*10.44*
BO	0.18*10.37*	0.15*10.27*	0.14*10.27*	-

d) Genetic differentiation (F_{ST}) of *C. gunnari* populations based on mtDNA. Below diagonal cytochrome *b*, above diagonal D-loop. BO=Bouvet Island, EI=Elephant Island, SG=South Georgia, SO=South Orkneys, *=significant differentiation at 5% level

	EI	SO	SG	BO
EI	-	0.01	0.20*	0.03
SO	-0.02	-	0.33*	0.01
SG	0.41*	0.44*	-	0.31*
BO	0.80*	0.77*	0.58*	-

Mitochondrial DNA data show significant differentiation between all populations but EI-SO for *cyt b*, whereas D-loop data suggest significant differences only for the populations at SG in both species. In general, the degree of differentiation between populations is approximately 2.5-fold higher in the benthopelagic *C. gunnari* compared to the benthic *C. aceratus*. The least population differentiation as measured by all markers was found at the southern Scotia Arc between EI and SO.

Although the haplotype genealogies revealed no strict separation of genetically distinct populations in any species, star-like genealogies with unique haplotypes in all populations except for *C. gunnari* at BO are indicative of local population expansions and self-recruitments. In general, the D-loop haplotype genealogies displayed only one or two central haplotypes of ancestral polymorphisms, depending on the species. Bayesian cluster analyses with STRUCTURE identified 2-3 clusters within each species, depending on the information given about the sampling localities (Table II-S5, Supporting information). Without sampling site information STRUCTURE suggested the existence of two clusters (BO and EI, SO, SG) for each species (Fig. II-4). The incorporation of sampling site information resulted in three clusters each, which correspond to BO, SG and the group of EI and SO. Excluding loci with null-alleles did not change the results in *C. gunnari*. Overall, the combined STRUCTURE analyses confirmed the pattern revealed by F_{ST} and Jost's D of genetic similarities in the southern Scotia Arc, but high divergence of BO populations.

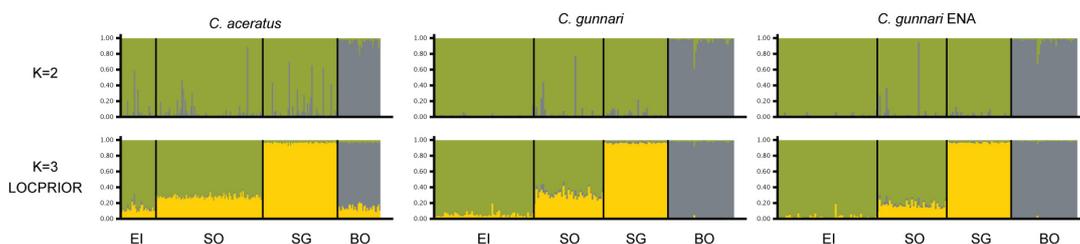


Fig. II-4 Individual probabilities of cluster assignments from the software STRUCTURE. All individuals are grouped according to their sampling locality (EI=Elephant Island, BO=Bouvet Island, SG=South Georgia, SO=South Orkneys). Each vertical line represents the probabilities for a single individual to belong to one of the clusters (K) used in the analysis. LOCPRIOR=analysis including data of sampling localities, ENA=excluding loci with null alleles

Demographic histories

Tests of neutrality delivered varying results between genes, species and populations (Table II-S6, Supporting information). Nonetheless, growing population sizes were evident for both species. Fu's F_s showed significant negative deviations from the neutral expectation on species levels, indicating an overall increase in population sizes, as supported by the star-like haplotype genealogies and increasing skyline plots (Fig. II-5). The BSPs mostly recovered the pattern of demographic expansions but show subtle differences between species and populations. While overall population sizes clearly increased since the beginning of the last warming period, this trend is less pronounced for D-loop data in *C. aceratus*. Remarkably, the populations at BO of both species seem to have suffered a drastic decline in population size, as indicated by *cyt b* in *C. aceratus* and the impossibility to reconstruct reliable BSPs in *C. gunnari* due to the lack of genetic diversity. Information gained from BSPs about population size changes ceases with time and is not informative for the time prior to the LGM.

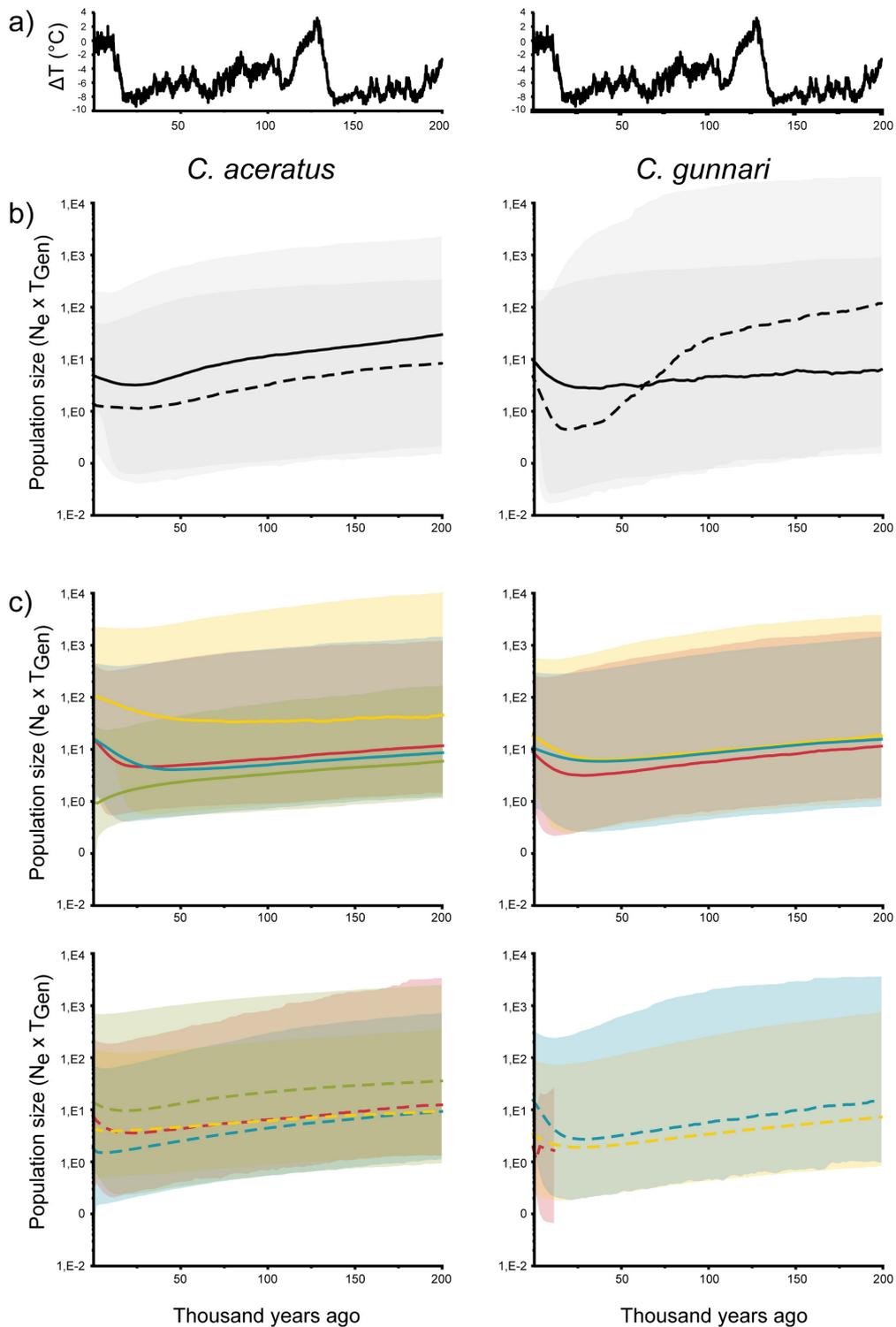


Fig. II-5 Bayesian Skyline Plots showing population size changes per species, population and genetic marker for the last 200,000 years. a) plots of relative temperature change ΔT ($^{\circ}\text{C}$) from Petit *et al.* (1999), b) BSPs for each species (including all individuals of a species), c) BSPs for each population (including all individuals of a population) and marker (cyt *b*=solid lines, D-loop=dashed lines). Population size changes are plotted as the product of effective population size (N_e) and generation time (T_{Gen}). Colour codes as in Figs. II-2 and II-3

Drifter trajectories

Drifters leaving the shelf areas of the four study locations floated consistently in northeasterly or easterly direction with the ACC (Fig. II-2). Every drifter leaving one of the

shelf areas has the potential to reach any island located downstream of its place of origin within 400 days. Once entrained in the ACC the majority of drifters remained within a core area of the current, although individual trajectories were highly variable. Drifters at the northern and southern edge of the ACC showed an undulating pathway probably caused by local eddy systems. Retention of drifters in lee-gyres behind islands was evident from the tip of the Antarctic Peninsula, SG, and to a lesser extent SO. Under the assumption of passive dispersal, larvae should have the potential to establish gene flow between all islands in the investigated area.

Discussion

Population genetic structures

Despite long PDs and oceanographic connectivity between islands through the ACC, the here presented data indicate substantial genetic population structure in both *C. aceratus* and *C. gunnari* in the study area. The highest levels of population differentiations are revealed between the Scotia Arc and BO, whereas the highest similarities were found in concordance with previous population genetic studies in the southern Scotia Arc area (Papetti *et al.* 2009; Van de Putte *et al.* 2012; Papetti *et al.* 2012; Damerou *et al.* 2012). Accordingly, the populations at SG and BO each represent discrete populations.

The identified population divergence between the northern (SG) and the southern (EI, SO) Scotia Arc populations conforms to previous non-genetic assessments based on meristic and morphological characters, such as dorsal fin ray counts, vertebra counts and proportional length measurements (Gubsch & Hoffmann 1981; Kock 1981; Gubsch 1982; Sosiński 1985) as well as parasite infestation rates (Siegel 1980b) for either one or both study species. In *C. aceratus* the finding of a population boundary between AP and SG was furthermore supported based on otolith chemistry (Ashford *et al.* 2010). However, a preliminary genetic study based on mitochondrial ND2 gene sequences and small sample sizes did not reveal signs of divergence in *C. aceratus* (Jones *et al.* 2008).

A larger number of molecular studies aimed at delineating the population structure of *C. gunnari*, a species of commercial interest, but the obtained results were incongruent. An early allozyme-based study suggested high similarities between SG in the north and SO in the south (Carvalho & Warren 1991 in Kock & Jones 2005), whereas a combination of mitochondrial and nuclear SNPs showed a clear separation (Kuhn & Gaffney 2006). Our measures of population differentiation now give further support for the subdivision of *C. gunnari* into northern and southern Scotia Arc populations.

Bouvet Island is the geographically most isolated island in our study, and, in contrast to previous investigations, we found that the populations of both species showed the highest genetic differentiations towards any other island in the study area (Kuhn & Gaffney 2006; Jones *et al.* 2008). Although significant differences between SSh and BO were found for *C. gunnari* based on SNPs, this difference was attributed to the population of SSh, as this was also significantly differentiated from Shag Rocks, SG and Heard Island (Kuhn & Gaffney 2006). However, the number of samples from BO used in both these studies was rather low (4 and 3 specimens, respectively), which might have resulted in unrepresentative estimates. Indeed, a study on *D. eleginoides* with 10 individuals from the area around BO showed significant differences towards SG with microsatellites (Rogers *et al.* 2006). The differences between the presented and

previous studies can therefore be explained by varying degrees of marker polymorphisms and sample sizes.

Gene flow by larval dispersal

The drifter trajectories show potential oceanographic connectivity among populations along the ACC and the high similarity of teleost species assemblages at SG and BO (Permitin 1973) suggest that larvae are the primary means for gene flow in demersal notothenioids. Regarding the vertical distribution of larvae of both study species, which are restricted to the upper 250 m of the water column, with high abundances in the surface layer around dusk (North 1991; Loeb *et al.* 1993), it can be assumed that passively dispersing larvae would roughly follow the direction of the ACC similar to the surface drifters. Moreover, the pelagic early developmental stages last long enough to passively reach islands downstream. Yet, our finding of pronounced genetic population structures shows that pelagic phases of more than one year do not necessarily imply high levels of gene flow. Therefore, effective larval retention mechanisms or low rates of larval survival in oceanic waters must be responsible for reduced gene flow among populations. Also, the chance of reaching downstream islands by passive dispersal seems rather low, given that most drifters passed downstream shelves outside the 500 m isobaths contours. Unfortunately, information about larval survival in oceanic waters is scarce, but it has been argued that the long PDs of notothenioids are generally at odds with a successful survival strategy (White 1998). Interestingly, it has repeatedly been shown that notothenioid larvae are primarily distributed in neritic waters, with decreasing abundances towards the outer shelf, whereas larvae of mesopelagic fishes, such as myctophids, are more abundant beyond the slope in oceanic waters (e.g. Efremenko 1983; Loeb *et al.* 1993). Retention mechanisms in the form of lee gyres at islands, in which the larvae can become entrapped, are known from the Scotia Sea (Efremenko 1983) and are also visible beyond the slope from the drifter trajectories near AP, SG and SO. Moreover, slow exchange of water masses across the shelf break may further limit offshore dispersal, as indicated by the zooplankton distribution at SG (Atkinson & Peck 1990).

Additional factors favouring larval retention are adult spawning and larval vertical migration behaviours. In a recent study, Young *et al.* (2012) compared the larval retention of *C. gunnari* and the nototheniid *Notothenia rossii* at SG with a numerical modelling approach and found mean retentions of 31.3% and 5.3%, respectively. The supposed main reasons for this difference are the longer PD of *N. rossii* eggs and larvae as well as the location of spawning sites (onshore-offshore). Further, diel vertical migrations enhanced retention of larvae in both species, whereas the time of spawning had no effect. Since both our study species are known to migrate inshore for spawning (Kock & Kellermann 1991) and larvae carry out diel vertical migrations (North 1988), these behaviours help to explain low levels of gene flow despite long PDs. Indeed, high levels of self-recruitment and growing population sizes are evident from the star-like haplotype genealogies with private haplotypes in most populations, possibly also reflecting local adaptations.

For both species, the SACCF is not a strict barrier to gene flow. Although SG is separated from any other study location oceanographically by the SACCF and the local populations were significantly differentiated to any other island with all markers, the distribution of haplotypes exclusively shared with SG populations indicates at least

temporally high levels of gene flow. For example in *C. aceratus*, the populations of SO and SG share 5 exclusive haplotypes, which is the highest number of exclusively shared haplotypes in our study. In addition, the degree of differentiation between these two populations is comparatively low. Therefore, the SACCF does not constitute a strong barrier to gene flow for the studied channichthyids, unlike the Polar Front (PF), which has been found to be a strong barrier to gene flow in *D. eleginoides* populations from the South American shelves and the area of Shag Rocks and SG (Rogers *et al.* 2006). In contrast to the PF, which is marked by sharp temperature and salinity gradients at the sea surface, the SACCF is characterized by a temperature maximum at 500 m depth (Orsi *et al.* 1995), which probably has little influence on larvae in the upper waters layers.

Based on our results, we conclude that the PD itself is not a reliable estimator for the level of gene flow. Unusually long PDs do not predict genetic homogeneity among populations, even though they are located within the trajectory of one of the world's strongest currents known. Instead, local oceanography as well as spawning and larval behaviours might regulate larval retention considerably.

Species-specific gene flow patterns

Although our results indicate the existence of three different populations in the study area for both studied icefishes, the species-specific degrees of differentiation and haplotype distributions suggest lower dispersal capabilities for *C. gunnari*. The cyt *b* haplotype genealogy of *C. gunnari* resembles a stepping-stone pattern in parallel with the direction of the ACC. Under the assumption that dispersal results in shared haplotypes, which would also be evident from colonization events, connections exist between all populations except EI and BO. Even when larvae from EI were dispersed with the ACC and transported past SG, the capabilities of young *C. gunnari* to survive such long distance dispersal appear low. This observation is further corroborated by the STRUCTURE analyses, when no sampling site information was provided for clustering (Fig. II-4). High population assignment probabilities of individual *C. gunnari* from the Scotia Arc to belong to BO, which indicate genetic similarities and, hence, potential origins of former migrants, vary between locations and are negligible for samples collected at EI, low for SG and highest for SO.

By contrast, the cyt *b* haplotype genealogy of *C. aceratus* is less indicative of a stepping-stone pattern and haplotypes are shared between all populations. The existence of a haplotype exclusively shared between EI and BO might indicate direct gene flow between these two distant populations. Nonetheless, additional sampling from SO, SG and preferably South Sandwich Islands could prove that this haplotype is also found in between. In addition, STRUCTURE assigns high probabilities for single *C. aceratus* to belong to BO in every cluster from the Scotia Arc. These findings and the lower genetic differentiation of *C. aceratus* populations as compared to *C. gunnari*, indicate higher dispersal capabilities of *C. aceratus*. However, the high degrees of genetic differentiation suggest that these distances are realized only rarely.

Since our study species' resemble each other in regard to adult inshore spawning, diel vertical larval migrations and the total time larvae spend in the water column, the underlying mechanisms causing the differences in population divergences are likely demographic.

Demographic factors influencing population divergence

In addition to the varying degrees of connectivity between the populations of *C. aceratus* and *C. gunnari* that can be explained by differences in successful larval dispersal, it is likely that the disparity in the extent of genetic differentiation is also caused by generation-time effects and differing effective population sizes (N_e).

The generation-time hypothesis predicts faster nucleotide substitution rates per unit time in species with shorter generation times, as DNA replication errors (probably during stem cell division) accumulate more often per absolute time (e.g. Laird *et al.* 1969; Li *et al.* 1996). Without gene flow, populations of species with short generation times will therefore diverge more rapidly. Our results are in congruence with this hypothesis and show elevated levels of divergence in populations of *C. gunnari*, whose generation time of 3 years is about half the generation time of *C. aceratus* in which individuals spawn for the first time after 6-8 years. However, the unknown times of colonization events do not allow a final conclusion and this point requires further examination.

The effective population sizes also influence population divergences, as genetic drift acts faster in small populations (Kimura & Ohta 1969). Data on census and effective population sizes in the study area are available as biomass estimates, but regional and temporal differences in biomass as well as differing sizes of specimen between species prohibit reliable estimates of population sizes within the scope of this study. It must be noted that biomass estimates for both species fluctuate heavily between years and are associated with large confidence intervals. For example, at EI the estimated biomass of *C. aceratus* from 1981 until 1996 varied from 768 t to 7619 t, while the biomass of *C. gunnari* varied from 502 t to 2059 t (Kock 1998). Estimates on total and spawning stock biomass at EI from the year 1998 indicate that a larger proportion of *C. aceratus* (40.3%) spawned as compared to *C. gunnari* (Jones & Kock 2005; Convey *et al.* 2009), but little is known about the temporal stability of these ratios. Unfortunately, N_e estimates based on our genetic data did not result in unambiguous estimates and depended upon methods (data not shown), leaving the relative importance of N_e on population divergences an open question to be resolved.

Our findings are important as they show that simple reductions of the underlying causes for genetic divergence among populations are neither single-parametric (such as the PD) nor are they restricted to certain life-history stages (such as the larval stage). Comparative studies among species are highly valuable for disentangling the complexity of factors shaping the genetic structure of populations, but the results will always be limited by our ecological knowledge of the investigated species.

Demographic responses to the LGM

Antarctic species encountered major challenges for survival during repeated glacial periods over the past million years (Convey *et al.* 2009). Data from ice-cores suggest cyclical temperature oscillations over a range of 10°C since about 400,000 years ago (Petit *et al.* 1999) with the last glacial maximum (LGM) at about 20-14 kya at the Antarctic Peninsula (Anderson *et al.* 2002). During glacial maxima the Antarctic ice-sheets expanded far over the continental shelf (Hambrey & Barrett 1993) and the maximum expansion of sea ice was displaced about 10° latitude to the north in the Atlantic Ocean sector as compared to its current state (Gersonde *et al.* 2005). Habitat

loss and changes in population size were the consequences for many Antarctic species and genetic imprints for such events are found in a diverse range of taxa including kelp (Fraser *et al.* 2009), shrimps (Dambach *et al.* 2012), krill (Zane *et al.* 1998), octopus (Strugnell *et al.* 2012) as well as notothenioid fishes (Patarnello *et al.* 2011).

For notothenioids there is evidence from genetic data that pelagic species were less affected by population bottlenecks caused by recent glaciation events than benthic species (Janko *et al.* 2007). For example, population expansion in the cryo-pelagic *Pagothenia borchgrevinki* could have started as early as 70 kya (Janko *et al.* 2007) and even earlier in the truly pelagic *Pleuragramma antarctica* at about 111-126 kya (Zane *et al.* 2006), but in the benthic notothenioids *Trematomus bernacchii*, *T. pennellii* and *G. gibberifrons* the onsets of possible population expansions were estimated to be more recently at about 50 kya, 25 kya and 33 kya, respectively (Janko *et al.* 2007; Matschiner *et al.* 2009). Also, population expansions in the benthopelagic *C. gunnari* occurred at an intermediate time of 37 kya or later (Kuhn & Gaffney 2006). Although these results are tempting to conclude a succession of population size changes from pelagic to benthic species, the large confidence intervals associated with them that range from thousands to even millions of years, do not allow an unambiguous conclusion. The difficulty in dating population size changes is the use of a correctly calibrated molecular clock, since substitution rates vary between taxa, lineages, loci and timescale (see Box 2 in Allcock & Strugnell 2012). The assumed substitution rates used for dating such events are hence only approximations to the 'real' molecular clock and it has recently been suggested that expansion events in notothenioids were more recent than stated above (Patarnello *et al.* 2011).

In contrast to previous studies, in which mismatch distributions were applied, our coalescent approach resulted in population expansion times starting 25 kya or later for both channichthyids. Although this corresponds to the lower CI bounds of previous estimations, our results suffer from large HPDs, too, and prevent a clear dating for the expansions. However, BSPs based on phylogenetically derived molecular clocks often over-estimate the timing of historical, post-glacial population size changes (Ho *et al.* 2005; Grant *et al.* 2012). We examined the effect of substitution rate on the BSPs by conducting further analyses using rates of 0.02, 0.04 and 0.08 substitutions/site/myr, which were all higher than our phylogenetically inferred rate of 0.01. Although the plots remained highly identical in shape (data not shown), the slopes of the population expansions steepened with increasing rates and population growths started between 18-12 kya. Our inferred estimate of the population expansion onset of 25 kya might therefore indeed be rather conservative.

Conclusions

The two channichthyids *C. aceratus* and *C. gunnari* show a clear population genetic structure despite prolonged pelagic development of more than one year and connectivity through one of the world's largest current systems. Nonetheless, occasional gene flow is evident even over thousands of kilometres possibly through larval dispersal with the ACC. A greater divergence between populations of *C. gunnari* as compared to *C. aceratus* may indicate both a reduced capability for successful larval dispersal as well as influences from demographic effects, but additional reasons such as local adaptations should be explored in future studies. The low levels of gene flow between sub-Antarctic island populations as well as the reduction of population sizes

during glacial maxima indicate that the notothenioid diversity is possibly driven by allopatric speciation.

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Data accessibility

All DNA sequences from this study are available on Genbank, Accession Numbers KC907761-KC907839. Fragment lengths of microsatellites can be accessed in the form of CONVERT formatted txt files from the Online Supporting Material (Data S1, S2).

Supporting information

Additional supporting information may be found in the online version of this publication:

Fig. II-S1 Statistical power to detect significant population differentiations per species and marker as inferred by POWSIM

Table II-S1 Sequence accession numbers used for provisional phylogenetic trees based on *cyt b* and D-loop data

Table II-S2 Genetic diversities of *cyt b* and D-loop mtDNA sequences per species, population and locus

Table II-S3 Species specific microsatellite properties

Table II-S4 Population specific microsatellite properties and inbreeding coefficient (F_{IS})

Table II-S5 Mean Ln P(D) values (SD) of 20 runs for K=1-6 from the software Structure per species

Table II-S6 Neutrality tests (Tajima's D and Fu's F) per species and population analysed for *cyt b* and D-loop

CHAPTER III

Population genetic structure of *Lepidonotothen larseni* revisited: *cyt b* and microsatellites suggest limited connectivity in the Atlantic sector of the Southern Ocean

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Abstract

Antarctic fishes (Notothenioidei) are characterized by unusually long pelagic larval stages of up to more than one year and population genetic studies on notothenioids often revealed insignificant population differentiations over large geographic scales. Hence, gene flow by passive larval dispersal with ocean currents is often assumed to be predominant among notothenioid populations. We re-examined the genetic population structure of the semi-pelagic Painted notothen *Lepidonotothen larseni* in the Atlantic sector of the Southern Ocean based on cytochrome *b* gene sequences and microsatellite markers, for which absence of population structure had been inferred in a preliminary study. Our new results suggest highly restricted gene flow between populations and low levels of successful dispersal with the currents. This incongruence among studies is related to differences in sample sizes and the types of genetic marker used, highlighting the need of adequate sample sizes and the application of multiple markers. Insufficiently resolved population structure may not only have severe consequences for conservation management, but also for our general understanding of speciation processes in Antarctic fishes. In addition, we provide evidences based on Bayesian Skyline Plots of increasing population sizes in this sub-Antarctic species since the last glacial maximum.

Introduction

Antarctic fishes (Notothenioidei) evolved in the constantly cold waters of the Southern Ocean and diversified into more than 130 species within a time frame of approximately 25-22 million years ago (Eastman 2005). Today, notothenioids constitute about 91% of the biomass and 77% of the species diversity on the shelves and slopes of the Antarctic continent and the sub-Antarctic islands (Eastman 2005). Their evolutionary success is related to key adaptations such as antifreeze glycoproteins, which prevent their body fluids from freezing at sub-zero temperatures (Cheng 1998; Matschiner *et al.* 2011), as well as ecological adaptations to vacant niches left void in the Antarctic ecosystem in response to ancient climate changes (Near *et al.* 2012). For example, pelagic niches in the water column were repeatedly filled through pelagization during the evolutionary history of notothenioids (Klingenberg & Ekau 1996; Rutschmann *et al.* 2011; Near *et al.* 2012), accompanied by increased lipid depositions and reduced skeletal ossifications to achieve buoyancy in these swimbladder-lacking fishes (Eastman 1993). Hence, the evolution of notothenioids is often referred to as a prime example of an adaptive radiation in the marine realm (see e.g. Salzburger 2008).

Speciation in most Antarctic organisms is supposedly allopatric triggered by periodical glaciation events (Kennett 1982; Eastman 1993; Petit *et al.* 1999). In particular, fragmentation of populations by ice, isolation in refugia during glacial maxima and recolonization of destructed habitat may have been key mechanisms for allopatric speciation in the Antarctic realm (Hewitt 1996; Rogers 2007). This is especially true for Antarctic marine bottom invertebrates, which are to a great extent characterized by high levels of endemism and scarcity of pelagic larval stages (Thorson 1950; Mileikovsky 1971; Thatje 2012). However, in the case of demersal notothenioids, of which most are confined to shelf and slope areas during their adult stage, gene flow between populations is assumed to be established via the unusually prolonged larval stage of up to more than one year (Kellermann 1986; 1989; North 2001), during which larvae might be subject to long-distance dispersal with the currents. Gene flow by larval dispersal might therefore counteract population divergences and allopatric speciation in notothenioids.

Given the large size of the oceans and small size of most marine propagules, direct observations of migration between populations are rather difficult in the marine environment and indirect measures of connectivity by genetic signatures are of great help to reveal the population structure and the degree of connectivity between populations (Cowen & Sponaugle 2009; Hellberg 2009). In addition, genetic imprints from past population size changes, as for example reductions of genetic diversity during bottlenecks, allow us to infer the demographic histories of species and populations, thereby elucidating the response of organisms to environmental and anthropogenic influences.

Population genetic studies on notothenioids have been performed for strictly benthic to pelagic species and resulted in a diverse pattern of connectivity. For example, the benthic Humped rockcod *Gobionotothen gibberifrons* and the circum-Antarctic distributed pelagic Antarctic silverfish *Pleuragramma antarctica* have both been shown to be only weakly genetically differentiated over their distribution range (Zane *et al.* 2006; Matschiner *et al.* 2009). Other pelagic circum-Antarctic species, such as the large Patagonian toothfish *Dissostichus eleginoides* are also genetically homogenous over vast distances of several thousand kilometers, but genetic breaks occur over

relatively short distances, where frontal systems like the polar front are present (Smith & Gaffney 2000).

In the Atlantic sector of the Southern Ocean, along the islands of the Scotia Arc and the Bouvet Island to their east, genetic homogeneity among populations is no exception to this predominant pattern (Kuhn & Gaffney 2006; Papetti *et al.* 2007; Jones *et al.* 2008; Matschiner *et al.* 2009; Papetti *et al.* 2009; 2012; Damerau *et al.* 2012). This led to the hypothesis that high levels of gene flow among populations, mediated via pelagic larval dispersal with the currents, are common for notothenioids. However, recent comparative population genetic studies based on multiple genetic markers have shown that populations might be more structured than previously thought (Lumpkin & Speer 2007; Van de Putte *et al.* 2012). This discrepancy is probably related to the availability of high-resolution markers together with more adequate sample sizes. Indeed, genetic homogeneity was often inferred based on single genetic markers or small sample sizes (e.g. less than ten samples per population) (e.g. Kuhn & Gaffney 2006; Jones *et al.* 2008; Matschiner *et al.* 2009). As a result, the level of connectivity among populations of notothenioid species may have been overestimated. Therefore, a re-evaluation based on alternative genetic markers and increased sample sizes appears useful to validate notothenioid population structures.

In this study, we re-examined the genetic population structure of the Painted notothen (*Lepidonotothen larseni*) in the Atlantic sector of the Southern Ocean, for which genetic homogeneity has been found in a preliminary study (Jones *et al.* 2008). This benthic (Eastman & Grande 1989; Eastman 1993) species inhabits the shelves of the Antarctic Peninsula as well as most sub-Antarctic islands around Antarctica in 30-550 m depths (DeWitt *et al.* 1990). In the Atlantic sector, this nototheniid occurs along the island chain from the South Shetlands (SSh) in the west to Bouvet (BO) in the east, including South Orkneys (SO), Shag Rocks (SR), South Georgia (SG) and South Sandwich (SS) Islands (DeWitt *et al.* 1990; Jones *et al.* 2008). *L. larseni* is highly abundant and constitutes up to 80% of bottom trawl catches at e.g. SG, SS and BO (Jones *et al.* 2008). But due to its small total lengths of maximum 24 cm (DeWitt *et al.* 1990), the total biomass of this species is rather low compared to other abundant notothenioids (Kock & Jones 2005). As a consequence, *L. larseni* has not been targeted by fisheries and its population sizes were relatively stable over recent time (e.g. Jones *et al.* 2000). The life history of *L. larseni* is characterized by one of the longest pelagic larval and juvenile developments among notothenioids (Kellermann 1989; North 2001). It becomes sexually mature at the age of four to five years (North & White 1987) and spawns between 1 815 and 9 745 small (1.6-2.0 mm) demersal eggs (Andriashev 1965; Permitin & Sil'yanova 1971; Kock 1989; DeWitt *et al.* 1990; Kock & Kellermann 1991). However, the exact spawning locations (e.g. nearshore or deeper shelf) are still unknown. Spawning takes place in June-July at SG (Sil'yanova 1981 in Kock 1989) and about one month later at SSh (Kock 1989; Kock *et al.* 2000). Larval hatching starts at SG in September (Efremenko 1983) and at EI/AP/SSh from mid September onwards (Kellermann 1986; Kock 1989; Kellermann 1990). Late larvae and early juveniles remain pelagic over their first winter and return to a demersal life style during their 2nd summer/autumn (see e.g. Efremenko 1983; Kellermann 1989). Hence, *L. larseni* has an exceptionally long pelagic development during early life stages lasting for more than

one year and is therefore highly interesting for studying the influence of larval dispersal on gene flow.

Materials and methods

Sampling and DNA extraction

Specimen were collected with bottom trawls at South Georgia, South Orkneys and Bouvet Island during two expeditions to the Southern Ocean: in February-March 2009 during the U.S. AMLR (Antarctic Marine Living Resources) finfish survey aboard RV Yuzhmorgeologiya and in February-April 2011 during ANT-XXVII/3 aboard RV Polarstern (Fig. III-1, Table III-1). Muscle tissues for genetic analyses were stored in 95% ethanol prior to DNA extraction. All DNA collected in 2006 was extracted with the BioSprint 96 workstation (QIAGEN) according to the manufacturers protocol, while DNA from the cruises in 2009 and 2011 was extracted in 300 μ l 5%-Chelex solution with 12 μ l Proteinase K (20 mg/ml). The incubation time was 3 h at 55°C, followed by 25 min at 98°C in a thermomix.

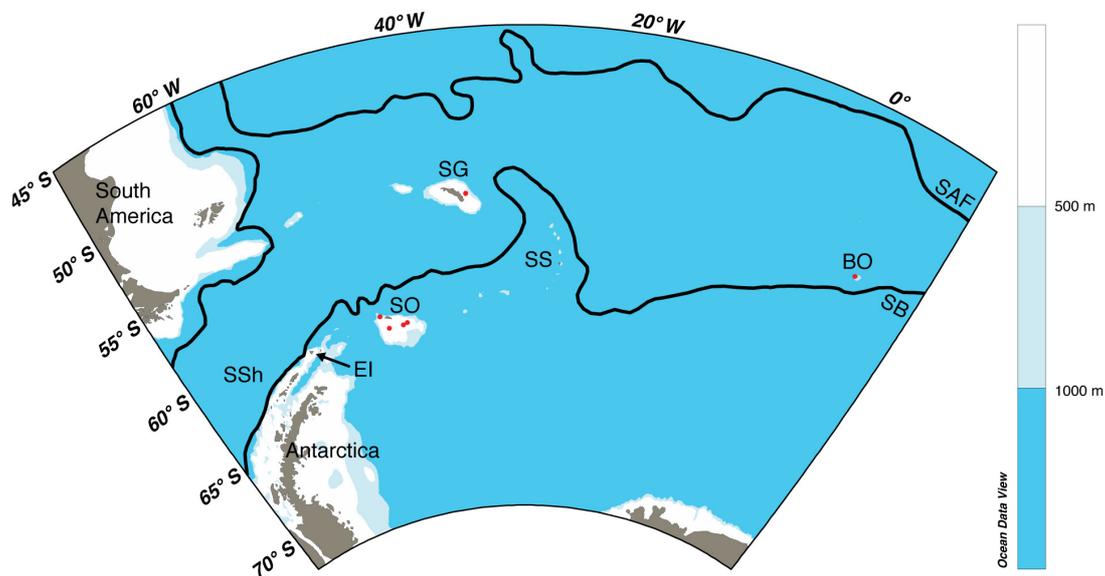


Fig. III-1 Map of the study area and sampling localities (red dots). Islands of the Atlantic sector of the Southern Ocean include South Shetlands (SSh), Elephant Island (EI), South Orkneys (SO), South Georgia (SG), South Sandwichs (SS) and Bouvet (BO). The Antarctic Circumpolar Current (ACC) flows in easterly direction around the Antarctic continent and is marked by the Subantarctic Front (SAF) to the north and the Southern Boundary (SB) to the south. Fronts adopted from Orsi *et al.* (1995)

Table III-1 *Lepidonotothen larseni* specimen sampled in the Atlantic sector of the Southern Ocean and analysed in this study. Data includes cruise, sampling location, depth and number of collected individuals (n)

Area	Cruise	Latitude	Longitude	Depth [m]	n
South Orkneys	U.S. AMLR 2009	60° 25.77' S	46° 26.16' W	142	2
	U.S. AMLR 2009	61° 09.38' S	43° 32.66' W	455	1
	ANT-XXVII/3 2011	61° 9.62' S	44° 2.37' W	354	7
	ANT-XXVII/3 2011	61° 10.61' S	45° 41.91' W	337	27
South Georgia	ANT-XXVII/3 2011	54° 24.47' S	35° 36.95' W	275	14
	ANT-XXVII/3 2011	54° 25.06' S	35° 37.94' W	279	25
Bouvet Island	ANT-XXVII/3 2011	54° 28.23' S	3° 11.10' E	250	25
	ANT-XXVII/3 2011	54° 28.84' S	3° 11.33' E	300	17

Mitochondrial DNA

Partial mitochondrial gene sequences of cytochrome *b* (cyt *b*) were amplified with the primers NotCytbF and H15915n (Matschiner *et al.* 2011) using Phusion polymerase (Finnzymes) and following the manufacturers PCR protocol at 57°C annealing temperature. Purification of PCR products was accomplished by adding 2 μ l ExoSAP-IT (USB Corporation) to 5 μ l of extracted DNA following the manufacturers manual.

Sequencing PCR was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), subsequently purified with BigDye XTerminator (Applied Biosystems) and finally sequenced on an AB3500 Genetic Analyzer (Applied Biosystems). All sequences were automatically aligned with CODONCODE ALIGNER (CodonCode Corp.) and manually corrected if necessary.

Basic sequence properties and polymorphisms such as nucleotide (Pi) and haplotype diversities (h) were examined with DNASP 5.10 (Librado & Rozas 2009). The genetic population structure was assessed by Analysis of Molecular Variance (AMOVA) based on traditional *F*-statistics as implemented in ARLEQUIN 3.5 (Excoffier & Lischer 2010). In addition, haplotype genealogies were constructed from unrooted parsimony trees inferred from DNAPARS 3.5c (Felsenstein 2005) and plotted with HAPLOVIEWER (Salzburger *et al.* 2011).

Past demographic histories of populations were tested for population size changes with Tajima's *D* and Fu's *F* neutrality tests (Tajima 1989; Fu 1997) as implemented in DNASP. Deviations from neutrality are expected under population expansions or bottlenecks and can be detected by either significant negative or positive deviations, respectively.

Past changes in population size were also explored based on Bayesian skyline plots using BEAST v1.7.2 (Drummond *et al.* 2005; 2012) and TRACER v1.5 (Rambaut & Drummond 2007). The underlying model of sequence evolution (HKY-G) was chosen according to the Bayesian information criterion (BIC) in JMODELTEST v0.1.1 (Posada 2008). Since the exact substitution rate for cyt *b* in *L. larseni* is unknown, we explored fixed rates of 0.01, 0.02, 0.04 and 0.08 substitutions/site/myr, which are in the range of rates found for other notothenioids (e.g. Damerou *et al.*, submitted). The best model was chosen according to Bayes Factors with standard errors calculated by 1000 bootstrap replicates as implemented in TRACER. For the analyses, the time to the most recent common ancestor (tMRCA) was directly inferred from the tree prior. First, each analysis was run comprising all specimens (species level) for 50 million MCMC generations with a 10% burn-in. The convergence of parameters and the presence of effective sampling sizes (ESS) of at least 200 were checked with TRACER. Afterwards, two replicate analyses were carried out to check for consistency for each population separately (population level).

Microsatellites

Six microsatellites originally isolated from *Trematomus newnesi* (Van Houdt *et al.* 2006) were successfully amplified for *L. larseni* during Multiplex-PCRs with final volumes of 10 μ l containing 5 μ l Multiplex Master Mix (QIAGEN), 0.2 μ l of 10 μ M primers, 0.8 μ l template DNA and water. PCR protocols started at 95°C for 15 min followed by 35 cycles of 30 s at 94°C, 90 s at 59°C, 90 s at 72°C, and final primer extension for 10 min at 72°C. Fragment lengths were determined on an AB3500 Genetic Analyzer referring to GeneScan LIZ 500 size standard and scored with

GENEMAPPER 4.0 (Applied Biosystems). Before carrying out data analyses, TANDEM (Matschiner & Salzburger 2009) was used for automatic allele binning.

Alleles were checked for stuttering, large allele dropout and null alleles with MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004). Basic allele properties like allele size ranges, number of alleles, private alleles, allelic richness, effective number of alleles, expected (H_e) and observed (H_o) heterozygosities and genotypic linkage disequilibrium were examined with GENALEX 6.5 (Peakall & Smouse 2006; 2012). All significances were tested with 999 permutations in GENALEX, except for population specific deviations from Hardy-Weinberg-Equilibrium (HWE), which was tested using FSTAT (Goudet 1995; 2001).

The population genetic structure was inferred by Analysis of Molecular Variance (AMOVA) based on traditional F -statistics in ARLEQUIN 3.5 (Excoffier & Lischer 2010). In addition, the standardized differentiation measurements G'_{ST} and D_{est} were calculated among populations in GENALEX, which are based on the effective number of alleles instead of allele frequencies (Hedrick 2005; Jost 2008).

Further assessment of population structure was done by using a Bayesian clustering method as implemented in STRUCTURE 2.3.1 (Pritchard *et al.* 2000). Simulations based on the admixture model and correlated allele frequencies for up to four clusters (K) with 20 iterations each were run for 100 000 MCMC replications after a burn-in of 10 000. The same analyses were run a second time while including *a priori* information about the sampling locations (LOCPRIOR). As indicator of the most likely K we calculated ΔK following Evanno *et al.* (2005) with STRUCTURE HARVESTER v0.6.93 (Earl & Holdt 2012). In addition to STRUCTURE, we performed a second clustering algorithm to assess genetic discontinuities in the sampling area taking into account the spatial dependence of individuals as implemented in the R package GENELAND (Guillot *et al.* 2005). The underlying Bayesian model makes explicit use of the spatial location of genotypes without *a priori* information on the number of populations or degree of differentiation among them. We ran 100 independent analyses of 1 000 000 iterations and a thinning of 100, while the number of K was free to vary between one to five. The parameters were set to correlated allele frequencies, 115 as the maximum rate of poisson process, a maximum number of 300 nuclei and the null allele model. Since all runs resulted in the same number of clusters, we chose the run with the highest posterior probability to produce maps of the study area with assigned spatial probabilities to belong to one of the identified clusters with a resolution of about 18 km² per pixel.

Populations were also tested for recent, severe declines in population size with BOTTLENECK 1.2.02 (Piry *et al.* 1999). A two-phase model of mutation with 90% single step mutation and 15% variance was applied in 1 000 iterations. Significance was examined with the sign test, standard differences test and Wilcoxon signed rank test.

Results

mtDNA

Overall, 79 specimen of *L. larseni* were successfully sequenced for 647 base pairs of cyt *b* (Accession numbers KF670785-KF670815). The sequences contained 31 segregating sites and haplotype diversities (h) ranged from 0.759 at SO to 0.806 at BO, whereas P_i was about equal in all populations (Table III-2). Private haplotypes were present in all populations and accounted for 87% of haplotypes.

Table III-2 Genetic diversities of *cyt b* per species and population. bp=base pairs, h=haplotype diversity, Pi=nucleotide diversity

	Sequences	Haplotypes	Private haplotypes	h	Pi
<i>L. larseni cyt b</i> (647bp)	79	31	-	0.778	0.003
South Orkneys	28	15	13	0.759	0.003
South Georgia	25	10	6	0.767	0.002
Bouvet Island	26	11	8	0.806	0.003

Table III-3 Results of Analysis of Molecular Variance (AMOVA) as calculated with Arlequin 3.5 based on *cyt b* sequences and microsatellites. Populations are from South Orkney Islands, South Georgia and Bouvet Island

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fst	P
Microsatellites						
Among pops	2	11.478	0.046	2.04	0.020	<0.000
Within pops	112	251.446	0.058	2.57		
Within individuals	115	245.000	2.130	95.39		
Total	229	507.943	2.234			
Cyt b						
Among pops	2	2.956	0.026	3.14	0.031	0.003
Within pops	76	60.664	0.798	96.86		
Total	78	63.620	0.824			

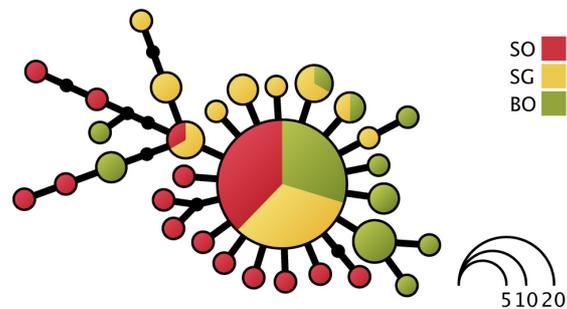
Table III-4 Genetic differentiation of *L. larseni* populations measured as F_{ST} based on microsatellites (below diagonal) and *cyt b* (above diagonal). SO=South Orkneys, SG=South Georgia, BO=Bouvet Island, *=sign. at 5% level, **=sign. at 1% level

	BO	SG	SO
BO	-	0.035*	0.248*
SG	0.031**	-	0.035*
SO	0.009**	0.021**	-

Table III-5 Tajima's D and Fu's F neutrality tests per population based on *cyt b* sequences. Significant deviation from H_0 for Tajima's D ($p < 0.05$) and Fu's F (< 0.02) are indicated by an asterisk

Population	Tajima's D	Fu's F
SO	-2.30*	-12.28*
SG	-1.45	-5.93*
BO	-1.64	-5.54*

Global AMOVA was highly significant with an F_{ST} of 0.031. Most variation was caused by within population variances (Table III-3). All pairwise population differentiations were significant for $p < 0.05$ with the highest degree of differentiation between BO and SO (Table III-4). The haplotype genealogy shows one ancestral haplotype shared by all three populations with a substantial amount of private haplotypes unique to either BO, SG or SO (Fig. III-2). Only three haplotypes are shared between two populations, always including SG. Neutrality tests based on Tajima's D and Fu's F were negative, indicating balancing selection or population growths, but significances varied among populations and tests (Table III-5). The only population for which both tests were significant was SO, while for the other two populations significant deviation from the null

**Fig. III-2** Unrooted haplotype genealogy constructed from *cyt b* sequence data, radii are proportional to haplotype frequencies. Sampling locations were South Orkneys (SO), South Georgia (SG) and Bouvet Island (BO)

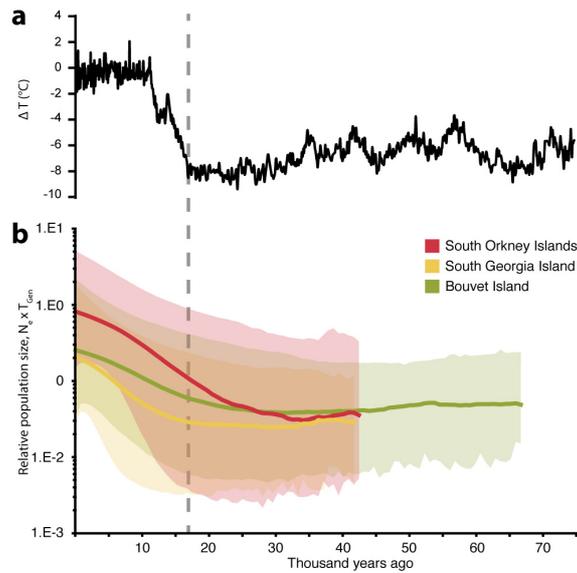


Fig. III-3 Bayesian Skyline Plots for each population based on a substitution rate of 0.08 substitutions/site/myr (b). Temperature changes according to Petit *et al.* (1999) (a). The vertical bar indicates the onset of temperature increase after the Last Glacial Maximum

hypothesis of constant population size and selective neutrality of mutations was only indicated by Fu's F. In addition, the Bayesian skyline plots indicate increasing population sizes at all study locations since approximately 35-17 ka (Fig. III-3) based on a substitution rate of 0.08 substitutions/site/ma, which was chosen according to Bayes Factors (Table III-S1). Since the log10 Bayes Factors values for each tested substitution rate were only slightly differing, additional BSPs were plotted for rates of 0.02 and 0.04 substitutions/site/myr, for which population sizes increase between 50-25 ka (Figure III-S1).

Microsatellites

The complete set of six microsatellites was successfully genotyped for 115 individuals and samples with missing data were omitted from further analyses. All loci were polymorphic and allelic richness per locus varied from 2.8 (Trne55) to 23.7 (Trne35, Table III-6). Deviations from HWE could be detected at four loci (BO: Trne35, Trne66; SG: Trne66; SO Trne37), but in total only BO deviates from HWE, whereas SO and SG are in balance (Table III-7). Furthermore, genotypic linkage disequilibrium was restricted to the locus pair Trne35 and Trne37 and null alleles were not present.

Table III-6 Microsatellite properties based on 115 samples. N_A=number of alleles, A_R=allelic richness standardized to the smallest sample size (35 individuals)

	Trne20	Trne35	Trne37	Trne53	Trne55	Trne66
Size Range (bp)	185-267	210-294	128-148	312-364	174-208	291-373
N_A	30	35	8	26	5	30
A_R	21.5	23.7	6.9	19.3	2.8	18.1

Table III-7 Population specific microsatellite properties and inbreeding coefficient (F_{IS}) of *L. larseni*. n=number of samples, F_{IS}=inbreeding coefficient, N_A=number of alleles, A_P=number of private alleles standardized to the smallest sample size, A_R=allelic richness standardized to the smallest sample size, n_e=effective number of alleles, H_o=observed heterozygosity, H_e=expected heterozygosity, *=sign. deviation from Hardy-Weinberg-Equilibrium (HWE; p<0.05), **=sign. deviation from HWE (p<0.01)

	SO	SG	BO
n	35	39	41
F_{IS}	0.03	-0.01	0.06
H_o (SE)	0.74 (0.14)	0.71 (0.14)	0.69* (0.13)
H_e (SE)	0.75 (0.14)	0.69 (0.14)	0.72 (0.13)
A_P	4.5	1.3	2.5
Allelic diversity	18.0	12.5	14.7

Trne20			
N_A	23	15	25
A_P	3	0	8.2
A_R	23.0	14.8	23.6
H_o	0.97	0.90	0.88
H_e	0.95	0.89	0.94
Ne	15.6	8.4	15.4
Trne35			
N_A	27	17	20
A_P	10	4.5	2.3
A_R	27.0	16.6	18.7
H_o	0.89	0.90	0.88*
H_e	0.94	0.86	0.89
Ne	16.0	6.9	9.2
Trne37			
N_A	8	6	7
A_P	1	0	0
A_R	8.0	5.9	6.8
H_o	0.69**	0.72	0.61
H_e	0.75	0.75	0.61
Ne	4.0	4.0	2.6
Trne53			
N_A	21	22	17
A_P	2	3.3	2.3
A_R	21.0	21.3	16.2
H_o	0.89	1.00	0.89
H_e	0.93	0.93	0.90
Ne	14.5	14.4	10.4
Trne55			
N_A	4	2	2
A_P	2	1.1	0
A_R	4.0	1.9	2.0
H_o	0.09	0.03	0.10
H_e	0.08	0.03	0.14
Ne	1.1	1.0	1.2
Trne66			
N_A	25	13	17
A_P	9	0	4.7
A_R	25.0	12.4	15.9
H_o	0.94	0.69**	0.78**
H_e	0.87	0.70	0.83
Ne	7.9	3.4	5.9

In congruence with *cyt b* data, AMOVA resulted in significant global differentiation ($F_{ST}=0.02$) among populations (Table III-3) and about 95% of the variance could be explained by within individual diversity. All pairwise F_{ST} comparisons were highly significant ($p<0.01$) between populations, but in contrast to *cyt b*, the population pair of BO and SO was least differentiated (Table III-4). Based on the standardized measures of genetic differentiation (G'_{ST} and D_{est}), the level of divergence among populations seems to be higher than suggested by F_{ST} (Table III-8).

Table III-8 Genetic differentiation of *L. larseni* populations based on microsatellites measured as Hedrick's G'_{ST} (below diagonal) and Jost's D_{est} (above diagonal). SO=South Orkneys, SG=South Georgia, BO=Bouvet Island, **=sign. at 1% level

	SO	SG	BO
SO	-	0.058**	0.025**
SG	0.068**	-	0.080**
BO	0.029**	0.095**	-

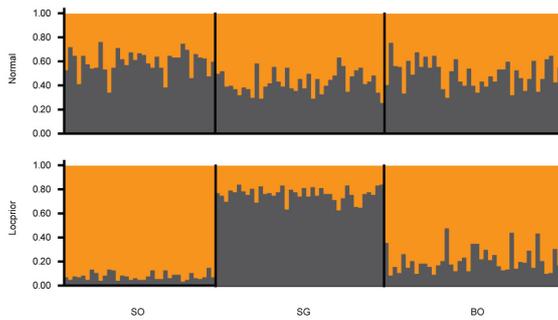


Fig. III-4 Assignment probabilities for each individual to belong to one of the two identified clusters in STRUCTURE. Analyses were run with (=locprior) and without (=normal) incorporation of sampling location data

The Bayesian inferences about population structure made with STRUCTURE resulted in two clusters, irrespective of whether information on the sampling location was incorporated or not (Table III-9). However, the graphical outputs for individual assignment probabilities differ between the two parameter sets and only the LOCPRIOR analyses identified spatial clusters (SG and SO-BO; Fig. III-4).

Table III-9 Mean Ln P(D) values (SD) and Delta K of 20 runs for K =1-4 from the software STRUCTURE. Locprior=including information about sampling site. Highest Delta K values in bold

Parameter Set	K			
	1	2	3	4
Normal				
Ln P(D)	-3058.48 (0.73)	-3181.12 (61.77)	-3180.64 (62.29)	-3299.79 (93.29)
Delta K	NA	1.99	1.92	NA
Locprior				
Ln P(D)	-3058.54 (0.56)	-3023.13 (7.91)	-3077.86 (61.25)	-3091.81 (54.29)
Delta K	NA	11.40	0.67	NA

The analyses with GENELAND, on the other hand, identified three clusters in each of 100 independent runs as the most likely number of clusters. Exemplified by the run with the highest posterior probability, Figure III-5 shows the number of clusters along the chain, which range between three to five clusters. In addition, maps of spatial assignment probabilities to belong to one of three clusters are depicted, while the probabilities to belong to cluster 4 or 5 were spatially indifferent and are therefore not presented.

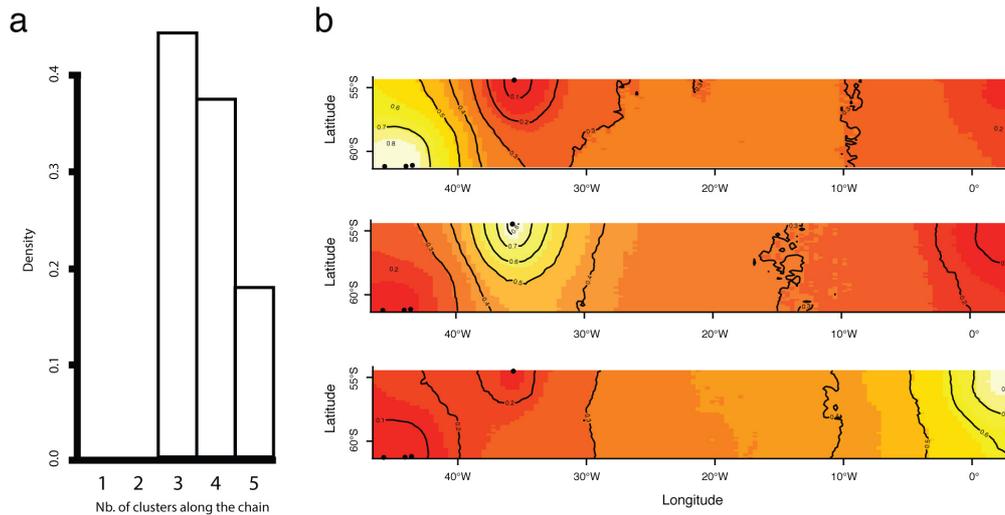


Fig. III-5 GENELAND analyses based on microsatellites with K varying from 1-5. Shown are the inferred number of clusters along the chain for the run with the highest posterior probability (a) and spatial assignment probabilities for individuals to belong to one of the identified clusters (b). The probabilities of cluster memberships increase from red to light-yellow. Black dots represent sampling localities. Maps for cluster 4 and 5 are not shown due to spatial homogeneities

The bottleneck analyses were not significant for the populations at SO and SG, but two out of three tests indicate a recent bottleneck at BO (Table III-10).

Table III-10 Results from BOTTLENECK analyses. P-values for bottleneck detection: - $P > 0.05$, + $P < 0.05$. SO=South Orkney Islands, SG=South Georgia, BO=Bouvet Island

Population	Test		
	Sign	Standard diff.	Wilcoxon signed rank
SO	-	-	-
SG	-	-	-
BO	-	+	+

Discussion

Genetic population structure

In this study, genetic discontinuities between *L. larseni* populations in the Atlantic sector of the Southern Ocean were evident from microsatellites and *cyt b* gene sequences. Although the global differentiation is rather low, the differences measured by F_{ST} and its relatives are nonetheless significant. Also, inferences from GENELAND suggest the existence of three genetically distinct populations in the study area. This result is in clear contrast to a previous study based on the mitochondrial ND2 gene, in which genetic homogeneity was inferred for populations in the study area (Jones *et al.* 2008). The observed incongruence between the two studies is probably caused by differences in sample sizes, as the genetic diversities of ND2 and *cyt b* are about equally high. Moreover, the suitability of both mtDNA markers in population genetic applications is supposed to be in the same order (Hwang & Kim 1999). The slightly higher h for ND2 over *cyt b* is very likely caused by longer sequences, which will result in more segregating sites and hence higher probabilities that two randomly chosen haplotypes differ. Under the assumption that gene flow between populations results in shared haplotypes, the constructed haplotype network based on ND2 in Jones *et al.* (2008) hints to highly self-sustained populations, as all haplotypes are unique to only one population. Hence, the high genetic diversities and the prevalence of private haplotypes for ND2 might indicate that the number of samples used to infer F_{ST} was too

low to yield reliable estimates. In order to examine the effect of small sample sizes for our own data set, we re-calculated global and pairwise F_{ST} s for *cyt b* with ten random subsets each containing sample sizes of ten, four and six individuals for BO, SG and SO, respectively, to match the sampling scheme of Jones *et al.* (2008). Of these, only two subsets showed significant global differentiation (significant F_{ST} =0.07-0.08, non-significant F_{ST} = 0.01-0.04) and only one pairwise significant differentiation was found between BO and SO (data not shown). Despite some randomness, smaller sample sizes resulted in elevated chances of recovering insignificant population differentiations. This highlights the need to be cautious about inferences of genetic population structure from small sample sizes based on single locus mtDNA. Our results based on two different marker types, which both showed significant differentiation in the study area, indicate that populations of *L. larseni* are higher structured than previously inferred. Since mtDNA and microsatellites evolve at different rates, the consistency of this pattern suggests restricted gene flow over both ecological and evolutionary time scales. Nonetheless, pairwise population comparisons indicate changes in connectivity over time.

The substitution rate of the mitochondrial *cyt b* varies between phylogenetic lineages and is in the order of 10^{-8} substitutions/site/generation in the nototheniid *P. antarctica* (recalculated from Zane *et al.* 2006), whereas microsatellites evolve at a much faster rate of about 10^{-2} to 10^{-6} mutations/generation (Schlötterer 2000). The information carried by these markers therefore allow inferences about demographic events on different time scales (Sunnucks 2000; Avise 2004). Based on *cyt b*, highly restricted gene flow is evident between the populations of SO and BO, but SG takes an intermediate position. This is not only indicated by *F*-statistics, but also the haplotype genealogy, in which SG is the only population sharing haplotypes with one of the other populations (despite the most common ancestral haplotype). This resembles a stepping-stone pattern along the latitudinal direction with limited dispersal between the islands. Given the high genetic diversity of the SO population compared to SG and BO, as well as the high number of private haplotypes and alleles at SO, it is reasonable to assume that the direction of gene flow follows the ACC, which might indicate dispersal of larvae with the currents. In contrast to *cyt b* sequences, microsatellite data measured by F_{ST} (and its relatives) revealed the least differentiation between SO and BO, but higher divergence of SG. Also, STRUCTURE identified two different clusters in the study area with high population assignment probabilities for individuals to belong either to SG or else SO and BO. Therefore, the direction of gene flow between SO and BO is probably not solely mediated via SG, but may during recent generations have been primarily established on a more southern route, possibly via South Sandwich Islands. Unfortunately, the lack of samples from South Sandwich Islands does not allow further conclusions about its role as a stepping-stone. It is notable, however, that temporal gene flow through larval dispersal is a very dynamic process.

Knowledge about the genetic exchange between populations is not only of interest for our understanding of evolution, but also important for species conservation and management purposes. Non-genetic assessments of nototheniid population structures in the Atlantic sector of the Southern Ocean based on meristic and morphological characters (Gubsch & Hoffmann 1981; Kock 1981; Gubsch 1982; Sosiński 1985), parasite infestation rates (Kock & Möller 1977; Siegel 1980b) and

otolith chemistry (Ashford *et al.* 2010) were mainly done on larger species, as e.g. *C. gunnari*, that are of interest to fisheries. The predominant pattern identified for the investigated species was a differentiation among populations in the northern (Shag Rocks, SG) and southern (South Shetlands, EI, SO) Scotia Sea, indicating little connectivity among populations separated over long distances as high as 900 km although connected by the ACC. However, this finding was questioned with the rise of genetic studies that examined the population structure along the Scotia Arc. Comparing the northern and southern Scotia Arc populations, only one out of six species, namely *C. gunnari*, showed a significant differentiation (Schneppenheim *et al.* 1994; Kuhn & Gaffney 2006; Jones *et al.* 2008; Matschiner *et al.* 2009). Moreover, high genetic similarity was also the predominant pattern between populations at the southern Scotia Arc for seven notothenioids (Papetti *et al.* 2007; 2009; 2012; Damerou *et al.* 2012) as well as between the northern Scotia Arc and BO for five investigated species (Kuhn & Gaffney 2006; Jones *et al.* 2008). This led to the notion that dispersal of larvae with the ACC during the prolonged pelagic development homogenizes populations genetically over long distances of open water. The discrepancy between genetic and non-genetic assessments of population structures may either indicate that the level of exchange of individuals between populations was underestimated by non-genetic approaches or overestimated by genetic ones. The results of the presented study and two recent publications based on large sample sizes and multiple genetic marker types on trematomids (Van de Putte *et al.* 2012) and channichthyids (Damerou *et al.*, submitted) do now suggest a more restricted exchange between populations, as it was also originally inferred from non-genetic approaches. Therefore, successful dispersal of pelagic notothenioid larvae between distant habitats seems to be scarce, although their general importance for gene flow in demersal species is evident. Consequently, allopatric speciation may also be the primary mode of speciation not only in Antarctic invertebrates, but also in notothenioids inhabiting sub-Antarctic islands, despite their long pelagic larval stages. Furthermore, the results of limited population connectivity should be taken into account when conservation strategies are developed to ensure sustainable fisheries, as overestimated external recruitment may have severe effects on targeted species.

Demographic history

Recent changes in population size of *L. larseni* were examined by bottleneck analyses based on microsatellite data. Unlike the larger fishes of *C. gunnari* and *Dissostichus* spp. that were targeted by commercial fisheries from 1969 onwards and regulated by CCAMLR since 1984, the small-sized *L. larseni* has never been targeted per se (Kock 1992), although its abundance at several locations along the Scotia Arc makes it prone to by-catch in bottom trawls. Estimation of biomass from finfish surveys indicate a relatively stable population size since 1985 at least at SO (Jones *et al.* 2000). Indeed, our microsatellite data indicate stable population sizes for the populations at SG and SO, whereas at BO two out of three tests suggest a recent bottleneck. This is surprising given that fishing activities at BO have in contrast to SG or SO never been commercial, but only exploratory (CCAMLR 2004; CCAMLR 2011a). Exploratory fishing around BO was reported since 2003 and concentrated on *Dissostichus* spp. by means of longlines with very low numbers of by-catches (CCAMLR 2011a; b). Therefore, the effect of fishing on small species like *L. larseni* should have been minimal. However,

recent reductions of population sizes were also inferred from genetic data at BO for *C. aceratus* and *C. gunnari* (Damerou *et al.*, data unpublished), of which only the former was reported as by-catch in the area although with less than one ton in total (CCAMLR 2011b). The congruence among the results for all three notothenioids suggests that this pattern is not species-specific, but more ubiquitous. Whether the causes are nonetheless related to fisheries or changes in the ecosystem is still an open question and needs further investigation. Given the geographic isolation of BO and the apparently low external recruitment from other locations, it seems likely that impacts from human exploitation will have strong lasting effects on the ichthyologic community at BO.

In addition to recent population size changes, we investigated the demographic history of *L. larseni* on the evolutionary time-scale based on mtDNA and our results indicate increasing population sizes in all three study areas. The neutrality tests of Tajima and Fu were both negative and indicate either population expansions or selective sweeps. Considering the results of the haplotype genealogy, which is constructed of one common and many tip-haplotypes, as well as the increasing BSPs since approximately 35-17 kya, the negative neutrality test values seem rather likely to be caused by population expansions instead of selective sweeps. Therefore, increasing population sizes of *L. larseni* are in congruence with the predominant demographic history of notothenioids examined with genetic markers. Population expansions were also reported for *Chionodraco* spp. (Patarnello *et al.* 2003), *C. gunnari* (Drummond *et al.* 2005; Kuhn & Gaffney 2006; Drummond *et al.* 2012), *Pleuragramma antarctica* (Zane *et al.* 2006), *Trematomus* spp. (Janko *et al.* 2007; Van de Putte *et al.* 2012), *Gobionotothen gibberifrons* (Matschiner *et al.* 2009) and *C. aceratus* (Damerou *et al.*, submitted). The only exception is *Trematomus nicolai*, for which stable population sizes were inferred from 14 individuals examined (Kuhn *et al.* 2009). Dating of these expansions ranged from about 24 ky in *G. gibberifrons* to 126 ky in *P. antarctica*, but large confidence intervals associated with these estimates and uncertainties with substitution rates make the exact dating difficult. It has already been noted that, for notothenioid fishes, most estimates on the time of population expansions predate the LGM and that they could have occurred more recently than suggested by the methods (Patarnello *et al.* 2011). Although during the LGM ice sheets expanded asynchronously around Antarctica (Anderson *et al.* 2002) and much debate exists about its exact dating, most authors assume it to be around 20 ka (for a review see Ingólfsson 2004). At the Antarctic Peninsula, for example, the estimates for the occurrence of the maximum ice extent vary between 13 ka to more than 30 ka, depending on the methods and geographic location (Sugden & Clapperton 1981; Banfield & Anderson 1995; Anderson *et al.* 2002; Weber *et al.* 2011). Our finding of increasing population sizes since 35-17 ka, therefore, coincides with the onset of the last interglacial period. However, the most likely BSP model according to Bayes Factors was based on a substitution rate of 0.08 substitutions/site/myr, which is rather high as compared to a rate of 0.004 to 0.0045 substitutions/site/myr inferred for *cyt b* in the nototheniid *P. antarctica* (Zane *et al.* 2006) or the divergence rate of 1% per million years generally assumed for teleost mtDNA (Martin & Palumbi 1993). But even for lower rates of 0.04 and 0.02 substitutions/site/myr the population sizes increased approximately 50-25 ka, which is on the lower end of the supposed range of population size expansions in

notothenioids. Regardless of the exact dating, it is noteworthy that most notothenioid species show increasing population sizes since the upper Pleistocene, irrespective of their benthic or pelagic life strategies (Janko *et al.* 2007) or distribution range. Species inhabiting sub-Antarctic islands, such as *L. larseni*, which have not been directly effected by expanding ice sheets from the Antarctic continent, seem to have reacted similar to high-Antarctic species to the environmental changes associated with glaciation cycles. Therefore, global climate changes are likely to effect sub-Antarctic and high-Antarctic organisms in a similar fashion, as their regional ecosystems are interconnected by oceanographic features as the ACC.

Conclusion

Our study shows highly restricted gene flow between populations of *L. larseni* in the Atlantic sector of the Southern Ocean, whose pelagic larval and juvenile development is among the longest known from notothenioids. This indicates that larval time is not a good estimator for gene flow in notothenioids and that successful larval dispersal between populations is rare. A comparison with a preliminary study based on a single mitochondrial marker and small sample sizes highlights the need for reasonable large sample sizes and shows the advantage of multiple genetic markers in the use of population genetics. Furthermore, analyses of the demographic history of *L. larseni* indicate growing population sizes since the last glacial maximum, and that recent activities of fisheries in the study area had low impact on the population sizes at South Orkneys and South Georgia.

Acknowledgements

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Supplemental Material

The following supplemental material can be accessed via the online journal:

Table III-S1: Log10 Bayes Factor model comparison for Bayesian Skyline Plots based on cytochrome *b* gene sequences with substitution rates of 0.01, 0.02, 0.04 and 0.08 substitutions/site/myr.

Figure III-S1: Bayesian Skyline Plots reconstructed from substitution rates of 0.02 and 0.04 substitutions/site/myr.

CHAPTER IV

Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation

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Abstract

Antarctic notothenioid fishes represent a rare example of a marine species flock. They evolved special adaptations to the extreme environment of the Southern Ocean including antifreeze glycoproteins. Although lacking a swim bladder, notothenioids have diversified from their benthic ancestor into a wide array of water column niches, such as epibenthic, semipelagic, cryopelagic and pelagic habitats. Applying stable carbon (C) and nitrogen (N) isotope analyses to gain information on feeding ecology and foraging habitats, we tested whether ecological diversification along the benthic–pelagic axis followed a single directional trend in notothenioids, or whether it evolved independently in several lineages. Population samples of 25 different notothenioid species were collected around the Antarctic Peninsula, the South Orkneys and the South Sandwich Islands. The C and N stable isotope signatures span a broad range (mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between -25.4‰ and -21.9‰ , and between 8.5‰ and 13.8‰ , respectively), and pairwise niche overlap between four notothenioid families was highly significant. Analysis of isotopic disparity-through-time on the basis of Bayesian inference and maximum-likelihood phylogenies, performed on a concatenated mitochondrial (*cyt b*) and nuclear gene (*myh6*, *Ptr* and *tbr1*) data set (3148 bp), showed that ecological diversification into overlapping feeding niches has occurred multiple times in parallel in different notothenioid families. This convergent diversification in habitat and trophic ecology is a sign of interspecific competition and characteristic for adaptive radiations.

Introduction

Adaptive radiation, the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage, is thought to be responsible for a great portion of the diversity of life (Simpson 1953; Schluter 2000). The most famous examples of adaptive radiations are the Darwin's finches on Galápagos, the Caribbean *Anolis* lizards and the East African cichlid fishes. One of the key features of an adaptive radiation is the correlation between the morphologically diverse phenotypes of the 'participating' species and the various habitats that these occupy (Schluter 2000). While it is conceivable how such an 'adaptive disparity' is fulfilled by the paradigmatic Darwin's

finches, anoles and cichlids with their characteristic adaptations in beaks, limbs and trophic structures, respectively, the inference of phenotype-environment correlation remains a challenge in other cases of adaptive radiation (Schluter 2000; Gavrillets & Losos 2009).

In fishes, most studies on adaptive radiation focus on freshwater systems, with the cichlid species flocks of the East African Great Lakes being the prime examples (Salzburger 2008; 2009). The Antarctic notothenioids represent a marine species flock that evolved under extreme environmental conditions (Eastman & Clarke 1998; Eastman 2000). The perciform suborder Notothenioidei diversified into at least 130 species in eight families, encompassing over 100 Antarctic species (Eastman 2005; Eakin *et al.* 2009). Three ancestral families, Bovichtidae, Pseudaphritidae and Eleginopidae, comprise eleven primarily non-Antarctic species, distributed around southern South America, the Falkland Islands, southern New Zealand and southeastern Australia (Eastman 1993). The remaining families Artedidraconidae, Bathydraconidae, Channichthyidae, Harpagiferidae and Nototheniidae are, with few exceptions, endemic to Antarctic waters and are usually referred to as the 'Antarctic clade' (e.g. Eastman 1993). Notothenioids dominate the Antarctic continental shelf and upper slope, accounting for approximately 46% of the species diversity and over 90% of the fish biomass (Eastman & Clarke 1998; Eastman 2005).

Antarctic waters are constrained by the Antarctic Circumpolar Current (ACC). The Antarctic Polar Front, the northern boundary of the ACC between 50°S and 60°S, acts as major oceanographic barrier, effectively isolating the Southern Ocean faunal assemblages from those of the Indian, Pacific and Atlantic oceans. Through the establishment of a thermally and oceanographically isolated area and the inhibition of faunal admixture, the Antarctic Polar Front is, hence, a likely driver of notothenioid evolution (Coppes Petricorena & Somero 2007). As a means to adapt to Southern Ocean environmental conditions, the Antarctic notothenioids evolved special anatomical and physiological features and, at the same time, lost traits no longer 'needed' in permanently cold waters: (i) The evolution of antifreeze glycoproteins is regarded as an evolutionary key innovation of notothenioids (Eastman 1993; Matschiner *et al.* 2011), facilitating permanent life in subzero temperate waters. (ii) All notothenioids lack a functional swim bladder. Several pelagic species, however, have evolved neutral buoyancy by a combination of skeletal mineralization and the accumulation of lipid deposits (Eastman 1993; Klingenberg & Ekau 1996). (iii) Some notothenioids have lost the classical heat-shock protein response (Place & Hofmann 2005; Clark *et al.* 2008). (iv) The Channichthyidae represent the only known vertebrate group that lacks erythrocytes in the adult state and that is unable to synthesize a functional version of the respiratory oxygen transporter haemoglobin (Ruud 1954; Near *et al.* 2006).

Here, we investigate niche evolution in notothenioids, using a set of 25 representative species (and 365 individuals) that belong to four of the five notothenioid families in the exceptionally species-rich Antarctic clade. Apparently, Antarctic notothenioids diversified along the benthic-pelagic axis in the absence of competition from other fish taxa (Eastman 1993; 2005). From a morphological perspective, this process termed 'pelagization' appears to have occurred independently in several clades (Klingenberg & Ekau 1996; Bargelloni *et al.* 2000).

We used isotopic signatures as indicators for ecological specialization to assess the diversity of lifestyles and feeding strategies/habits of the Antarctic clade, as has been done for adaptively radiating rockfishes (Ingram 2011), and to further test whether these strategies/habits evolved clade-specifically and unidirectionally or independently in several lineages. Stable isotope analysis (SIA) makes use of the fact that the C and N stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of organisms are directly related to their diet. In general, the ratio of the heavier over the lighter stable isotope is greater in consumers than in food material and thus continuously increases with trophic level (TL; e.g. Hobson & Welch 1992; Hobson *et al.* 1994). This is particularly true for nitrogen, where N isotope fractionation leads to trophic shifts of 3–5‰ (DeNiro & Epstein 1978; Minagawa & Wada 1984; Post 2002). The C isotope fractionation is less pronounced during food chain processing, with a typical 1‰ increase per TL (Hobson & Welch 1992). Yet, carbon isotopic values can often be used to assess constraints on the primary carbon source, which can vary strongly between different feeding grounds (e.g. inshore vs. offshore and pelagic vs. benthic). Thus, while N isotope ratios can be used to predict the relative TL of an organism, its C isotopic composition yields valuable information with regard to its habitat (e.g. Hobson *et al.* 1994).

To reconstruct the evolution of ecological specialization in notothenioids, which has not been studied in detail, we established a new phylogeny of the studied species based on mitochondrial and nuclear markers [3148 base pairs (bp) in total]. This phylogeny extends previous work (e.g. Near & Cheng 2008) by the use of multiple nuclear markers and by the longest total sequence length used in notothenioid phylogenetics to date. Phylogeny and time estimation were fully integrated with SIA by the application of a disparity-through-time (DTT) analysis.

According to the results of earlier studies (Klingenberg & Ekau 1996; Eastman & McCune 2000), we expected to find evidence for independent colonization of ecological niches in different lineages. Furthermore, should previous descriptions of the notothenioid diversification as an adaptive radiation be appropriate, the pattern of average subclade disparity throughout the radiation could be expected to resemble those found in other adaptive radiations like *Liolaemus* lizards (Harmon *et al.* 2003) or Tanganyikan cichlid fishes (Gonzalez-Voyer *et al.* 2009) and to be different from patterns observed in putative non-adaptive radiations, such as rats (Rowe *et al.* 2011).

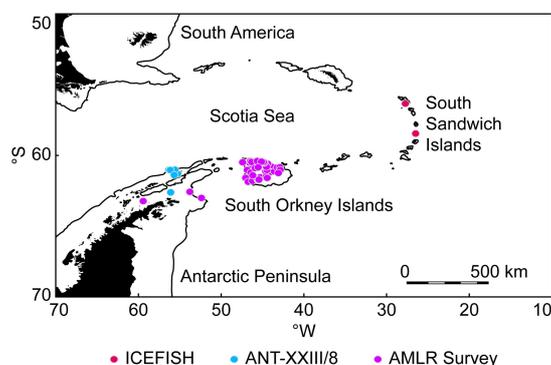


Fig. IV-1 Sampling sites off the northern Antarctic Peninsula, the South Orkney Islands and the South Sandwich Islands. The *solid line* indicates the 1000-m depth contour

Materials and methods

Sample collection

Sampling took place during three expeditions in the austral summer to the Scotia Sea: The ICEFISH 2004 cruise with RV Nathaniel B. Palmer (Jones *et al.* 2008), cruise ANT-XXIII/8 with RV Polarstern, and the 2008/09 US AMLR Survey with RV Yuzhmorgeologiya (Jones *et al.* 2009) (Fig. IV-1 and Table IV-1, Tables IV-S1 and IV-S2, Supporting information). White muscle

tissue samples were preserved in 95% ethanol and stored at -20 °C for subsequent investigations. A total of 365 adult individuals of 25 Antarctic notothenioid species were processed for SIA. Molecular analyses were performed with 39 individuals of the same 25 species and three representatives of non-Antarctic notothenioid families serving as outgroups (Table IV-1).

Table IV-1 Sampled species with collection site; sample size for stable isotope analysis (n) and lifestyle of adult individuals. Lifestyle descriptions are often based on trawl-depth and may not be definite

Sample	Location (n)	Lifestyle of adults
<u>Bovichtidae</u>		
<i>Bovichtus diacanthus</i>	Tristan da Cunha	
<u>Pseudaphritidae</u>		
<i>Pseudaphritis urvillii</i>	Victoria, Australia	
<u>Eleginopidae</u>		
<i>Eleginops maclovinus</i>	South America	
<u>Nototheniidae</u>		
<i>Aethotaxis mitopteryx</i>	AP (4), SO (7)	pelagic ^{a,b,c,d} , benthopelagic ^e
<i>Dissostichus mawsoni</i>	AP (2), SO (5)	pelagic ^{b,d}
<i>Gobionotothen gibberifrons</i>	AP (10), SO (10)	benthic ^{b,c}
<i>Lepidonotothen larseni</i>	SO (10), SSI (10)	semipelagic ^b
<i>Lepidonotothen nudifrons</i>	SO (10)	benthic ^{b,d}
<i>Lepidonotothen squamifrons</i>	AP (10), SO (10)	benthic ^b
<i>Notothenia coriiceps</i>	AP (10), SO (11)	benthic ^d
<i>Notothenia rossii</i>	SO (11)	semipelagic ^b
<i>Pleuragramma antarcticum</i>	AP (10), SO (10)	pelagic ^{a,b,e}
<i>Trematomus eulepidotus</i>	AP (10), SO (10)	epibenthic ^{a,b,c}
<i>Trematomus hansonii</i>	SO (11)	benthic ^{b,c}
<i>Trematomus newnesi</i>	AP (10), SO (10)	cryopelagic ^b
<i>Trematomus nicolai</i>	SO (6)	benthic ^{a,b,c,f,g} , benthopelagic ^h
<i>Trematomus tokarevi</i>	SO (11)	benthic ^g
<u>Artedidraconidae</u>		
<i>Pogonophryne barsukovi</i>	SO (8)	benthic ⁱ
<i>Pogonophryne scotti</i>	SO (10)	benthic ^{b,i}
<u>Bathydraconidae</u>		
<i>Gymnodraco acuticeps</i>	AP (15)	benthic ^b
<i>Parachaenichthys charcoti</i>	SO (11)	benthic ^b
<u>Channichthyidae</u>		
<i>Chaenocephalus aceratus</i>	AP (10), SO (10)	benthic ^{b,j}
<i>Chaenodraco wilsoni</i>	AP (10)	pelagic ^k
<i>Champscephalus gunnari</i>	AP (11), SO (10)	pelagic ^{b,j}
<i>Chionodraco rastrospinosus</i>	AP (10), SO (10)	benthic ^b , benthopelagic ^l
<i>Cryodraco antarcticus</i>	AP (10), SO (10)	pelagic ^b , benthic ^j
<i>Neopagetopsis ionah</i>	AP (6), SO (6)	pelagic ^j
<i>Pseudochaenichthys georgianus</i>	SO (10)	pelagic ^{b,j} , semipelagic ^b

^aDeWitt *et al.* (1990); ^bEastman (1993); ^cKlingenberg & Ekau (1996); ^dKock (1992); ^eKunzmann & Zimmermann (1992); ^fKuhn *et al.* (2009); ^gLa Mesa *et al.* (2004); ^hBrenner *et al.* (2001); ⁱLombarte *et al.* (2003); ^jKock (2005b); ^kKock *et al.* (2008); ^lHureau (1985a)

AP, Antarctic Peninsula, SO, South Orkney Islands, SSI, South Sandwich Islands

DNA extraction, amplification, sequencing and alignment

Genomic DNA from approx. 10 mm³ white muscle tissues was extracted by proteinase K digestion, followed by sodium chloride extraction and ethanol

precipitation. Marker selection was based on the genome-wide marker comparison of Li *et al.* (2007). We included a fast-evolving gene (*myh6*), a gene evolving at intermediate rates (*Ptr*) and a slowly evolving gene (*tbr1*). As a representative mitochondrial marker (mtDNA), we used cytochrome b (*cyt b*), which had previously been proven suitable for phylogenetic analyses in notothenioids (Chen *et al.* 1998; Matschiner *et al.* 2011). Nuclear markers were amplified with the following primer pairs: *myh6_F507/myh6_R1325*, *Ptr_F458/Ptr_R1248* and *tbr1_F86/tbr1_R820* (Li *et al.* 2007); the amplification of *cyt b* was performed using the primers *NotCytBf* and *H15915n* (Matschiner *et al.* 2011). Sequences of the three outgroup species and *Pogonophryne scotti*, as well as *Ptr* sequences of *Notothenia coriiceps* and *Trematomus newnesi* were obtained from GenBank (see Data accessibility and Table IV-S4, Supporting information).

The gene fragments were amplified using different polymerase chain reaction (PCR) protocols. *Cyt b*, *myh6* and *Ptr* PCR products were achieved using the Finnzymes' Phusion[®] High-Fidelity DNA Polymerase (Finnzymes). Individual reaction volumes contained 8.6 μL ddH₂O, 10.0 μL 2 x Phusion[®] Master Mix with HF Buffer [containing 0.04 U/ μL Phusion[®] DNA Polymerase, 2 x Phusion[®] HF Buffer, 400 μM of each deoxynucleotides (dNTP)], 0.2 μL forward primer, 0.2 μL reverse primer and 1.0 μL DNA template. The PCR profiles included initial denaturation (30 s, 98 °C), followed by 30 (*cyt b*) or 40 cycles (*myh6*, *Ptr*) of denaturation (10 s, 98 °C), annealing (30 s, 56 °C) (53 °C for *Ptr*), extension (30 s, 72 °C) and a final extension phase (10 min, 72 °C). *Tbr1* amplification was achieved using REDTaq[®] DNA Polymerase (Sigma-Aldrich). The PCR mixes contained 5.5 μL ddH₂O, 1.25 μL 10x Taq buffer (Sigma-Aldrich), 1.0 μL MgCl₂, 1.25 μL dNTP mix, 1.0 μL forward primer, 1.0 μL reverse primer, 0.5 μL REDTaq[®] DNA Polymerase (Sigma-Aldrich) and 1.0 μL DNA template. Amplifications of *tbr1* were carried out using the following temperature profile: initial denaturation (2 min, 94 °C) followed by 32 thermocycles of denaturation (30 s, 94 °C), annealing (30 s, 57 °C), extension (1 min, 72 °C) and a final extension phase (7 min, 72 °C). All amplification products were purified using the ExoSAP-IT (USB) standard protocol, adding 0.5 μL ExoSAP-IT and 3.5 μL ddH₂O to 2.5 μL PCR templates, incubating (15 min, 37 °C; 15 min, 80 °C) and, in some cases, using the GenElute[™] Gel Extraction Kit (Sigma-Aldrich). The purified PCR products were used as templates for cycle sequencing reactions with the BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's instructions. The reaction volumes included 0.5 μL primer, 1.0 μL BigDye[®] Terminator Reaction Mix (Applied Biosystems) and 3.0–6.5 μL purified DNA in a total volume of 8 μL . The nuclear markers were sequenced with one forward and reverse primer each. Sequencing of *cyt b* was additionally performed with two different forward primers: *NotCytBf* (Matschiner *et al.* 2011) and *cytbcentralF* (5'-CYA CCC TNA CYC GYT TCT TTG C -3'), which was newly designed to bind at a central position of *cyt b* (bases 518–539 in *cyt b* of *Chionodraco rastrospinosus*). The reaction conditions were as follows: initial denaturation (1 min, 94 °C) followed by 25 cycles of denaturation (10 s, 94 °C), annealing (20 s, 52 °C) and elongation phase (4 min, 60 °C). Unincorporated BigDye[®] terminators were removed with the BigDye[®] XTerminator[™] Purification Kit (Applied Biosystems). To this end, 14.5 μL ddH₂O, 22.5 μL SAM[™] solution and 5.0 μL XTerminator[™] beads were added to the sequencing products, then shaken (30 min, 2000 rpm), and finally centrifuged (2 min, 211 g). All sequences were read with an

ABI3130x/ Capillary Sequencer (Applied Biosystems). Sequence reads were verified by eye, and forward and reverse fragments were assembled using CODONCODE ALIGNER v.3.5.6 (CodonCode Corporation).

All sequences were aligned per locus with the multiple sequence alignment program MAFFT v.6.717b (Kato & Toh 2008). The alignments were trimmed in MESQUITE v.2.72 (Maddison & Maddison 2009) so that each alignment started and ended with codon triplets, and we also checked for stop codons. Alignments were concatenated and partitioned by molecule type and codon position to account for heterogeneity in evolutionary rates and substitution patterns. Thus, the first and second codon positions of mitochondrial *cyt b* ('mit12'), the third codon positions of mitochondrial *cyt b* ('mit3'), the first and second codon positions of nuclear genes ('nuc12') and the third positions of nuclear genes ('nuc3') were used as separate partitions. In a second partitioning scheme, the data set was partitioned with respect to the four genes. The best-fitting models of molecular evolution for each of the eight partitions were estimated with the computer program JMODELTEST v.0.1.1 (Posada 2008), using the Bayesian information criterion (BIC; Schwarz 1978). Selected models were TPM2uf+G (*myh6*), K80+G (*Ptr*), HKY+I (*tbr1*), TrN+G+I (*cyt b*), HKY+I+G (mit12), K80+I (nuc12) and TrN+G (mit3, nuc3).

Phylogenetic analysis

Phylogenetic tree reconstructions were carried out using maximum-likelihood (ML) and Bayesian inference (BI) approaches. Maximum-likelihood phylogenetic inference was performed with both partitioning schemes, applying the respective models of molecular evolution for each partition, in a partition-enabled version of GARLI, GARLI-PART v.0.97 (Zwickl 2006). Heuristic searches were used to find the topology with the best likelihood score. The searches were conducted using automatic termination, after a maximum of 5 million generations, or, alternatively, after 10 000 generations without significant ($P < 0.01$) improvement in scoring topology. Bootstrap (BS) analysis was performed with 100 BS replicates, which were summarized using PAUP* v.4.0a110 (Swofford 2003). The non-Antarctic notothenioid species *Bovichtus diacanthus* was defined as outgroup on the basis of well-supported phylogenetic information (e.g. Near & Cheng 2008; Matschiner *et al.* 2011).

Bayesian phylogenetic analyses were performed with the software BEAST v.1.5.3 (Drummond & Rambaut 2007). For divergence date estimation, the separation of Bovichtidae, Pseudaphritidae and Eleginopidae from the Antarctic lineage (nodes A, B, and C in Fig. IV-3), as well as the initial diversification of the Antarctic clade (node D) were temporally constrained according to the results of Matschiner *et al.* (2011). Specifically, normal prior distributions were used for each of these splits to approximate highest posterior density (HPD) intervals found by Matschiner *et al.* (2011). Thus, the root of Notothenioidei (node A) was constrained with a mean divergence prior to 71.4 million years ago (Ma; 2.5% quantile: 89.1 Ma, 97.5% quantile: 53.8 Ma), and nodes B-D were constrained at 63.0 (79.5–46.6) Ma, 42.9 (56.5–29.4) Ma and 23.9 (31.3–16.4) Ma, respectively. While these time constraints generally agree with the interpretation of *Proeleginops grandeastmanorum* from the La Meseta Formation on Seymour Island (~40 Ma; Eastman & Grande 1991) as an early representative of the eleginopid lineage (Balushkin 1994), we deliberately avoided using it as a time constraint owing to its debated taxonomical assignment

(Near 2004). With the exception of outgroup relationships, which were used for time calibration, no topological constraints were applied. Divergence dates were estimated using the uncorrelated lognormal relaxed molecular clock and the reconstructed birth-death process as a tree prior (Gernhard 2008). Following Shapiro *et al.* (2006), we implemented the codon position-specific model of sequence evolution HKY₁₁₂ + CP₁₁₂ + Γ_{112} , but we furthermore tested GTR₁₁₂ + CP₁₁₂ + Γ_{112} and the model combination selected by BIC for codon-specific partitions. For each of the three combinations, 10 independent analyses were performed with 20 million generations each. Replicates were combined in LOGCOMBINER v.1.5.3 (Drummond & Rambaut 2007) after removing the first 2 million generations of each run as burn-in. Convergence of run replicates was verified by effective sample sizes > 1200 for all parameters and by comparison of traces within and between replicates in TRACER v.1.5 (Drummond & Rambaut 2007). The three settings were compared with Bayes factors (BF), using the harmonic mean approach as implemented in TRACER. While we acknowledge that the harmonic mean estimator may be biased towards more parameter-rich models (Lartillot & Philippe 2006), we chose this approach owing to the lack of suitable alternatives. As the inclusion of multiple individuals per species may violate assumptions of constant diversification implicit in the birth–death tree prior, BI analyses were repeated with a reduced data set containing only one individual of each species.

Stable isotope analysis

In this study, approximately 10 mm³ of white muscle tissue was used for the SIA. White muscle tissue is less variable with regard to the carbon and nitrogen isotope composition and has a longer retention time than other tissue types (Pinnegar & Polunin 1999; Quevedo *et al.* 2009). Samples were dried (24 h, 60 °C) and then ground in a Zirconia bead mill (30 min, 1800 bpm). Then, the sample powder was rinsed from the beads using 1 mL 99% ethanol, and the supernatant was evaporated (24 h, 60 °C). The ethanol treatment had no effect on subsequent carbon isotope analyses (e.g. Syväranta *et al.* 2008). For C and N isotope measurements, between 0.5 and 0.8 mg sample powder was filled into 5 x 9 mm tin capsules and introduced into an elemental analyser (Thermo Finnigan) coupled to a Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer, with standard setup for N₂ and CO₂ analysis. Measurements were replicated for about 10% of the samples (42 samples). The isotopic composition is expressed in the conventional delta notation as permil (‰) deviation vs. atmospheric N₂ (AIR) and carbonate standards (V- PDB): $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, with R representing the ratio of the heavy to the light isotope (i.e. ¹³C/¹²C and ¹⁵N/¹⁴N) in the sample and in the standard material, respectively. EDTA ($\delta^{13}\text{C} = -30.25\text{‰}$, $\delta^{15}\text{N} = -1.1\text{‰}$) and ammonium oxalate ($\delta^{13}\text{C} = -17.02\text{‰}$, $\delta^{15}\text{N} = 32.7\text{‰}$) were used as internal standards, calibrated against international nitrogen (IAEA-N1, IAEA-N2) and carbon (NBS22) standards. The analytical reproducibility based on replicate sample and standard measurements was better than 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Isotope values are presented as mean \pm standard deviation (SD). Variable lipid content can have a biasing effect on the interpretation of bulk C and N stable isotope data. In marine fish samples, this effect seems to be minor (Kiljunen *et al.* 2006; Logan *et al.* 2008), and hence, we did not perform a lipid removal step. Nevertheless, we performed a posteriori ‘mathematical lipid correction’ after the study of Logan *et al.* (2008). The correction, however, did not

affect the species distribution pattern, and thus, only the uncorrected values are presented in this study. (The corrected data set is available upon request.)

Statistical analysis

The correlation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was tested with a Pearson correlation, whereby we accounted for phylogenetic non-independence using phylogenetic independent contrast ('pic' function in the R package 'ape'; Paradis *et al.* 2004; R Development Core Team 2009). We tested for the effect of geographic sites on isotopic signatures by comparison of pooled $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between AP and SO (*t*-test). Here, only values from species with similar sample sizes at both locations were considered. Pairwise niche overlap between all families and additional comparisons of the nototheniid *Lepidonotothen–Trematomus* clade with the other families were tested with a multivariate analysis of variance (MANOVA). To assess the group overlap in isotopic signatures, we calculated Wilk's lambda (Wilk's λ) for each comparison.

We analysed the subdivision of ecological niche space throughout the radiation using the BI phylogeny (Fig. IV-3) and the averaged stable isotope data for each species. Average subclade disparity was calculated at each splitting event and plotted against time. A Brownian motion (BM) model of trait evolution was employed for comparison. Disparity-through-time analyses were conducted in R using the package 'geiger' (Harmon *et al.* 2008). Using 475 trees drawn from the posterior distribution of the BI analysis and 500 permutations of the stable isotope data, we assessed the robustness of the observed pattern against phylogenetic uncertainty and intraspecific variation.

Results

Phylogenetic analysis

The alignments had lengths of 1099 bp (*cyt b*), 705 bp (*myh6*), 702 bp (*Ptr*) and 642 bp (*tbr1*), resulting in a total of 3148 bp with only 0.3% missing data. The *myh6* alignment contained a short insertion (6 bp) in the non-Antarctic outgroup *B. diacanthus*; these 6 bp were excluded from the following phylogenetic analyses. Sequences are available at GenBank under the accession numbers JF264479–JF264629. Bayes factors provided 'very strong' (Kass & Raftery 1995) evidence that the codon position-specific combination of substitution models selected by BIC yielded a better fit than both the $\text{HKY}_{112} + \text{CP}_{112} + \Gamma_{112}$ (log 10 BF 6.215) and $\text{GTR}_{112} + \text{CP}_{112} + \Gamma_{112}$ (log 10 BF 19.19) models.

Our ML and BI phylogenetic analyses produced identical topologies and confirmed the monophyly of the Antarctic clade with high support values (BS 100%; Fig. IV-2, Fig. IV-S1, Supporting information). Yet, BS support and Bayesian posterior probability (BPP) were low at the base of the diversification of the Antarctic clade (but high at species-level relationships). In all cases, clustering of individuals from different populations of the same species was strongly supported (BS \geq 93% and BPP = 1.00). The three families Artedidraconidae, Bathydraconidae and Channichthyidae were recovered as monophyletic, while the Nototheniidae appeared paraphyletic. An ancestral position was assigned to *Aethotaxis mitopteryx*. The monophyly of a clade containing *Lepidonotothen* and *Trematomus* was highly supported (BS 100% and BPP 1.00), and *Notothenia* appeared as the sister group to the more derived 'high-Antarctic clade', comprising the families Artedidraconidae, Bathydraconidae and

Channichthyidae. Both the high-Antarctic clade and the channichthyid family were found monophyletic with BS 100% and BPP 1.00. The two artedidraconids, *P. barsukovi* and *P. scotti*, grouped together in all analyses (with high support values). Monophyly of the two bathydraconid representatives was weakly supported (BS 35% and BPP 0.67). Within the family of Channichthyidae, *Champocephalus gunnari* was placed as sister species of all other representatives followed by a clade containing *Pseudochaenichthys georgianus* and *Neopagetopsis ionah* and a clade containing the four genera *Chionodraco*, *Chaenodraco*, *Chaenocephalus* and *Cryodraco*. The ML reconstruction with gene-specific partitions resulted in minor topological differences (Fig. IV-S1, Supporting information). Reduction in the data set to one individual per species did not change the tree topology with the exception of *Dissostichus mawsoni*, which appeared basal to a group containing the high-Antarctic clade as well as *Notothenia*, *Pleuragramma* and *Gobionotothen* and the relationships within the *Trematomus* genus (Fig. IV-S1, Supporting information).

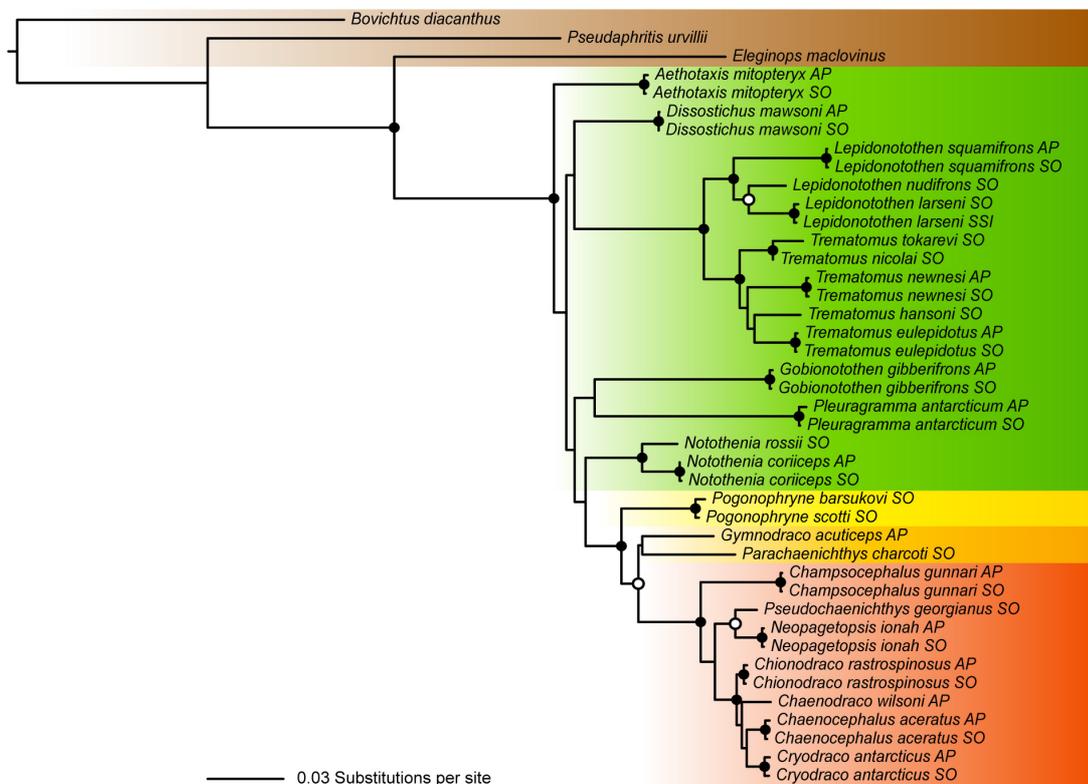


Fig. IV-2 Maximum-likelihood tree of the nototheniid phylogeny based on the codon position-specific partitioning scheme. *Filled circles* indicate strongly supported nodes, and moderately supported nodes are marked by *open circles*. Bootstrap (BS ≥ 95 and BS ≥ 70). All species are coloured according to family: *brown* = non-Antarctic species, *green* = Nototheniidae, *yellow* = Artedidraconidae, *orange* = Bathydraconidae and *red* = Channichthyidae

According to our time-calibrated phylogeny, diversification of the well-supported nototheniid clade combining *Lepidonotothen* and *Trematomus* began 12.0 Ma (95% HPD 16.4–7.9 Ma; node H) (Fig. IV-3). The high-Antarctic clade separated from the Nototheniidae around 18.6 Ma (95% HPD 24.0–13.4 Ma; node E). Within the high-Antarctic clade, artedidraconids separated from bathydraconids and channichthyids around 14.6 Ma (95% HPD 15.5–7.0 Ma; node F). The split between Bathydraconidae and Channichthyidae occurred around 2 million years later (12.5 Ma; 95% HPD 16.7–

8.5 Ma; node G). The radiation of Channichthyidae, the most derived notothenioid family, began 7.7 Ma (95% HPD 10.6–5.0 Ma; node I).

Stable C and N isotope ratios

The stable carbon and nitrogen isotope composition for the 25 notothenioid species exhibited a comparatively large variability, with values between -27.8‰ and -19.7‰ for $\delta^{13}\text{C}$ and between 7.3‰ and 15.6‰ for $\delta^{15}\text{N}$ (Fig. IV-3). Mean values ranged between -25.4‰ and -21.9‰ for $\delta^{13}\text{C}$ (SD: 0.3‰ to 1.8‰) and 8.5‰ to 13.8‰ for $\delta^{15}\text{N}$ (SD: 0.2‰ to 1.7‰ ; Fig. IV-4). Intraspecific ranges of isotopic signatures span from 1.0‰ to 8.1‰ for $\delta^{13}\text{C}$ and from 0.4‰ to 5.7‰ for $\delta^{15}\text{N}$. Overall, mean intraspecific ranges ($\delta^{13}\text{C}$: 2.79‰ , $\delta^{15}\text{N}$: 2.80‰) were small compared to interspecific ranges of isotopic signatures ($\delta^{13}\text{C}$: 8.12‰ , $\delta^{15}\text{N}$: 8.29‰). The isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ correlated significantly (0.69 ; $P < 0.001$), and the correlation remained significant ($P < 0.01$) after correcting for phylogenetic non-independence. No significant difference between values from AP and SO locations was found ($P > 0.16$; t -test), even though the mean values differed slightly (AP $\delta^{13}\text{C}$: -24.37‰ , SO $\delta^{13}\text{C}$: -24.13‰ ; AP $\delta^{15}\text{N}$: 11.30‰ , SO $\delta^{15}\text{N}$: 10.99‰).

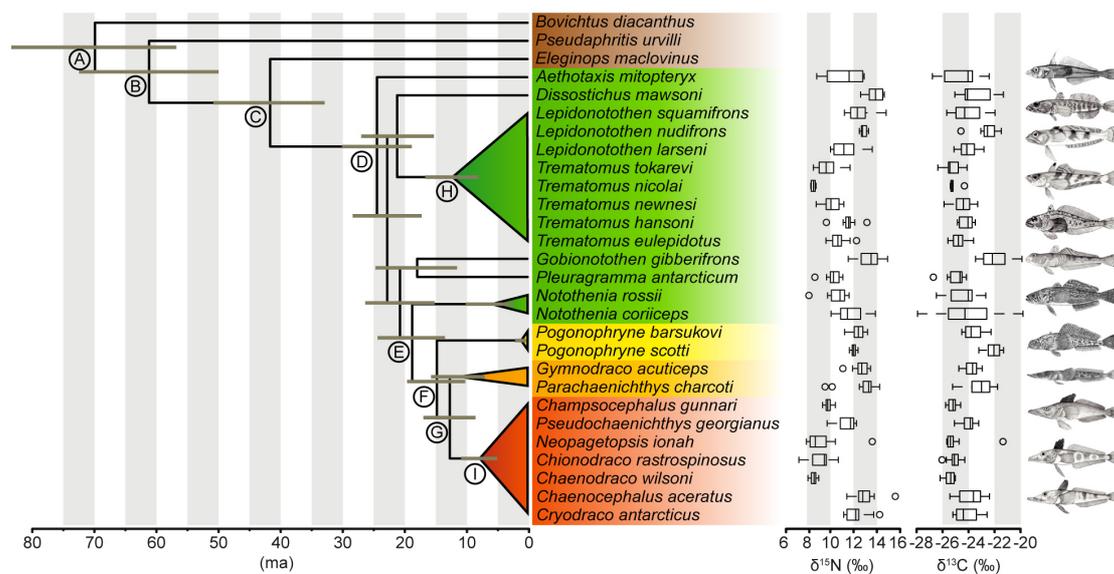


Fig. IV-3 Left: Time-calibrated phylogeny based on codon-specific partition, inferred with Bayesian inference. Time axis is given in million years ago and nodes labelled A-I are mentioned in the text. Grey node bars indicate upper and lower 95% HPD. All species are coloured according to family: *brown* = non-Antarctic species, *green* = Nototheniidae, *yellow* = Artedidraconidae, *orange* = Bathydraconidae and *red* = Channichthyidae. Right: Boxplot of stable isotope values of all included notothenioids. Representative habitus are illustrated at the right, from top to bottom: *Aethotaxis mitopteryx*^d, *Dissostichus mawsoni*^d, *Lepidonotothen nudifrons*^d, *Lepidonotothen larseni*^d, *Trematomus tokarevi*^d, *Gobionotothen gibberifrons*^d, *Notothenia rossii*^b, *Pogonophryne barsukovi*^c, *Gymnodraco acuticeps*^a, *Parachaenichthys charcoti*, *Champocephalus gunnari*, *Pseudochaenichthys georgianus*^e, *Neopagetopsis ionah*, *Chionodraco rastrospinosus*^e and *Chaenocephalus aceratus*^e. ^aBoulenger (1902); ^bDeWitt *et al.* (1990); ^cEakin (1990); ^dHureau (1985a); ^eHureau (1985b)

With regard to inferred lifestyle patterns, our SIA data are consistent with previous studies (Hobson *et al.* 1994; Post 2002) in that species that are commonly classified as pelagic clustered around lower $\delta^{13}\text{C}$ values, while benthic species possessed

relatively higher $\delta^{13}\text{C}$ signatures. However, there are notable exceptions to this: *D. mawsoni*, *C. rastropinosus*, *Trematomus nicolai* and *T. tokarevi* (Fig. IV-4, Table IV-1

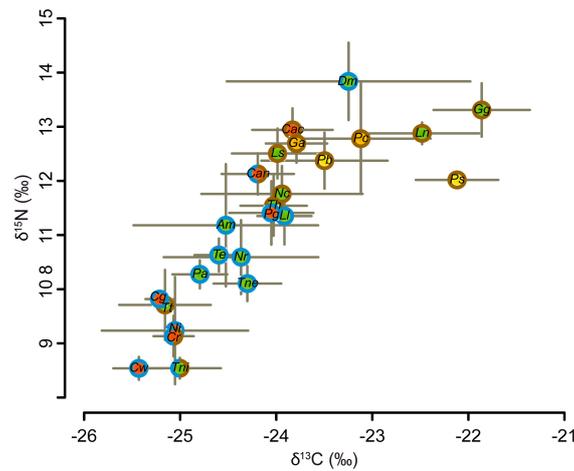


Fig. IV-4 Scatter plot of carbon and nitrogen isotopic values. Grey bars indicate 95% confidence intervals. All species are coloured according to family (*brown*: non-Antarctic species, *green*: Nototheniidae, *yellow*: Artedidraconidae, *orange*: Bathydraconidae, *red*: Channichthyidae), and strokes indicate corresponding lifestyle [*blue* = pelagic, benthopelagic, semipelagic and epibenthic; *brown* = benthic; and semicircles when references (Table V-1) disagree]

and Data IV-S1, Supporting information). Most species had relatively high $\delta^{15}\text{N}$ signatures, indicating feeding at upper TL. The two well-represented families Nototheniidae and Channichthyidae covered a wide range of isotopic signatures, while bathydraconids and artedidraconids displayed a relatively low variability in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (although the number of individuals was significantly lower). Overlap of the C and N isotope compositions as proxies for niche space was found in all pairwise comparisons (MANOVA) of the four Antarctic notothenioid families (Table IV-2). Wilk's λ was largest for comparisons of Nototheniidae with all other families ($\lambda > 0.91$; Table IV-2),

and lower values were found for comparisons including the lesser-represented families Artedidraconidae and Bathydraconidae ($\lambda > 0.68$). Notably, within-family variation resulted mostly from interspecific variation, instead of intraspecific variation, and closely related species with small intraspecific variation could be found at both ends of the ranges (e.g. *T. nicolai* and *Lepidonotothen nudifrons*; Fig. IV-3).

Table IV-2 Pairwise niche overlap comparisons for the four Antarctic notothenioid families, performed with MANOVA (Wilk's λ)

Family 1	Family 2	Wilk's λ
Artedidraconidae	Nototheniidae	0.936
	<i>Lepidonotothen-Trematomus</i> clade	0.791
Bathydraconidae	Nototheniidae	0.913
	<i>Lepidonotothen-Trematomus</i> clade	0.818
Channichthyidae	Nototheniidae	0.930
	<i>Lepidonotothen-Trematomus</i> clade	0.932
Artedidraconidae	Bathydraconidae	0.681
Artedidraconidae	Channichthyidae	0.629
Bathydraconidae	Channichthyidae	0.781

Using the DTT method, we assessed how the stable isotope space (as a proxy for ecological niche space) used by the whole clade was subdivided by smaller and smaller subclades as the radiation proceeded. We find positive deviations from the averaged neutral-evolution BM model, indicating larger overlap in niche space between subclades than would be expected if evolution proceeded neutrally (Fig. IV-5). This result was found to be robust against phylogenetic uncertainty and intraspecific variation by visual inspection of repeated DTT analyses.

Discussion

Phylogenetic relationships

Previous molecular phylogenetic analyses of notothenioids were based on mitochondrial DNA sequences (Bargelloni *et al.* 2000; Stankovic *et al.* 2002; Near 2004; Near *et al.* 2004), on a combination of mtDNA with a single nuclear gene (Near & Cheng 2008) or on morphological characters in addition to molecular data (Derome *et al.* 2002; Sanchez *et al.* 2007). The family-level phylogeny of notothenioids is thus relatively well established. Several questions remain, however, such as the position of the genus *Gobionotothen* (Near *et al.* 2004; Sanchez *et al.* 2007; Near & Cheng 2008) or whether Bathydraconidae are mono- or paraphyletic (e.g. Derome *et al.* 2002; Near & Cheng 2008).

In agreement with most previous studies (e.g. Near 2004; Near & Cheng 2008), our results support paraphyly of the family Nototheniidae. The low support values at the beginning of the Antarctic diversification are characteristic for rapid diversifications. Consequently, the basal position of *D. mawsoni* and the sister species relationships of

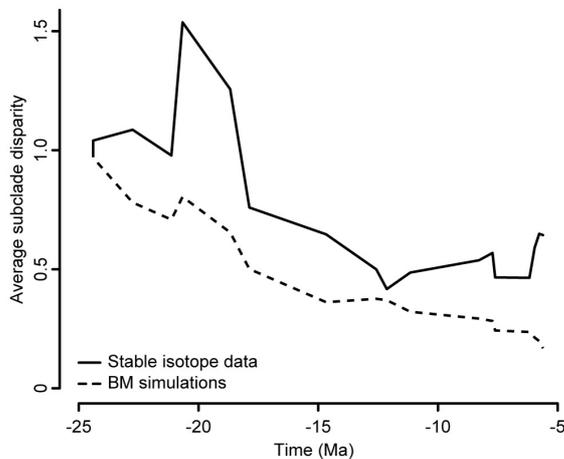


Fig. IV-5 Disparity-through-time plot for the stable isotopic signatures of Antarctic notothenioid fishes and Brownian motion simulations of character evolution. Time axis is given in million years ago

(Sanchez *et al.* 2007; Kuhn & Near 2009), except for *T. tokarevi* and *T. nicolai*, which appeared at basal positions in the phylogeny based on codon position-specific substitution models (Fig. IV-2, Fig. IV-S1, Supporting information). The early split of the two included bathydraconid species relative to the divergence between Bathydraconidae and Channichthyidae could indicate paraphyly of the former, as was concluded in previous studies (e.g. Derome *et al.* 2002; Near *et al.* 2004; Near & Cheng 2008). Resulting support values within the channichthyids were high, and the recovered topology was in complete agreement with the study of Derome *et al.* (2002). The three genera *Champscephalus*, *Neopagetopsis* and *Pseudochaenichthys* seem to be well established as the most basal channichthyids (Chen *et al.* 1998; Near *et al.* 2003). In disagreement with former findings, *C. rastrospinosus* and *Chaenodraco wilsoni* did not cluster monophyletically (Chen *et al.* 1998). Near *et al.* (2003) also recovered these two species as paraphyletic but placed *Chaenocephalus aceratus* as the sister taxon to the genera *Cryodraco*, *Chaenodraco* and *Chionodraco*, which disagrees with our findings. Near & Cheng (2008) determined *C. aceratus* as the closest related species of *C. rastrospinosus*.

G. gibberifrons and *Pleuragramma antarcticum* remain questionable. As in previous studies (Near 2004; Near & Cheng 2008), the three neutrally buoyant species *A. mitopteryx*, *D. mawsoni* and *P. antarcticum* diverged early within the Antarctic clade but did not cluster together. Phylogenetic relationships of the two genera *Notothenia* and *Lepidonotothen* are consistent with former studies (Bargelloni *et al.* 2000; Near & Cheng 2008). Also, the topology of the nototheniid subfamily Trematominae agrees with previous findings

Inferred split dates (Fig. IV-3) roughly agree with those found by Near (2004) and Matschiner *et al.* (2011): Divergence estimates for the *Lepidonotothen–Trematomus* clade and the high-Antarctic clade were 12.0 (95% HPD 16.4–7.9) Ma and 18.6 (95% HPD 24.0–13.4) Ma, respectively, while Near (2004) reported them to be 14 ± 0.4 Ma and Matschiner *et al.* (2011) found these splits at 10.3 (95% HPD 15.2–6.1) Ma and 14.7 (95% HPD 20.0–9.9) Ma. According to our estimates, the radiation of the Channichthyidae began 7.7 (95% HPD 10.6–5.0) Ma ago, in good agreement with the estimates of Near (2004) (8.5 ± 0.3 Ma) and Matschiner *et al.* (2011) (6.2 Ma; 95% HPD 9.4–3.4 Ma).

Foraging ecology of notothenioids

So far, it has been shown that some particular feeding strategies are poorly represented or even absent in notothenioids, such as active skeleton-breaking predation (Clarke *et al.* 2004) or planktivory (Eastman & Grande 1989; Eastman 1993). The latter is probably due to restricted phytoplankton production during the austral winter (Clarke *et al.* 2004). The drawback of traditional dietary proxies (stomach content analyses and foraging observations) is that they only capture a snapshot of food uptake. Contrarily, SIA provides time-integrated information on the feeding ‘ecology’ for a period of weeks to years (McIntyre & Flecker 2006). Isotopic signatures could theoretically be influenced by geographic differences, sampling season and the age of sampled individuals, especially when ontogenic shifts occur in the investigated species. However, our sampling design accounted for these potential problems, as only adult specimens were collected, and all expeditions took place during austral summers. Also, most species were collected at the same two sampling locations, AP and SO, and populations from these two sites did not differ in isotopic signatures. Thus, the observed interspecific differences suggest ecological specialization rather than effects of geographical distribution or life history traits.

Our SIA data confirm that notothenioids occupy a wide variety of ecological niches (Figs IV-3 and IV-4). Comparatively high $\delta^{15}\text{N}$ values suggest that most investigated species reside at a high TL and may be considered tertiary consumers (see also Dunton 2001; Pakhomov *et al.* 2006). The wide range of the carbon stable isotope signatures reflects the notothenioids’ variety in habitats along the benthic-pelagic axis (Fig. IV-4). However, our results are only partly congruent with the lifestyles and feeding reports based on stomach content analyses (Fig. IV-4, Table IV-1, Table IV-S3 and Data IV-S1, Supporting information).

At the family level, Nototheniidae are – in terms of habitat and feeding strategies – the most diverse clade among Antarctic notothenioids (La Mesa *et al.* 2004, this study) and include plankton, nekton and benthos feeders, as well as species that combine several feeding modes (Gröhsler 1994). The five included *Trematomus* species were differentiated in both isotopic signatures, thus indicating trophic niche separation (see also Brenner *et al.* 2001). Artedidraconids and bathydraconids represent the most benthic families among notothenioids (Fig. IV-4; Olaso *et al.* 2000; La Mesa *et al.* 2004). Their $\delta^{15}\text{N}$ values suggest feeding habits at higher TL (Olaso *et al.* 2000; Jones *et al.* 2009). The well-studied channichthyids clustered into three groups according to their diet (Kock 2005b). Carbon signatures indicated a rather pelagic lifestyle for most channichthyid species, with the exception for *C. aceratus*, which we

can classify as benthic top predator, in agreement with previous findings (Kock 2005b; Reid *et al.* 2007).

The DTT plot (Fig. IV-5) indicates larger overlap of subclades in niche use than expected from a model of neutral evolution. This is characteristic for adaptive radiations (Harmon *et al.* 2003; Gonzalez-Voyer *et al.* 2009) and differs from patterns of putative nonadaptive radiations, which show a negative deviation from the averaged neutral-evolution BM model (e.g. Rowe *et al.* 2011). Taking into account the considerable variation in stable isotope signatures found in notothenioids as a whole (Fig. IV-4) – basically ruling out stasis in the evolution of niche use – as well as the robustness of this pattern against intraspecific variation, these results suggest convergent evolution in niche use between species of notothenioid subclades, especially between those clades separating around 20 Ma (Figs IV-3 and IV-5). This emphasizes the importance of ecological niche differentiation in the adaptive radiation of notothenioids.

Adaptive radiation and ecological diversification in notothenioids

Our integrative analyses, combining both the phylogenetic relationships and the isotopic signatures of 25 notothenioid species, reveal that ecological diversification into overlapping feeding niches has occurred multiple times in parallel in different notothenioid families (Figs IV-3 and IV-5). Using carbon and nitrogen stable isotope ratios as indicators of TL, feeding strategy and macrohabitat, we find great variation within, and substantial overlap between the more basal nototheniids and the derived channichthyids. The representatives of the benthic artedidraconids and bathydraconids also overlap and cluster at high TLs and $\delta^{13}\text{C}$ values. Our results further confirm partitioning of habitat and trophic resources within notothenioid fishes, indicating that diversification along the benthic–pelagic axis and to different TLs took place independently in at least two of five notothenioid families of the Antarctic clade (Nototheniidae and Channichthyidae; Fig. IV-3 and Table IV-2).

Convergent diversification in habitat and trophic ecology suggests interspecific competition and is a characteristic of adaptive radiations (e.g. Losos 1995; Schluter 2000). For example, *Anolis* lizards of the Caribbean have independently evolved four to six so-called ecomorphs on each of the four large islands of the Greater Antilles, including species specialized to live on grass, twigs, trunks and tree crowns. Variation in limb lengths of anole ecomorphs supports these different lifestyles, so that e.g. the trunk-ground ecomorph possesses relatively long legs adapted to running and jumping on broad surfaces, while the twig ecomorph has short legs and moves slowly on narrow surfaces (Losos 2009). In this context, diversification of notothenioids along the benthic-pelagic axis, as evidenced by their isotopic composition, and the respective adaptations in buoyancy (Eastman 1993) can be considered analogous to the *Anolis* diversification along the ground-tree axis. The notothenioid adaptive radiation shows further analogies to that of Caribbean anoles in terms of species richness (both around 120 species) and age (about 24 and 15–66 Ma, respectively) (Fig. IV-3; Eastman 2005; Nicholson *et al.* 2005; Losos 2009; Matschiner *et al.* 2011). Not all descendents of the *Anolis* radiation remained within the confined area of the radiation (Nicholson *et al.* 2005), and neither did the notothenioids: *Notothenia angustata*, *N. microlepidota* and the genus *Patagonotothen* secondarily escaped Antarctic waters and occur in New Zealand and South America (Eastman 2005).

Moreover, both radiations were probably triggered by key innovations: subdigital toepads support the particular arboreality of *Anolis* lizards, whereas antifreeze glycoproteins in blood and tissues allow notothenioid survival in ice-laden Antarctic waters (Chen *et al.* 1997; Losos 2009; Matschiner *et al.* 2011).

Compared to another well-studied adaptive radiation, that of cichlid fishes in East African lakes, the rate at which lineage formation seems to have occurred is much smaller in Antarctic notothenioids. In the Great Lakes of East Africa, cichlid fishes have diversified into at least 1500 species that differ greatly in naturally and sexually selected traits, including body shape, mouth morphology and colouration (Salzburger 2009). Comparison of cichlid species flocks between East African lakes, as well as mathematical models, have shown that larger habitats effectuate higher diversification rates, as they provide greater habitat heterogeneity and facilitate isolation by distance ('area effect'; Salzburger & Meyer 2004; Gavrillets & Vose 2005; Seehausen 2006). Different adaptive radiations may not be directly comparable as they depend on many ecological, genetic and developmental factors, with an important contribution of historical contingencies (Gavrillets & Losos 2009). Cichlids are known for their philopatry and low dispersal abilities (Danley & Kocher 2001; Salzburger & Meyer 2004), whereas most notothenioids have prolonged pelagic larval stages, enhancing long-range migration (Eastman 1993). Notothenioid populations are characterized by fragmented habitat, historical demographic fluctuations (Patarnello *et al.* 2011) and the absence of genetic structuring over large distances (Matschiner *et al.* 2009; and references therein), whereas many cichlid species possess significant population structuring even on extremely small scales (e.g. Arnegard *et al.* 1999; Rico & Turner 2002). Genetic differentiation over small scales has rarely been found in notothenioids (but see Clément *et al.* 1998). Eastman & McCune (2000) suggested that the smaller species number of notothenioids, compared with cichlid species flocks, could be explained by the absence of certain prime inshore habitats in the Southern Ocean. Alternatively, the notothenioid adaptive radiation may not yet have entered its final stage, namely the diversification with respect to communication. Streelman & Danley (2003) suggested a three-stage model of adaptive radiation (see also Danley & Kocher 2001), in which diversification first occurs with respect to macrohabitats, then with respect to microhabitats and finally with respect to communication (e.g. mating traits such as colouration; see also Gavrillets & Losos 2009). Full species richness would only be achieved through this final step. Streelman & Danley (2003) further suggested that divergence of habitat and trophic morphology is driven by natural selection, whereas diversification along the axis of communication is forced by sexual selection. It is as of yet unclear whether the radiation of notothenioids followed discrete stages. Here, we provide conclusive evidence that the species are separated along the benthic-pelagic axis (i.e. according to macrohabitats; Figs IV-3 and IV-4) and probably also as a function of bottom topography and sediment types (Kock & Stransky 2000). Much less is known about microhabitat diversification, although our data suggest that closely related species do differ with respect to foraging strategies (e.g. genera *Lepidonotothen* and *Trematomus*; Figs IV-3 and IV-4). Recent evidence further indicates the possibility of divergence along Streelman and Danley's axis of communication, as egg guarding and parental care were observed in all major notothenioid lineages except within the Artedidraconidae (Kock *et al.* 2006; Barrera-Oro & Lagler 2010 and references therein).

On the other hand, because of the paucity of the Antarctic fossil record, it cannot be excluded that the notothenioid radiation has already surpassed its maximum species richness. It is an important characteristic that young adaptive radiations often ‘overshoot’ in terms of species number and that, generally, niche filling causes declining speciation rates (e.g. Seehausen 2006; Gavrillets & Losos 2009; Meyer *et al.* 2011). That notothenioids already underwent periods of ‘overshooting’ and niche filling could possibly explain the smaller diversity of Notothenioidei compared to the younger cichlid radiation in the East African Lakes. However, in this case, an early burst of diversification should have left its footprint in a ‘bottom-heavy’ phylogeny (Gavrillets & Vose 2005). A more extensive study, including many more representatives of the notothenioids, would be necessary to reconstruct the succession of their adaptive radiation.

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Data accessibility

All DNA sequences from this study are available under GenBank accessions: JF264479–JF264516 (cyt *b*); JF264517–JF264554 (myh6); JF264555–JF264590 (Ptr); and JF264591–JF264629 (tbr1). GenBank accession numbers for sequences of other studies are the following: *B. diacanthus* (HM049936; HM050034; HM050153; HM050214); *E. maclovinus* (DQ526429; HM050045; HM050163; HM050225); *N. coriiceps* (HM050183); *P. urvillii* (HM049963; HM050074; HM050195; HM050258); *P. scotti* (HM049962; HM050072; HM050193); and *T. newnesi* (HM050204) (see Table IV-S4, Supporting information).

Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. IV-S1 Maximum-likelihood tree based on the codon position- specific partitioning with numbered nodes (1–19).

Table IV-S1 Antarctic notothenioid samples with corresponding collection id (Table IV-S2) and sample size (n) for stable isotope analysis.

Table IV-S2 Collection id for all Antarctic notothenioid samples.

Table IV-S3 Lifestyle and feeding for all included Antarctic notothenioid species.

Table IV-S4 GenBank accession numbers for all used samples.

Table IV-S5 Stable isotope values of all investigated species.

Data IV-S1 Discussion of stable isotope analysis results of individual species.

GENERAL DISCUSSION

The aim of this thesis was to assess population genetic structures of notothenioid fishes and to elucidate the potential of larval dispersal for gene flow and its consequences for evolutionary processes. The role of larval dispersal for gene flow was investigated in a comparative approach with sympatric sub-Antarctic species inhabiting the Atlantic sector of the Southern Ocean, to identify factors influencing gene flow and population divergences and to validate the generality of the observed patterns. Moreover, all population genetic analyses were based on multiple genetic marker types, to account for potential gene specific biases. Furthermore, it has been tested whether the ecological diversification of notothenioids resembles an adaptive radiation.

Population genetic structures and gene flow in notothenioids

Restricted gene flow despite long pelagic larval development

The most important insight from this thesis comes from the significant genetic differentiation among populations at sub-Antarctic islands separated by long stretches of deep-ocean areas, which disproves the hypothesis that the long pelagic larval stages generally result in high levels of gene flow in notothenioids. The significant population differentiations among island groups are in contrast to a large number of population genetic studies, which found non-significant population differentiations not only in the study area, but also on a circumpolar scale (see Table 1). This discrepancy can mostly be attributed to differences in the variability of employed markers and sample sizes. However, the congruent results among different marker types and species presented in this thesis, as well as reasonable sample sizes of 22-102 individuals, make the results relatively robust against gene specific biases and stochasticity. Moreover, the results from this thesis are supported by a recent comparative population genetic study on 3 trematomids with circum-Antarctic distributions based on microsatellites and mtDNA, which also strongly indicates that elevated levels of gene flow are not as common as often hypothesized for notothenioids (Van de Putte *et al.* 2012).

However, a relationship between the duration of pelagic developmental stages and population connectivity may exist. This conclusion arises from two observations: (1) significant population differentiations were mostly detected between populations separated by more than 350 km (chapters I, II, III), and (2) an isolation-by-distance (IBD) relationship exists for most species, except for *L. larseni* whose early development duration is the longest known from notothenioids (chapters II and III; Van de Putte *et al.* 2012). Table 3 shows the relationship between genetic distance (F_{ST}) and geographic distance (km), calculated by Mantel tests for the species sampled from at least three locations.

GENERAL DISCUSSION

Table 3 Isolation-by-distance results from Mantel tests with genetic distance (F_{ST}) and geographic distance (km) for microsatellites, *cyt b* and D-loop data as implemented in ARLEQUIN v3.5. Samples included for *C. aceratus* and *C. gunnari* originate from Elephant Island (EI), South Orkneys (SO), South Georgia (SG) and Bouvet Island (BO). Samples for *L. larseni* are from SO, SG and BO. R=correlation coefficient, p=probability of R based on 1000 permutations. Bold values indicate significance at the 5% level

	Microsatellites		<i>cyt b</i>		D-loop	
	R	p	R	p	R	p
<i>C. aceratus</i>	0.949	0.035	-0.014	0.495	-0.452	0.863
<i>C. gunnari</i>	0.982	0.038	0.770	0.049	-0.0309	0.646
<i>L. larseni</i>	-0.209	0.676	-0.633	0.842	-	-

For the species investigated in this thesis a relationship between geographic distance and the degree of population differentiation is only evident for *C. aceratus* (microsatellites) and *C. gunnari* (microsatellites, *cyt b* sequences), but not *L. larseni*. The last species has a 1-4 months longer larval stage duration than *C. aceratus* and *C. gunnari*, depending on the region, and a particularly longer overall pelagic development until juveniles begin a demersal life (about 4 months longer, Fig. 3). Therefore, a relationship between the duration of pelagic development of early life stages and population connectivity might exist. However, a re-examination of the IBD analyses from *C. aceratus* and *C. gunnari* with the populations of EI removed from the data set (matching the available sampling sites of *L. larseni*) resulted in insignificant IBD correlations (data not shown). Hence, further studies with an increased number of sampling sites are needed for a final conclusion on the relationship between gene flow and geographic distance. Nonetheless, geographical isolation seems to be an important factor in regulating gene flow, as populations in close proximity, for example in the southern Scotia Arc between the AP and SO, proved to be genetically similar for a large number of species (Papetti *et al.* 2009; 2012). This observation points to a minimum distance of 350 km over which pelagic developmental stages are able to successfully disperse in relatively high numbers. The rejection of the hypothesis that long pelagic larval stages generally result in high levels of gene flow, leads to the question which additional oceanographic and biological factors influence gene flow and the genetic differentiation of populations.

The ACC promotes larval dispersal

The Antarctic Circumpolar Current (ACC) connects the sub-Antarctic islands in the study area as evident from the trajectories of drifting buoys (chapters I, II). Within the time of 3.5 months (105 days, the approximate duration of pelagic larval development) drifters from any island shelf are able to reach the neighboring shelf located downstream. Hence, under the assumption that pelagic larvae drift passively near the water surface, gene flow can theoretically be established between the sub-Antarctic islands of the Atlantic sector in a stepping-stone pattern (Figs. I-2 and II-2). However, small scale oceanography is highly variable and passive particles may cross the Scotia Sea (from the Antarctic Peninsula to South Georgia) within a time frame of two to eight months, as has been shown by drifter trajectories of the World Ocean Circulation Experiment (WOCE) and models of krill transport (Thorpe *et al.* 2004; Murphy *et al.* 2004). Given the vertical distribution of notothenioid larvae in the water column, of which many carry out diurnal vertical migrations, the transport times may be prolonged due to decreasing current speeds at increasing depths. For example, based on krill

transport models, the passive drift of particles at about 323 m depth takes on average 1.07 times longer than at the surface, and the exact trajectories may differ due to Ekman drift as well as small-scale spatial and temporal complexity of currents (Murphy *et al.* 2004; Fach & Klinck 2006; Gawarkiewicz *et al.* 2007). However, several deep reaching frontal systems exist in the study area (Orsi *et al.* 1995) and the associated jet streams (Tomczak & Godfrey 2002) may accelerate dispersal also at greater depths (e.g. Sands *et al.* 2003). As a conclusion, it can be assumed that the oceanographic currents in the study area allows at least temporally the directed and successful dispersal of pelagic larvae between islands. This may especially hold true when the complete pelagic developmental phase, including larvae and young juveniles, is considered.

The general importance of oceanographic dispersal of pelagic developmental stages for colonizations and gene flow in otherwise demersal notothenioids is demonstrably strong, given the similarities between demersal fish species assemblages (Permitin 1973) and the present distribution of notothenioids at sub-Antarctic islands of the Atlantic sector (Miller 1987). That larval dispersal is the most likely means for gene flow is suggested by the isolation-with-migration analyses (chapter I; Matschiner *et al.* 2009), which have shown that the direction of gene flow, and hence dispersal, is in congruence with the flow pattern of the ACC in eastward direction (but see Papetti *et al.* 2012). These results support evidence for eastward dispersal with the ACC for many teleost species with circum-Antarctic distributions based on zoogeographic analyses (DeWitt 1971). Therefore, the ACC plays a prominent role for the population connectivity and biogeography of notothenioids, similar to observations from a wide variety of organisms from plants to crustaceans distributed on sub-Antarctic islands and southern hemisphere continents (Fell 1962; Sanmartin *et al.* 2006; Nikula *et al.* 2010).

Moreover, the results of chapter II and III have shown that the Southern Antarctic Circumpolar Current Front (SACCF), which runs south of South Georgia (SG), is not a strong barrier to gene flow among notothenioid populations, as opposed to the Polar Front (PF), which has been demonstrated to be an effective barrier between populations of the Patagonian toothfish *D. eleginoides* (Shaw *et al.* 2004). Overall, these findings suggest that the oceanography in the study area rather promotes than hinders larval dispersal; yet, the population genetic results mostly show significant differentiations among sub-Antarctic islands.

The found significant population differentiations indicate that no interbreeding occurred for a long time, despite a theoretical connectivity by larval dispersal with oceanic currents. This points to either low larval survival rates in the open-ocean or effective retention mechanisms for larvae at their natal shelf. Although a combination of both factors is most likely responsible for regulating gene flow among populations their relative importance remains unresolved. Since quantitative estimates of larval survival rates are not available for notothenioids, the following discussion is restricted to factors influencing larval retention.

Factors limiting gene flow by larval dispersal

Factors influencing population dynamics and gene flow by larval dispersal are both biological and physical (Huntley & Niiler 1995). Complex interactions between these

factors prohibit a clear delineation of their relative importance, but the comparative population genetic studies of this thesis give valuable insights into likely oceanographic and biological factors that influence retention and dispersal of pelagic larvae.

Regional oceanography

Regional oceanography indicates physical retention mechanisms for larvae over their natal shelves. The larval distribution of demersal notothenioids is mostly restricted to neritic waters, whereas larvae of mesopelagic fishes, such as myctophidae, have higher abundances in oceanic waters (Efremenko 1983; Ślósarczyk 1986; Loeb *et al.* 1993). Moreover, the abundance of notothenioid larvae has regularly been found to decrease with increasing distance from the coast at the islands of the Scotia Arc (Ślósarczyk 1986; North 1988). Possible oceanographic retention mechanisms are gyres that form behind islands, eddies within fjords, and shelf-break frontal systems, which have been reported from the Antarctic Peninsula and South Georgia (Loeb *et al.* 1993; White 1998, and references therein). However, the shelves around South Orkney Islands and Bouvet Island are much smaller on the lee side of the current, and oceanographic retention, if present, may be less pronounced. But in consideration of the population genetic differentiation presented in this thesis there is no indication that gene flow, and, hence, the magnitude of advected larvae, is higher in populations inhabiting narrower shelves. Overall, it can be inferred from the genetic analyses that pelagic larvae must to a considerable amount be retained near their spawning sites. The increase in population sizes since the last glacial maximum inferred from Bayesian Skyline Plots (Figs. II-5 and III-2) and the starlike haplotype genealogies with many private haplotypes within most populations (Figs. II-3 and III-3) indicate high levels of self-recruitment.

Nevertheless, these retention mechanisms are not strict barriers to gene flow. Eggs and larvae of notothenioids have also been infrequently found past the shelf in oceanic waters (Kellermann 1991; White *et al.* 1996) and the spatio-temporal variability of currents (Thorpe *et al.* 2004; Meredith *et al.* 2005) likely results in a break-up of otherwise relatively stable retention mechanisms, allowing larvae to be advected past the shelves into the open ocean (Efremenko 1983). Increased rates of larval dispersal, and, hence, increased chances for gene flow, may generally occur during weather extremes, such as storms, as observed in fishes from the North Pacific and Indian Ocean. For example, wind-driven transport during storms has been shown to flush larvae of the walleye pollock away from their habitat (Bailey *et al.* 1997) and lee gyres behind islands may detach as eddies, in which neritic waters including the entrained plankton are transported into the open ocean (Heywood *et al.* 1996). In the Southern Ocean, frequent storms during the winter caused a homogenization of neritic and oceanic water masses around the Kerguelen Islands, thereby leading to unpredictable patterns of larval distributions of mesopelagic myctophid fish larvae, which were distributed in oceanic waters during summer (Koubbi *et al.* 1991). Likewise, neritic notothenioid larvae may be displaced by storms beyond the shelf.

Although these environmental retention mechanisms are likely to influence many species in a similar way, the influence on larval dispersal is also highly dependent on the spatio-temporal variability not only of the oceanographic dynamics, but also the occurrence and distribution of larvae.

Biological factors – spawning

Biological characteristics may enhance retention of larvae over the shelf. Foremost, the sites of spawning and egg development seem to be one of the primary factors in determining the advection of eggs and larvae. In most marine fishes the egg type (demersal vs. pelagic) has been found to correlate with dispersal (Bradbury *et al.* 2008) and species with demersal eggs tend to have smaller scales of genetic connectivity (Sclafani *et al.* 1993; but see Shulman & Bermingham 1995). The same may apply for notothenioids in which demersal eggs predominate (e.g. Permitin 1973; Kock & Kellermann 1991), but direct inferences on the influence of the egg type for gene flow from the study species are stymied by the uniform spawning of demersal eggs (Table 2). The only difference is between species spawning unguarded eggs (*C. gunnari*, *C. rastrospinosus*, *G. gibberifrons*, *L. squamifrons*, *T. newnesi*) and those for which egg dispersal is hindered by either laying guarded nests (*C. aceratus*), sticking eggs to a substrate (*T. eulepidotus*) or using sheltered cavities for spawning such as spongocoels (*L. larseni*; Konecki & Targett 1989). With regard to the population genetic structures identified within this thesis, the magnitude of differentiation is not correlated with the different types of demersal spawning strategies. For example, the genetic differentiation measured as global F_{ST} across the Atlantic Sector of the Southern Ocean is in contrast to expectations highest for *C. gunnari* (unguarded, demersal eggs), intermediate for *C. aceratus* (guarded, attached to a substrate) and lowest for *L. larseni* (demersal, partly sheltered within spongocoels).

In contrast, pelagic eggs may promote gene flow. Of all 22 species investigated by population genetic studies, only 4 species are known to have (at least for some period of time) either pelagic (*Notothenia coriiceps*, *N. rossii*, *Dissostichus eleginoides*) or cryo-pelagic egg stages (*Pleuragramma antarctica*) (Kellermann 1990; Evseenko *et al.* 1995; White *et al.* 1996; Sapota 1999; Vacchi *et al.* 2012). Indeed, neither *Notothenia* species exhibited genetic population divergences within ocean sectors (Duhamel *et al.* 1995; Jones *et al.* 2008). However, these findings may change with future studies based on increased sample sizes and more sensitive markers, as was the case in the often-studied *D. eleginoides* (see Table 1). Although populations of *P. antarctica* sampled around the Antarctic continent were not significantly differentiated as well (Zane *et al.* 2006), no inferences on the influence of cryo-pelagic eggs on gene flow can be made, because adult specimen are truly pelagic and highly migratory. Moreover, it is not clear to what magnitude the cryo-pelagic eggs of *P. antarctica* are subject to dispersal by currents, as they are often found within a layer of platelet ice forming on the underside of ice-floes (Vacchi *et al.* 2012).

The *spawning site* determines where larvae hatch and has a large influence on larval dispersal, especially during the early development, when swimming capacities are still undeveloped. Larvae hatching in the proximity of coasts (inshore) have higher probabilities to be retained at their spawning grounds, than larvae hatching near the outer shelf and slope (offshore), where they are more prone to advection by oceanic currents. Based on a numerical modeling approach, it has been shown that the spawning site, besides pelagic larval duration and diurnal vertical migrations, is a major factor influencing larval retention at South Georgia, as the mean retention of *C. gunnari*, which spawns inshore, is about ten times higher than that of *N. rossii*, which spawns at the deeper parts of the shelf (Young *et al.* 2012). The spawning site might also partly explain why populations of the nototheniid *G. gibberifrons* around the Scotia

Sea are not genetically differentiated (Matschiner *et al.* 2009) despite a shorter pelagic development than the investigated channichthyids *C. aceratus* and *C. gunnari*, because *G. gibberifrons* spawns at offshore shelf areas and the two channichthyids migrate inshore for spawning (sources in Table 2). Moreover, the higher *potential fecundity* of *G. gibberifrons* with up to 143,620 eggs, as compared to about 22,626 eggs of *C. aceratus* and 31,045 eggs of *C. gunnari* might numerically increase the chances that individual larvae successfully disperse towards suitable habitats downstream.

It is interesting to note that in contrast to the spawning sites, the *time of spawning* seems to have less influence on the retention of larvae. As it has been shown by Young *et al.* (2012) at South Georgia for *C. gunnari* and *N. rossii* based on their modeling approach, the dispersal differences between early, middle and lately released propagules with respect to the complete hatching (*C. gunnari*) or spawning (*N. rossii*) periods were only marginal. Nonetheless, seasonal variations in oceanography (e.g. Klinck *et al.* 2004; Meredith *et al.* 2005; Boehme *et al.* 2008) may have a more pronounced influence on larval dispersal, but this needs further investigations in future studies. For example, larvae hatching in winter may be more prone to advection caused by storms than those hatching in summer.

Biological factors – larval behavior

Larval behavior is an additional factor influencing retention and dispersal of pelagic larvae (Kingsford *et al.* 2002; Sponaugle *et al.* 2002; Leis 2006) and may partly explain notothenioid larval retention in neritic waters (Hubold 1990). Although sensory and swimming capacities are generally expected to increase with age and size of fish larvae (Eastman 1993; Leis 2006), the net avoidance behavior during day time of notothenioid larvae with sizes as small as 10-20 mm (North & Murray 1992) indicates that already early larvae are capable of directed movements, at least over short time periods. In this regard, the larger hatching sizes of channichthyid larvae (11-17 mm for the study species) as compared to nototheniid larvae (7-11 mm; sources in Table 2) may give channichthyids a higher swimming capacity during early larval stages. Anyhow, larvae of many notothenioids carry out diurnal vertical migrations (North 1990; Loeb 1992; North & Murray 1992), which have been shown to positively affect larval retention at South Georgia (Young *et al.* 2012). Diurnal vertical migrations during larval development are known among others for the investigated species *C. aceratus*, *C. gunnari*, *L. larseni* and *G. gibberifrons* (sources in Table 2), as well as the nototheniid *Trematomus hansonii*, with the most distinct migration pattern evident for *C. gunnari* (North 1990). But given the results of significant population differentiations along the Scotia Arc islands for all aforementioned species except *G. gibberifrons* (chapters II, III; Matschiner *et al.* 2009; Van de Putte *et al.* 2012), it seems unlikely that diurnal vertical migrations of larvae are the primary factor for restricted gene flow among populations.

Additional factors influencing population structure

Effective population sizes

The investigated population genetic structures in this thesis were primarily examined to assess the role of pelagic larval dispersal for gene flow, but the pattern of connectivity is also inevitably a result of past and present demographic factors (e.g. Avise 2004;

Crame 2004; McDowall 2004; Wiens & Donoghue 2004). Foremost, the effective population sizes (N_e) have a large influence on the genetic divergence of populations, as genetic drift acts faster in small populations (Kimura & Ohta 1969). For notothenioids, reductions in population size seem to be a frequent phenomenon on different time scales, thereby accelerating population divergences. On a more evolutionary time scale, glacial cycles have been shown to reduce notothenioid population sizes substantially (chapters II, III). On an ecological time scale, regular census surveys in the Southern Ocean during the last 30 years revealed considerable, but probably natural, fluctuations in population size for many notothenioids by one order of magnitude (e.g. Kock 1998). In addition, commercial fishing reduced population sizes of targeted species substantially in the late 1970s, as for example *C. gunnari* at SG to less than 20% of its primordial size (Kock 1992). Anyway, the frequent population size reductions imply rather low N_e for notothenioids, as the loss of genetic diversity at population size minimums will be remained over generations. These frequent fluctuations may also be the reason why estimates of N_e based on genetic data failed to deliver congruent results for both *C. aceratus* and *C. gunnari* (chapter II). Nonetheless, relatively low N_e help to explain the significant differentiation of populations investigated within this thesis.

Generation-time effects

Another identified potential factor influencing population divergences of notothenioids is the generation time (chapter II). The comparison of the population structures of *C. aceratus* and *C. gunnari* revealed an at least two-fold higher magnitude of population differentiation (measured as global F_{ST} s) of *C. gunnari* over *C. aceratus*. This observation is in line with the generation-time effect hypothesis, because the generation time of *C. aceratus* is twice as high as the generation time of *C. gunnari* (6 and 3 years, respectively). However, a comparison with data from *L. larseni*, which spawns for the first time at the intermediate age of 4 (chapter III, Table 2), questions this simple and direct correlation, as its global F_{ST} s are lower than those of *C. aceratus*. But in consideration of the different sampling schemes used for chapters II and III, a direct comparison of the global F_{ST} s between these studies might be skewed. Hence, the relative importance of generation-time effects on population divergences remains unresolved and needs additional studies.

Adaptive radiation and mode of speciation in notothenioids

The findings of significant population divergences despite long pelagic developmental stages, severe population size reductions during the last glacial maximum (LGM) at sub-Antarctic islands (chapter II and III) as well as the evidence of ecological speciation in the course of an adaptive radiation (chapter IV) give new insights into speciation processes of notothenioids especially in the sub-Antarctic realm. In general, the evolution of polar taxa including notothenioids is hypothesized to have been enhanced by climatic “diversity pumps” (Clarke & Crame 1992), which mainly relates to range shifts and distributional fragmentation of populations in the course of glacial cycles and consequent allopatric speciation (Valentine 1968; Crame 1993). This theory is especially appealing for high-Antarctic species that are distributed along the shelves of the Antarctic continent, which were repeatedly subject to massive icesheet expansions

during glacial maxima (e.g. Ingólfsson 2004; Gersonde *et al.* 2005). The accompanying periodic depletion of whole Antarctic communities further allowed for subsequent recolonizations and the utilization of open ecological niches during warmer periods, resulting in bursts of speciation events not only in Antarctic benthic invertebrates (Thatje *et al.* 2005; Thatje 2012), but also notothenioids (Near *et al.* 2012; Laurédou *et al.* 2012). Therefore, the interaction of glacial and inter-glacial periods is a driver of speciation at the Antarctic continent for many taxa. However, the influence of glacial cycles on speciation of species with sub-Antarctic distributions, such as notothenioid fishes, which are characterized by long pelagic developmental stages and, thus, high potential for gene flow by dispersal, were important, yet, unresolved questions.

The results from this thesis give evidence that sub-Antarctic notothenioids were influenced during the last glacial maximum (LGM) by severe reductions of population sizes similar to high-Antarctic species. Although population size increases after climatic cooling events have already been inferred previously based on mismatch distributions, the exact dating of such demographic events were often weakened by large confidence intervals or the lack of a precise molecular clock (see Volckaert *et al.* 2012 and references therein). The Bayesian Skyline Plots constructed in chapter II and III based on phylogenetically calibrated, relaxed molecular clocks allowed for a relatively good temporal fit between demographic and environmental events. The inferred population size reductions during the LGM show that sub-Antarctic notothenioid populations have been severely affected by past climate changes, thereby raising the chances for allopatric speciation, since small populations are stronger subject to genetic drift, which increases the rate of genetic divergence among populations (e.g. Wright 1931). In combination with the restricted gene flow among populations despite long pelagic developmental stages this result suggests that sub-Antarctic islands may play an important role for the diversification of notothenioids. This may especially be true for regions that are influenced by oceanography from both the high-Antarctic and the sub-Antarctic regimes, as for example the southern Scotia Arc, where waters from the Weddell Sea and ACC mix, as this mixing results in highly productive zones with increased ecological opportunities, allowing for ecological speciation (see chapter IV). Indeed, the high species diversity along the Scotia Arc islands, especially in the southern part, suggests a prominent role for speciation not only in notothenioids (Permitin 1977), but also other taxa such as mollusks (Linse *et al.* 2007).

In summary, it can be inferred from the presented data that (1) the long pelagic developmental stages are not in conflict with the hypothesis that allopatric speciation is the predominant mechanism for speciation in Notothenioidei, and (2) the speciation at sub-Antarctic islands is likely triggered by population size reductions during glacial maxima and increased ecological opportunities due to changing environmental conditions in the course of repeated glacial cycles.

Methodology – possibilities and constraints

The comparative approach of this thesis has proven useful to identify factors influencing gene flow. Nonetheless, some caution must be exercised in using population genetic structures and the distribution of genetic diversity as proxies for gene flow. It is not possible to distinguish clearly between historical and contemporary

causes for gene flow. Hence, genetic signatures originating from colonizations and occasional gene flow by dispersal might be obscured. In this regard, methods for estimating gene flow through modeling, such as done with IMA in chapter I, would have been desirable for all population genetic studies. Unfortunately, these simulations are very time consuming and do not always result in reliable estimates as they require 'proper' parameters for a good model, which might not always be available. This was the case for the analyses of chapter II and results of these simulations have therefore been omitted.

One alleged weakness of this dissertation is probably the sparse presentation of statistical tests for correlating ecological and genetic observations. My decision to refrain from presenting any statistical proofs for my conclusions on potential factors influencing gene flow and population structures has two reasons. First, studies differing in their sampling schemes and marker types can statistically not be compared, thereby reducing the number of comparable studies to a minimum. This applies for correlations of global genetic differentiation measures as well as local population differentiations. For example, the maximum number of correlations between the genetic divergence of two island populations in the Atlantic sector of the Southern Ocean (e.g. F_{ST}) and any independent variable (e.g. spawning location or number of eggs) would be between 6 (EI-BO) and 18 (EI-SO), when replicate species are excluded (Matschiner *et al.* 2009; Van de Putte *et al.* 2012). As a result of low sample numbers and associated large variances, the statistical power is low, especially in consideration of the high number of co-variates. The second reason is based on the more or less obvious observation that the genetic differentiation of populations is not a result of a single factor, but a product of multiple factors without knowledge about their relative contributions. Moreover, the individual influence of a single factor on gene flow is inevitably dependent on other factors. For example, the magnitude of gene flow between populations in close geographic distance will probably be higher in species with a high number of propagules, but for gene flow between populations located far apart the ability of propagules to survive in the open ocean may be more important. For this reason, the results from the seven species investigated in chapter I were often excluded for discussion, as the high genetic similarities between populations do not allow the identification of regulating factors other than geographic proximity, which outweighs any additional factors, especially in consideration of the significant differentiations detected among other populations.

Although the comparative approach based on sympatric species and different genetic markers is able to set a baseline for our understanding of gene flow in notothenioids, the statistical issues show that the relative contributions of single factors can only be elucidated with increased numbers of species for which detailed ecological and biological data is available. Therefore, additional population genetic studies on sympatric species should be pursued and their ecological monitoring continued.

CONCLUSIONS

The presented thesis shows substantial population genetic structure in fishes that are characterized by long pelagic developmental stages, whose dispersal is favored by one of the strongest currents known worldwide. The results of the comparative approach indicate that the relative importance of dispersing pelagic developmental stages for gene flow is often overestimated. Instead, other biological characteristics seem to have great influence on retention or dispersal of propagules, such as egg type, location of spawning site and larval behavior. In future studies, species that differ in respect to the aforementioned biological characteristics should be compared to further elucidate the relative importance of each factor. Moreover, the presented thesis indicates that demographic factors, such as the effective population sizes, likely influence the divergence of populations to a great extent, but their relative importance also needs to be explored. Nonetheless, the investigated populations were genetically not completely isolated, indicating that occasional gene flow exists (or existed) at least temporally.

From an evolutionary perspective, the results of this thesis are in congruence with a vicariance and dispersal scenario, which explains the relatively high species diversity of notothenioids in the Southern Ocean. The long pelagic developmental stages allow for dispersal and the (re-) colonization of distant habitats. This may be especially important during environmental changes, such as glacial cycles, which have been shown to reduce population sizes during glacial maxima. These population size reductions further benefit speciation processes as they facilitate population divergences. Last but not least, the ecological diversity of notothenioids has been shown to be a result of an adaptive radiation, probably caused by interspecific competition for resources. Since this thesis dealt primarily with notothenioids from sub-Antarctic islands, it would be interesting to compare their population genetic structures and responses to glacial maxima with those species inhabiting the high-Antarctic continental shelf. Such a comparison would further allow identifying centres of notothenioid evolution.

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LIST OF PUBLICATIONS

The chapters of this thesis are either published (Chapter I and IV) or submitted for publication (Chapter II and III) to peer-reviewed scientific journals. Journal-specific format styles were applied to the published and submitted manuscripts and may vary to the format used in this thesis. The individual chapter references are combined in the final references of this thesis.

Chapter I

Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica

Authors: Malte Damerou, Michael Matschiner, Walter Salzburger, Reinhold Hanel.
Published in *Polar Biology*, 35:1073-1086 (2012)

Chapter II

Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae)

Authors: Malte Damerou, Michael Matschiner, Walter Salzburger, Reinhold Hanel.
Submitted for publication to *Molecular Ecology* (under review)

Chapter III

Population genetic structure of *Lepidonotothen larseni* revisited: cyt *b* and microsatellites suggest limited connectivity in the Atlantic sector of the Southern Ocean

Authors: Malte Damerou, Walter Salzburger, Reinhold Hanel.
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Chapter IV

Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation

Authors: Sereina Rutschmann, Michael Matschiner, Malte Damerou, Moritz Muschick, Moritz F. Lehmann, Reinhold Hanel, Walter Salzburger.
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CONTRIBUTIONS OF AUTHORS

The following list clarifies my personal contributions to the manuscripts presented in this thesis.

Chapter I - Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica

The study was initiated by Reinhold Hanel and Walter Salzburger. Study design was planned by all authors. Malte Damerou performed sampling in 2009, laboratory analyses as well as statistical analyses, except for the Isolation-with-Migration analyses, construction of haplotype genealogies and drifter maps, which were done by Michael Matschiner. Malte Damerou wrote the manuscript and all co-authors contributed helpful comments before submission of the final version.

Chapter II - Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae)

The study was designed by Malte Damerou, Walter Salzburger and Reinhold Hanel. Malte Damerou performed sampling in 2009 & 2011, laboratory as well as statistical analyses and wrote the manuscript. Michael Matschiner helped in sampling in 2011 and provided the drifter maps, graphical haplotype genealogies and inferred branching rates for the phylogenetic tree. All co-authors contributed helpful comments on the manuscript before submission of the final version.

Chapter III - Population genetic structure of *Lepidonotothen larseni* revisited: *cyt b* and microsatellites suggest limited connectivity in the Atlantic sector of the Southern Ocean

Malte Damerou designed the study, performed sampling in 2009 & 2011, laboratory & statistical analyses and wrote the manuscript. All co-authors contributed helpful comments on the manuscript before submission of the final version.

Chapter IV - Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation

The study was designed by Michael Matschiner, Reinhold Hanel and Walter Salzburger and primarily executed by Sereina Rutschmann and Michael Matschiner. Malte Damerou performed sampling in 2009, helped in sample preparations and selections, and contributed ecological data. Moritz Muschick performed the disparity through time analyses; Moritz Lehmann assisted with the stable isotope analyses. Sereina Rutschmann and Michael Matschiner wrote the manuscript and all co-authors contributed helpful comments on the manuscript before submission of the final version.

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Hiermit erkläre ich, dass die vorliegende Dissertation selbständig von mir angefertigt wurde. Die Dissertation ist nach Form und Inhalt meine eigene Arbeit und es wurden keine anderen als die angegebenen Hilfsmittel verwendet. Diese Arbeit wurde weder ganz noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Biologische Meereskunde erfolgen.

Hamburg, den 22. Oktober 2013

Malte Damerau