

Population genetic structure of *Lepidonotothen larseni* revisited: *cyb* and microsatellites suggest limited connectivity in the Southern Ocean

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ABSTRACT: Antarctic fishes (Notothenioidei) are characterized by unusually long pelagic larval stages of up to more than 1 yr, and population genetic studies on notothenioids have 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 restricted gene flow between populations and low levels of successful dispersal with the currents. Hence, long pelagic larval phase durations do not translate into high genetic exchanges. In addition, we provide evidence based on Bayesian skyline plots of increasing population sizes in this sub-Antarctic species since the last glacial maximum.

KEY WORDS: Notothenioidei · Antarctic icefishes · Larval dispersal · Gene flow · Bayesian skyline plots

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INTRODUCTION

Antarctic icefishes (Notothenioidei) have evolved in the constantly cold waters of the Southern Ocean and diversified into more than 130 species over approximately 22 to 25 million years (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 anti-freeze glycoproteins, which prevent their body fluids from freezing at sub-zero temperatures (Cheng 1998, Matschiner et al. 2011), as well as ecological adaptations to available 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), 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 periodic glaciation events (Allcock & Strugnell 2012). In particular, fragmentation of populations by ice, isolation in refugia during glacial maxima, and re-colonization of destructed habitat may have been key mechanisms for allopatric speciation in the Antarctic realm (Rogers 2007). This is especially true for Antarctic marine bottom invertebrates, which are to a great extent characterized by high levels of endemism and scar-

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city of pelagic larval stages (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 pelagic larval stage of up to more than 1 yr (Kellermann 1989, North 2001), during which larvae might be subject to long-distance dispersal with the currents. Gene flow by larval dispersal might therefore facilitate survival during glacial periods and counteract population divergences as well as allopatric speciation in notothenioids.

Population genetic studies on notothenioids have been performed for both benthic and pelagic species and have revealed diverse patterns 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 Bouvet Island to their east, genetic homogeneity among populations has also been observed (Kuhn & Gaffney 2006, Papetti et al. 2007, Jones et al. 2008, Matschiner et al. 2009, Papetti et al. 2009, 2012, Damerou et al. 2012). These results have 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 (nuclear microsatellites and mtDNA sequences) have shown that populations might be more structured than previously thought (Van de Putte et al. 2012, Damerou et al. 2014). 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. <10 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 based on the mitochondrial *ND2* gene (Jones et al. 2008). This benthic (Eastman 1993) species inhabits the shelves of the Antarctic Peninsula as well as most sub-Antarctic islands at depths of 30 to 550 m (DeWitt et al. 1990). In the Atlantic sector, this nototheniid occurs along the island chain from the South Shetland Islands in the west to Bouvet Island in the east, including the South Orkney Islands, Shag Rocks, South Georgia Island, and South Sandwich Islands (DeWitt et al. 1990). *L. larseni* is highly abundant and constitutes up to 80% of bottom trawl catches at e.g. South Georgia Island, South Sandwich Islands and Bouvet Island (Jones et al. 2008); however, due to its small total length 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 have been 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 4 to 5 yr (North & White 1987) and spawns between 1815 and 9745 small (1.6–2.0 mm) demersal eggs (Andriashv 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 to July in South Georgia and about 1 mo later at the South Shetland Islands (Kock 1989). Larval hatching starts in South Georgia in September (Efremenko 1983) and at Elephant Island/Antarctic Peninsula/South Shetland Islands from mid-September onwards (Kellermann 1986). Late larvae and early juveniles remain pelagic over their first winter and return to a demersal lifestyle during their second summer/autumn (Efremenko 1983, Kellermann 1989). Hence, *L. larseni* has an exceptionally long pelagic development during early life stages lasting for more than 1 yr and is therefore highly interesting for studying the influence of larval dispersal on gene flow. If pelagic larval duration is a main determinant for gene flow between populations, for *L. larseni* we expect to find only weakly structured populations, especially in comparison to other notothenioids with shorter larval developments.

MATERIALS AND METHODS

Sampling and DNA extraction

Specimens were collected with bottom trawls at South Georgia, South Orkneys, and Bouvet Island during 2 expeditions to the Southern Ocean: February to March 2009 during the US Antarctic Marine Living Resources (AMLR) finfish survey aboard RV 'Yuzhmoregeologiya' and in February to April 2011 during ANT-XXVII/3 aboard RV 'Polarstern' (Fig. 1, and see Table S1 in the Supplement at www.int-res.com/articles/suppl/m517p251_supp.pdf). Muscle tissues for genetic analyses were stored in 95% ethanol prior to DNA extraction. All DNA was extracted in 300 μ l 5% Chelex solution with 12 μ l Proteinase K (20 mg ml⁻¹). The incubation time was 3 h at 55°C, followed by 25 min at 98°C in a thermomix.

Mitochondrial DNA

Partial mitochondrial gene sequences of cytochrome *b* (*cyb*) were amplified with the primers Not-CytbF and H15915n (Matschiner et al. 2011) using Phusion polymerase (Finnzymes) and following the manufacturer's PCR protocol at 57°C annealing temperature. Purification of PCR products was accomplished by adding 2 μ l ExoSAP-IT (USB) to 5 μ l of extracted DNA following the manufacturer's manual.

Sequencing PCR was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), subsequently purified with BigDye XTer-

minator (Applied Biosystems), and finally sequenced on an AB3500 Genetic Analyzer (Applied Biosystems). All sequences were automatically aligned with CodonCode Aligner (CodonCode) and manually corrected if necessary.

Basic sequence properties and polymorphisms such as nucleotide (π) 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 (BSPs) using Beast v1.72 (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 in J Model-Test v0.1.1 (Posada 2008). Since the exact substitution rate for *cyb* in *Lepidonotothen larseni* is unknown, we explored fixed rates of 0.01, 0.02, 0.04, and 0.08 substitutions per site per million years,

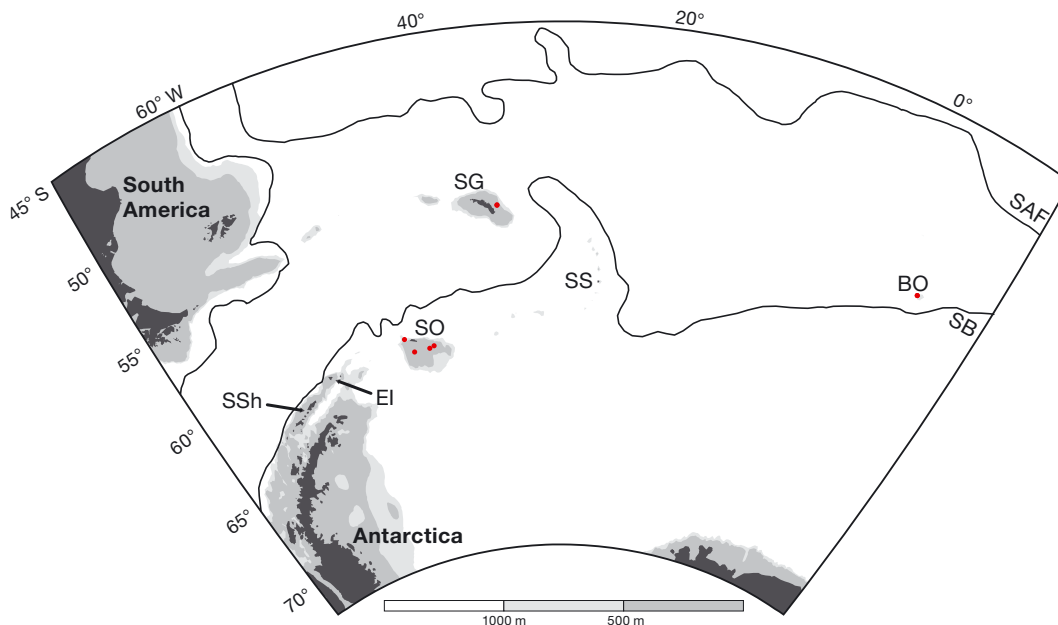


Fig. 1. Study area and sampling locations (red dots). Islands of the Atlantic sector of the Southern Ocean include the South Shetland Islands (SSh), Elephant Island (EI), South Orkney Islands (SO), South Georgia Island (SG), South Sandwich Islands (SS), and Bouvet Island (BO). The Antarctic Circumpolar Current flows in an easterly direction around the Antarctic continent and is marked by the Subantarctic Front (SAF) to the north and its Southern Boundary (SB) to the south. Fronts adapted from Orsi et al. (1995)

which are in the range of rates found for other notothenioids (e.g. Damerau et al. 2014). 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 was directly inferred from the tree prior. First, each analysis was run comprising all specimens (species level) for 50 million Markov chain Monte Carlo (MCMC) generations with a 10% burn-in. The convergence of parameters and the presence of effective sampling sizes of at least 200 were checked with Tracer. Afterwards, 2 replicate analyses were carried out to check for consistency for each population separately (population level).

Microsatellites

Six microsatellite loci 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 standards 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 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 AMOVA based on traditional F -statistics in Arlequin 3.5. 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 4 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 an indicator of the most likely K , we calculated ΔK following Evanno et al. (2005) with Structure Harvester v0.6.93 (Earl & von 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 1 and 5. 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 pixel⁻¹.

RESULTS

mtDNA

Overall, 79 specimens of *Lepidonotothen larseni* were successfully sequenced for 647 base pairs of *cyb* (accession numbers KF670785–KF670815). The sequences contained 31 segregating sites, and haplotype diversities (h) ranged from 0.759 at the South Orkney Islands to 0.806 at Bouvet Island, whereas π was about equal in all populations (Table 1). Private haplotypes were present in all populations.

Global AMOVA was highly significant, with an F_{ST} of 0.031. Most variation was caused by within-population variances (Table 2). All pairwise population differentiations were significant for $p < 0.05$, with the highest degree of differentiation between South Orkney Islands and Bouvet Island (Table 3). The haplotype genealogy shows 1 ancestral haplotype shared

Table 1. Genetic diversities of *Lepidonotothen larseni* based on a 647 base pair long fragment of the cytochrome *b* mitochondrial DNA locus per species and population; *h*: haplotype diversity, π : nucleotide diversity

	Sequences	Haplotypes	Private haplotypes	<i>h</i>	π
<i>L. larseni</i>	79	31	–	0.778	0.003
South Orkney Islands	28	15	13	0.759	0.003
South Georgia Island	25	10	6	0.767	0.002
Bouvet Island	26	11	8	0.806	0.003

by all 3 populations, with a substantial number of private haplotypes found in every population (Fig. 2). Only 3 haplotypes are shared between 2 populations, always including South Georgia Island. 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 4). The only population for which both tests were significant was from the South Orkney Islands, while for the other 2 populations, significant deviation from the null hypothesis of constant population size and selective neutrality of mutations was only indicated by Fu's *F*. In addition, the BSPs indicate increasing population sizes at all study locations since approximately 35 to 17 thousand years ago (Fig. 3) based on a substitution rate of 0.08 substitutions per site per million years, which was chosen according to Bayes Factors (see Table S2 in the Supplement at www.int-res.com/articles/suppl/m517p251_supp.pdf). Since the \log_{10} Bayes Factors values for each tested substitution rate differed only slightly, additional BSPs were plotted for rates of 0.02 and 0.04 substitutions per site per million years, for which population sizes show increases between 50 and 25 thousand years ago (Fig. S1 in the Supplement).

Table 2. Results of analysis of molecular variance for *Lepidonotothen larseni* as calculated with Arlequin 3.5 based on cytochrome *b* (*cyb*) sequences and microsatellites. Populations are from the South Orkney Islands, South Georgia Island, and Bouvet Island

Source of variation	df	Sum of squares	Variance components	Percentage of variation	F_{ST}	p
Microsatellites						
Among populations	2	11.478	0.046	2.04	0.020	<0.000
Within populations	112	251.446	0.058	2.57		
Within individuals	115	245.000	2.130	95.39		
Total	229	507.943	2.234			
<i>cyb</i>						
Among populations	2	2.956	0.026	3.14	0.031	0.003
Within populations	76	60.664	0.798	96.86		
Total	78	63.620	0.824			

Microsatellites

The complete set of 6 microsatellites was successfully genotyped for 115 individuals. Samples with missing data for any locus were omitted from analyses. All loci were polymorphic, and allelic richness per locus varied from 2.8 (*Trne55*) to 23.7 (*Trne35*, Table S3 in the Supplement). Deviations from HWE could be detected at

4 loci (Bouvet Island: *Trne35*, *Trne66*; South Georgia Island: *Trne66*; South Orkney Islands: *Trne37*), but across all loci, only the population of Bouvet Island deviated from HWE (Table 5). Furthermore, genotypic linkage disequilibrium was restricted to the locus pair *Trne35* and *Trne37*, and null alleles were not present.

Table 3. Pairwise genetic differentiation of *Lepidonotothen larseni* populations. (a) F_{ST} based measurements for microsatellites (below diagonal) and cytochrome *b* (above diagonal). (b) Hedrick's G'_{ST} (below diagonal) and Jost's D_{est} (above diagonal) for microsatellites. SO: South Orkney Islands, SG: South Georgia Island, BO: Bouvet Island; * $p < 0.05$, ** $p < 0.01$

	SO	SG	BO
(a)			
SO	–	0.035*	0.248*
SG	0.021**	–	0.035*
BO	0.009**	0.031**	–
(b)			
SO	–	0.058**	0.025**
SG	0.068**	–	0.080**
BO	0.029**	0.095**	–

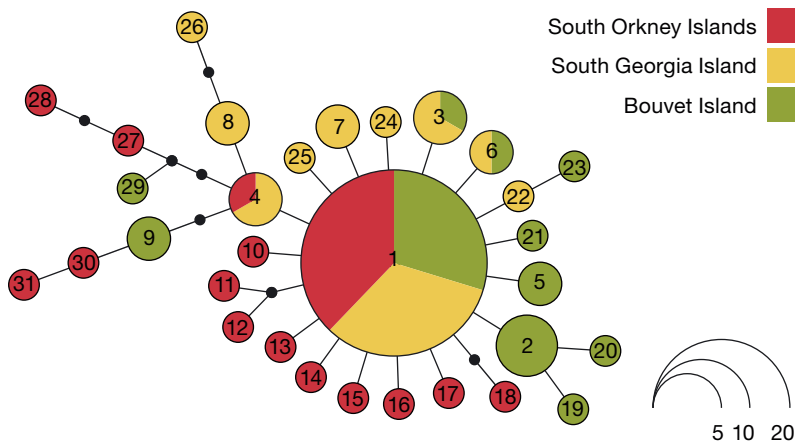


Fig. 2. Unrooted haplotype genealogy constructed from cytochrome *b* sequence data for *Lepidonotothen larseni*. Radii are proportional to haplotype frequencies. Sampling locations were South Orkney Islands, South Georgia Island, and Bouvet Island

Table 4. Tajima's *D* and Fu's *F* neutrality tests for *Lepidonotothen larseni* per population based on cytochrome *b* sequences. Significant deviation from H_0 for Tajima's *D* ($p < 0.05$) and Fu's *F* ($p < 0.02$) are indicated by an asterisk. SO: South Orkney Islands, SG: South Georgia Island, BO: Bouvet Island

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

In congruence with *cyb* data, AMOVA resulted in significant global differentiation ($F_{ST} = 0.02$) among populations (Table 2), 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 *cyb*, the population pair of Bouvet Island and South Orkney Islands was least differentiated (Table 3). 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 3).

The Bayesian inferences about population structure made with Structure resulted in 2 clusters, irrespective of whether information on the sampling location was incorporated or not (Table S4 in the Supplement). However, the graphical outputs for individual assignment probabilities differ between the 2 parameter sets and only the LOC-

PRIOR analyses identified spatial clusters (South Georgia Island and the pair of South Orkney Islands-Bouvet Island; Fig. 4). The analyses with Geneland, on the other hand, identified 3 clusters in each of 100 independent runs as the most likely number of clusters. Exemplified by the run with the highest posterior probability, Fig. S2 (in the Supplement) shows the number of clusters along the chain, which range between 3 and 5. In addition, maps of spatial assignment probabilities to belong to 1 of 3 clusters are depicted, while the probabilities to belong to cluster 4 or 5 were spatially indifferent and are therefore not presented.

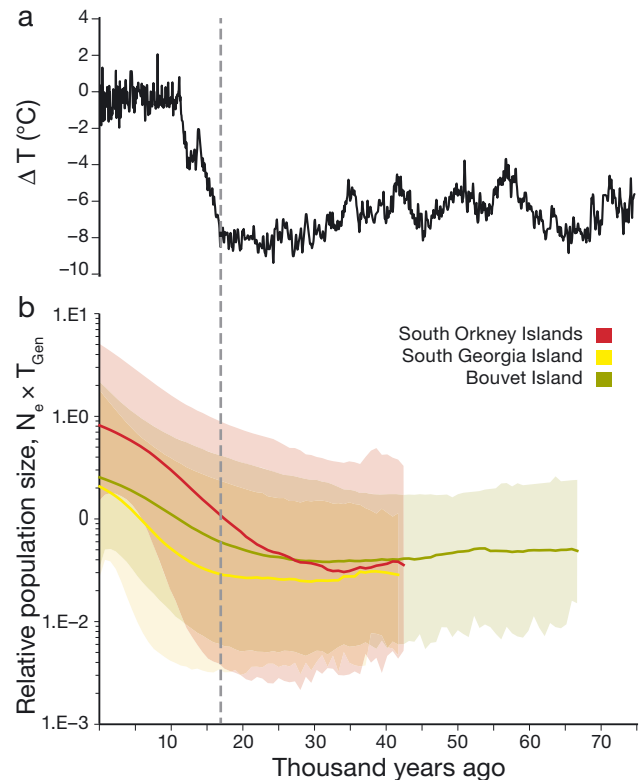


Fig. 3. (a) Relative temperature changes according to Petit et al. (1999). The vertical dashed line indicates the onset of temperature increase after the Last Glacial Maximum. (b) Bayesian skyline plots of *Lepidonotothen larseni* for each sampled population based on a substitution rate of 0.08 substitutions per site per million years. The median estimates (solid lines) and 95% highest probability densities (shaded areas) are shown. The relative population sizes are the products of the effective population size (N_e) and generation time (T_{Gen})

Table 5. Population-specific microsatellite properties and inbreeding coefficient (F_{IS}) of *Lepidonotothen larseni*; n: number of samples, 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. Asterisks indicate significant deviation from Hardy-Weinberg-Equilibrium (* $p < 0.05$, ** $p < 0.01$). SO: South Orkney Islands, SG: South Georgia Island, BO: Bouvet Island

	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	12.5	14.7
<i>Trne20</i>			
N_A	23	15	25
A_P	3	0	8.2
A_R	23	14.8	23.6
H_o	0.97	0.9	0.88
H_e	0.95	0.89	0.94
N_e	15.6	8.4	15.4
<i>Trne35</i>			
N_A	27	17	20
A_P	10	4.5	2.3
A_R	27	16.6	18.7
H_o	0.89	0.9	0.88*
H_e	0.94	0.86	0.89
N_e	16	6.9	9.2
<i>Trne37</i>			
N_A	8	6	7
A_P	1	0	0
A_R	8	5.9	6.8
H_o	0.69**	0.72	0.61
H_e	0.75	0.75	0.61
N_e	4	4	2.6
<i>Trne53</i>			
N_A	21	22	17
A_P	2	3.3	2.3
A_R	21	21.3	16.2
H_o	0.89	1	0.89
H_e	0.93	0.93	0.9
N_e	14.5	14.4	10.4
<i>Trne55</i>			
N_A	4	2	2
A_P	2	1.1	0
A_R	4	1.9	2
H_o	0.09	0.03	0.1
H_e	0.08	0.03	0.14
N_e	1.1	1	1.2
<i>Trne66</i>			
N_A	25	13	17
A_P	9	0	4.7
A_R	25	12.4	15.9
H_o	0.94	0.69**	0.78**
H_e	0.87	0.7	0.83
N_e	7.9	3.4	5.9

DISCUSSION

Genetic population structure

In this study, genetic discontinuities between *Lepidonotothen larseni* populations in the Atlantic sector of the Southern Ocean were evident from microsatellites and *cyb* 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 3 genetically distinct populations in the study area. This result is in 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 2 studies is probably caused by differences in sample sizes, as the genetic diversities of *ND2* and *cyb* are about equally high. Moreover, the suitability of both mtDNA markers in population genetic applications is assumed to be similar (Hwang & Kim 1999). The slightly higher h for *ND2* over *cyb* is very likely caused by longer sequences, which will result in more segregating sites and hence higher probabilities that 2 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 at highly self-sustained populations, as all haplotypes are unique to only 1 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} for *cyb* with 10 random subsets each containing sample sizes of 10, 4, and 6 individuals for Bouvet Island, South Georgia Island, and South Orkney Islands, respectively, to match the sampling scheme of Jones et al. (2008). Of these, only 2 subsets showed significant global differentiation (significant $F_{STs} = 0.07-0.08$, non-significant $F_{STs} = 0.01-0.04$) and only 1 pairwise significant differentiation was found between Bouvet Island and South Orkney Islands (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 2 different marker types, which both showed significant differentiation in the study area,

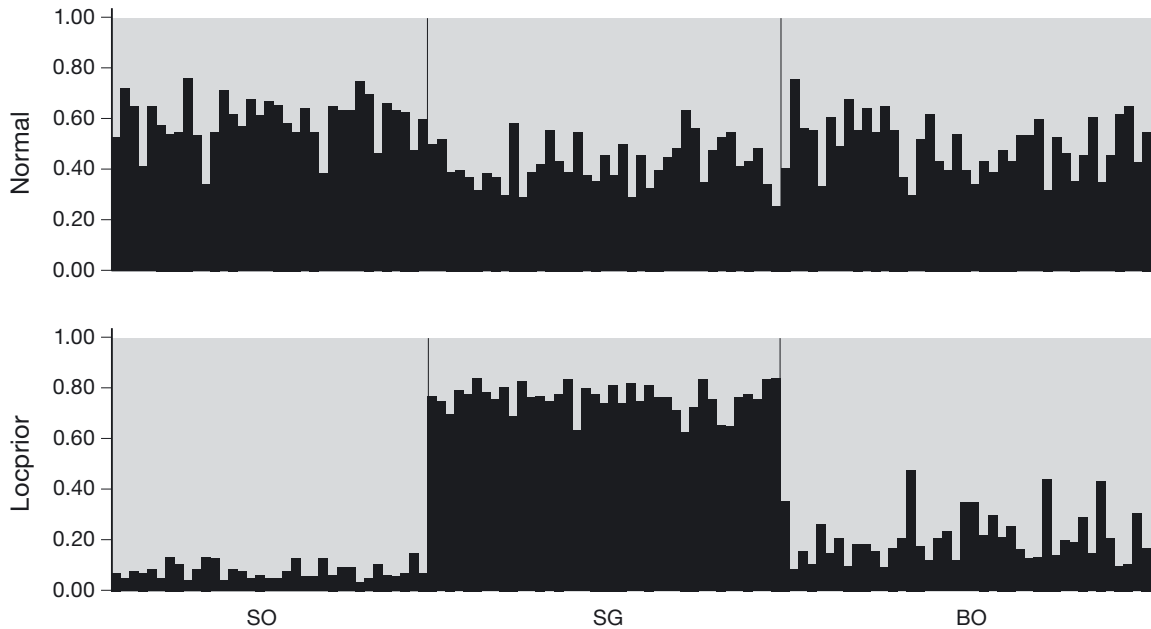


Fig. 4. Assignment probabilities of each individual *Lepidonotothen larseni* belonging to 1 of the 2 clusters identified in Structure. Analyses were run with (= locprior) and without (= normal) incorporation of sampling location data. SO: South Orkney Islands, SG: South Georgia Island, BO: Bouvet Island

indicate that populations of *L. larseni* are more highly 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.

The finding of significant differentiation among populations of *L. larseni*, the notothenioid species with the longest known larval development, questions the popular notion that long pelagic larval durations result in high levels of gene flow. Non-genetic assessments of notothenioid 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 1980), and otolith chemistry (Ashford et al. 2010) were mainly conducted for larger species that are of interest to fisheries, such as *Champsocephalus gunnari*. The predominant pattern identified for the investigated species was a differentiation among populations in the northern (Shag Rocks, South Georgia Island) and southern (South Shetland Islands including Elephant Island, South Orkney Islands) Scotia Sea, indicating little connectivity among populations separated over distances up to 900 km, although connected by the Antarctic Circumpolar Current (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 1 out of 6 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 7 notothenioids (Papetti et al. 2007, 2009, 2012, Damerou et al. 2012), as well as between the northern Scotia Arc and Bouvet Island for 5 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 structure 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 present study and 2 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. 2014) suggest now a more restricted exchange between populations, as was also originally inferred from non-genetic approaches. Our finding of significant population structure in a species with unusually long pelagic larval development indicates that the pelagic larval duration per se is not a good indicator of gene flow.

In fact, the molecular genetic signatures of many Antarctic marine organisms revealed a pattern of restricted connectivity among populations independent of individual dispersal abilities. For example, highly restricted gene flow is not only apparent according to expectations in the direct-developing sea slug *Doris kerguelenensis* (Wilson et al. 2009), but also in a range of taxa with pelagic developmental stages and circum-Antarctic distributions, including krill (Zane et al. 1998), squid (Sands et al. 2003), or even notothenioid fishes (Volckaert et al. 2012). Nonetheless, genetic homogeneity over large spatial scales could be inferred for many species with pelagic developmental stages, including shrimps (Raupach et al. 2010) and benthic notothenioid fishes (Matschiner et al. 2009). Hence, no universal pattern exists which can explain population connectivity by a single factor such as the presence and duration of pelagic developmental stages.

Gene flow between populations may be restricted by a diverse range of physical and biological factors, independent of the pelagic larval duration. Physical retention mechanisms such as shelf-break frontal systems or gyres on the lee side of islands, which are known to be present in the Scotia Sea, may retain larvae near their natal sites (Efremenko 1983). Predominant biological factors influencing larval dispersal include the spawning location and larval behaviour. Based on a modeling approach for notothenioid fishes, it has been shown that larvae hatching near the coast are more likely to be retained on the shelf, while those hatching on the outer shelf area are more prone to advection by currents (Young et al. 2012). Although spawning migrations towards the inner shelf area and parental care have been observed for many notothenioid fishes (Kock 1992, Jones & Near 2012), no such information exists for the study species. However, larval *L. larseni* carry out diel vertical migrations (North 1988), which can at least partly explain limited offshore dispersal (Young et al. 2012).

Apart from the possible retention mechanisms discussed above, it remains an interesting but open question how larvae cope with their environment once they get advected off the shelf into the open ocean.

Demographic history

Analyses of the demographic history of *L. larseni* based on mtDNA indicate increasing population sizes in all 3 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 1 common and many tip-haplotypes, as well as the increasing BSPs since approximately 17 to 35 thousand years ago, the negative neutrality test values likely result from 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 and indicate a recovery from glacial habitat disturbances during the last glacial maximum (LGM). Population expansions were also reported for *Chionodraco* spp. (Patarnello et al. 2003), *C. gunnari* (Kuhn & Gaffney 2006), *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. 2014). The only exception is *T. nicolai*, for which stable population sizes were inferred from 14 individuals examined (Kuhn et al. 2009). Dating of these expansions ranged from about 24 thousand years ago in *G. gibberifrons* to 126 thousand years ago 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 for notothenioid fishes that most estimates of the time of population expansions predate the LGM and that they could have occurred more recently than calculated (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 thousand years ago (for a review, see Ingólfsson 2004). At the Antarctic Peninsula, for example, the estimates for the occurrence of the maximum ice extent vary from 13 to >30 thousand years ago, depending on the methods and geographic location (Sugden & Clapperton 1981, Banfield & Anderson 1995, Anderson et al. 2002, Weber et al. 2011). Our finding that increasing population sizes began 17 to 35 thousand years ago 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 per site per million years, which is rather high as compared to a rate of 0.004 to 0.0045 substitutions per site per million years inferred for *cyb* 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). However, even for lower

rates of 0.04 and 0.02 substitutions per site per million years, the population sizes increased approximately 50 to 25 thousand years ago, which is on the lower end of the supposed range of population size expansions in notothenioids.

The BSPs indicate that population sizes of this cold-adapted species were negatively impacted by the LGM and recovered after the onset of the present interglacial, similar to most benthic Antarctic organisms. In the Southern Ocean, past climate changes recurrently challenged the extant Antarctic shelf community for survival (Thatje et al. 2008). During glacial maxima, grounded continental ice sheets extended asynchronously around Antarctica towards the shelf edge, thereby eradicating marine benthic communities over large spatial scales (Thatje et al. 2005). In addition, multiannual sea-ice led to reduced primary production along with negative impacts for population growth for the whole Antarctic food web, including sub-Antarctic islands. Nonetheless, species may have found refuge in ice-free polynyas on the shelf, periglacial regions, or the deep sea (Fraser et al. 2012).

The star-like haplotype genealogy of the study species with 1 dominant (ancestral) haplotype and many rare haplotypes is typical for species with a dispersal stage which survived the LGM in glacial refugia on the shelf (Allcock & Strugnell 2012). Since *L. larseni* primarily inhabits island shelves in the sub-Antarctic region, an alternative explanation for the star-like haplotype genealogy is the survival on the island shelves at its northern distribution range with post-glacial re-colonization of southern areas by dispersal of pelagic larvae. However, in contrast to our results, we would expect under this scenario a reduced genetic diversity in the southern populations due to the probably small number of founders. In addition, the high number of private haplotypes in the South Orkney Islands population renders this theory rather unlikely. Therefore, our results suggest that the benthic *L. larseni* survived the LGM locally in glacial shelf refugia. Moreover, our data show that species inhabiting sub-Antarctic islands, such as *L. larseni*, which have not been directly affected by expanding ice sheets from the Antarctic continent, seem to have reacted similarly to high-Antarctic species to the environmental changes associated with glaciation cycles. Therefore, global climate changes are likely to affect sub-Antarctic and high-Antarctic organisms in a similar fashion, as their regional ecosystems are interconnected by oceanographic features such as the ACC.

CONCLUSIONS

Our study shows restricted gene flow between populations of *Lepidonotothen 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 reasonably large sample sizes and shows the advantage of using multiple genetic markers in population genetics. Furthermore, analyses of the demographic history of *L. larseni* indicate survival of glacial cycles in shelf refugia and a recovery from reduced population sizes since the onset of the present interglacial.

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LITERATURE CITED

- Allcock AL, Strugnell JM (2012) Southern Ocean diversity: new paradigms from molecular ecology. *Trends Ecol Evol* 27:520–528
- Anderson J, Shipp S, Lowe A, Wellner J, Mosola A (2002) The Antarctic Ice Sheet during the Last Glacial Maximum and its subsequent retreat history: a review. *Quat Sci Rev* 21:49–70
- Andriashev AP (1965) A general review of the Antarctic fish fauna. In: van Oye P, van Mieghen J (eds) *Monographs in biology. Biogeography and ecology in Antarctica*. Junk Publishers, The Hague, p 491–550
- Ashford J, La Mesa M, Fach BA, Jones CD, Everson I (2010) Testing early life connectivity using otolith chemistry and particle-tracking simulations. *Can J Fish Aquat Sci* 67:1303–1315
- Banfield LA, Anderson JB (1995) Seismic facies investigation of the Late Quarternary glacial history of Bransfield Basin, Antarctica. *Antarct Res Ser* 68:123–140
- Cheng C (1998) Origin and mechanism of evolution of antifreeze glycoproteins in polar fishes. In: di Prisco G, Pisano E, Clarke A (eds) *Fishes of Antarctica: a biological overview*. Springer-Verlag, Milan, p 311–328
- Damerou M, Matschiner M, Salzburger W, Hanel R (2012)

- Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica. *Polar Biol* 35:1073–1086
- Damerou M, Matschiner M, Salzburger W, Hanel R (2014) Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae). *Mol Ecol* 23:284–299
- DeWitt HH, Heemstra PC, Gon O (1990) Nototheniidae. In: Gon O, Heemstra PC (eds) Fishes of the Southern Ocean. JLB Smith Institute of Ichthyology, Grahamstown, p 279–380
- Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol Biol Evol* 22:1185–1192
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 29:1969–1973
- Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4:359–361
- Eastman JT (1993) Antarctic fish biology: evolution in a unique environment. Academic Press, San Diego, CA
- Eastman JT (2005) The nature of the diversity of Antarctic fishes. *Polar Biol* 28:93–107
- Efremenko V (1983) Atlas of fish larvae of the Southern Ocean. *Cybiurn* 7:1–74
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611–2620
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10:564–567
- Felsenstein J (2005) PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, WA
- Fraser CI, Nikula R, Ruzzante DE, Waters JM (2012) Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends Ecol Evol* 27:462–471
- Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915–925
- Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. *J Hered* 86:485–486
- Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. Available at <http://www2.unil.ch/popgen/softwares/fstat.htm> (accessed November 2013)
- Gubsch G (1982) Zur Verbreitung und zur Biologie der Eisfische (Chaenichthyidae) im atlantischen Sektor der Antarktis. *Fisch-Forsch* 20:39–47
- Gubsch G, Hoffmann U (1981) Forschungsreise eines Zubringertrawlers in die Antarktis. *Fisch-Forsch* 19:31–34
- Guillot G, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. *Mol Ecol Notes* 5:712–715
- Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* 59:1633–1638
- Hwang UW, Kim W (1999) General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics. *Korean J Parasitol* 37:215–228
- Ingólfsson Ó (2004) Quaternary glacial and climate history of Antarctica. In: Ehlers J, Gibbard PL (eds) Quaternary glaciations—extent and chronology, Part III. Elsevier, Amsterdam, p 3–43
- Janko K, Lecointre G, DeVries A, Couloux A, Cruaud C, Marshall C (2007) Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity. *BMC Evol Biol* 7:220
- Jones CD, Near TJ (2012) The reproductive behaviour of *Pogonophryne scotti* confirms widespread egg-guarding parental care among Antarctic notothenioids. *J Fish Biol* 80:2629–2635
- Jones CD, Kock KH, Balguerías E (2000) Changes in biomass of eight species of finfish around the South Orkney Islands (Subarea 48.2) from three bottom trawl surveys. *CCAMLR Sci* 7:53–74
- Jones CD, Anderson E, Balushkin AV, Duhamel G and others (2008) Diversity, relative abundance, new locality records and population structure of Antarctic demersal fishes from the northern Scotia Arc islands and Bouvetøya. *Polar Biol* 31:1481–1497
- Jost L (2008) GST and its relatives do not measure differentiation. *Mol Ecol* 17:4015–4026
- Kellermann AK (1986) Zur Biologie der Jugendstadien der Notothenioidei (Pisces) an der Antarktischen Halbinsel. *Ber Polarforsch* 31:1–155
- Kellermann AK (1989) The larval fish community in the zone of seasonal pack-ice cover and its seasonal and inter-annual variability. *Arch FischWiss* 39(Beih 1):81–109
- Klingenberg C, Ekau W (1996) A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). *Biol J Linn Soc* 59:143–177
- Kock KH (1981) Fischereibiologische Untersuchungen an drei antarktischen Fischarten: *Champscephalus gunnari* Lönnberg, 1905, *Chaenocephalus aceratus* (Lönnberg, 1906) und *Pseudochaenichthys georgianus* Norman, 1937 (Notothenioidei, Channichthyidae). *Mitt Inst Seefisch Hambg* 32:1–226
- Kock KH (1989) Reproduction in fish around Elephant Island. *Arch FischWiss* 39(Beih 1):171–210
- Kock KH (1992) Antarctic fish and fisheries. Cambridge University Press, Cambridge
- Kock KH, Jones CD (2005) Fish stocks in the southern Scotia Arc region—a review and prospects for future research. *Rev Fish Sci* 13:75–108
- Kock KH, Kellermann AK (1991) Reproduction in Antarctic notothenioid fish. *Antarct Sci* 3:125–150
- Kock KH, Möller H (1977) On the occurrence of the parasitic copepod *Eubrachiella antarctica* on some Antarctic fish. *Arch FischWiss* 28:149–156
- Kuhn KL, Gaffney PM (2006) Preliminary assessment of population structure in the mackerel icefish (*Champscephalus gunnari*). *Polar Biol* 29:927–935
- Kuhn KL, Near TJ, Jones CD, Eastman JT (2009) Aspects of the biology and population genetics of the Antarctic nototheniid fish *Trematomus nicolai*. *Copeia* 2009:320–327
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452

- Martin AP, Palumbi SR (1993) Body size, metabolic rate, generation time, and the molecular clock. *Proc Natl Acad Sci USA* 90:4087–4091
- Matschiner M, Salzburger W (2009) TANDEM: integrating automated allele binning into genetics and genomics workflows. *Bioinformatics* 25:1982–1983
- Matschiner M, Hanel R, Salzburger W (2009) Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. *Mol Ecol* 18:2574–2587
- Matschiner M, Hanel R, Salzburger W (2011) On the origin and trigger of the notothenioid adaptive radiation. *PLoS ONE* 6:e18911
- Near TJ, Dornburg A, Kuhn KL, Eastman JT and others (2012) Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *Proc Natl Acad Sci USA* 109:3434–3439
- North AW (1988) Distribution of fish larvae at South Georgia: horizontal, vertical and temporal distribution and early life-history relevant to monitoring year-class strength and recruitment. *CCAMLR Select Sci Pap WG-FSA-87-19*:105–141
- North AW (2001) Early life history strategies of notothenioids at South Georgia. *J Fish Biol* 58:496–505
- North AW, White MG (1987) Reproductive strategies of Antarctic fish. In: Kullander SO, Fernholm B (eds) *Proceedings 5th Congress of European Ichthyologists, Stockholm 1985*. Swedish Museum of Natural History, Stockholm, p 381–390
- Orsi A, Whitworth T, Nowlin WD (1995) On the meridional extent and fronts of the Antarctic Circumpolar Current. *Deep-Sea Res I* 42:641–673
- Papetti C, Susana E, La Mesa M, Kock KH, Patarnello T, Zane L (2007) Microsatellite analysis reveals genetic differentiation between year-classes in the icefish *Chaenocephalus aceratus* at South Shetlands and Elephant Island. *Polar Biol* 30:1605–1613
- Papetti C, Susana E, Patarnello T, Zane L (2009) Spatial and temporal boundaries to gene flow between *Chaenocephalus aceratus* populations at South Orkney and South Shetlands. *Mar Ecol Prog Ser* 376:269–281
- Papetti C, Pujolar JM, Mezzavilla M, La Mesa M, Rock J, Zane L, Patarnello T (2012) Population genetic structure and gene flow patterns between populations of the Antarctic icefish *Chionodraco rastrospinosus*. *J Biogeogr* 39:1361–1372
- Patarnello T, Marcato S, Zane L, Varotto V, Bargelloni L (2003) Phylogeography of the *Chionodraco* genus (Perciformes, Channichthyidae) in the Southern Ocean. *Mol Phylogenet Evol* 28:420–429
- Patarnello T, Verde C, di Prisco G, Bargelloni L, Zane L (2011) How will fish that evolved at constant sub-zero temperatures cope with global warming? Notothenioids as a case study. *BioEssays* 33:260–268
- Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research — an update. *Bioinformatics* 28:2537–2539
- Permitin YY, Sil'yanova ZS (1971) New data on the reproductive biology and fecundity of fishes of the genus *Notothenia* Rich. in the Scotia Sea (Antarctica). *J Ichthyol* 11:693–705
- Petit JR, Jouzel J, Raynaud D, Barkov NI and others (1999) Climate and atmospheric history of the past 420 000 years from the Vostok ice core, Antarctica. *Nature* 399:429–436
- Posada D (2008) jModelTest: phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Rambaut A, Drummond AJ (2007) TRACER v.1.5. Available at <http://beast.bio.ed.ac.uk/tracer> (accessed July 2012)
- Raupach MJ, Thatje S, Dambach J, Rehm P, Misof B, Leese F (2010) Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. *Mar Biol* 157:1783–1797
- Rogers AD (2007) Evolution and biodiversity of Antarctic organisms: a molecular perspective. *Philos Trans R Soc Lond B Biol Sci* 362:2191–2214
- Rutschmann S, Matschiner M, Damerau M, Muschick M, Lehmann M, Hanel R, Salzburger W (2011) Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. *Mol Ecol* 20:4707–4721
- Salzburger W (2008) To be or not to be a hamlet pair in sympatry. *Mol Ecol* 17:1397–1399
- Salzburger W, Ewing GB, Haeseler Von A (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Mol Ecol* 20:1952–1963
- Sands CJ, Jarman SN, Jackson GD (2003) Genetic differentiation in the squid *Moroteuthis ingens* inferred from RAPD analysis. *Polar Biol* 26:166–170
- Schneppenheim R, Kock KH, Duhamel G, Janssen G (1994) On the taxonomy of the *Lepidonotothen squamifrons* group (Pisces, Perciformes, Notothenioidei). *Arch Fish Mar Res* 42:137–148
- Siegel V (1980) Parasite tags for some Antarctic channichthyid fish. *Arch FischWiss* 31:97–103
- Smith PJ, Gaffney PM (2000) Toothfish stock structure revealed with DNA methods. *Water Atmos* 8:17–18
- Sosiński J (1985) Some data on taxonomy and biology of Antarctic icefish, *Champscephalus gunnari* Lönnberg 1905. *Acta Ichthyol Piscat* 15:3–54
- Sugden DE, Clapperton CM (1981) An ice-shelf moraine, George IV Sound, Antarctica. *Ann Glaciol* 2:135–141
- Tajima F (1989) The effect of change in population size on DNA polymorphism. *Genetics* 123:597–601
- Thatje S (2012) Effects of capability for dispersal on the evolution of diversity in Antarctic benthos. *Integr Comp Biol* 52:470–482
- Thatje S, Hillenbrand CD, Larter R (2005) On the origin of Antarctic marine benthic community structure. *Trends Ecol Evol* 20:534–540
- Thatje S, Hillenbrand CD, Mackensen A, Larter R (2008) Life hung by a thread: endurance of Antarctic fauna in glacial periods. *Ecology* 89:682–692
- Van de Putte AP, Janko K, Kasparova E, Maes GE and others (2012) Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species. *Mar Genomics* 8:23–34
- Van Houdt JKJ, Hellemans B, Van de Putte AP, Koubbi P, Volckaert FAM (2006) Isolation and multiplex analysis of six polymorphic microsatellites in the Antarctic notothenioid fish, *Trematomus newnesi*. *Mol Ecol Notes* 6:157–159
- Van Oosterhout C, Hutchinson W, Wills D, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538
- Volckaert FAM, Rock J, Van de Putte AP (2012) Connectivity and molecular ecology of Antarctic fishes. In: di Prisco

- G, Verde C (eds) Adaptation and evolution in marine environments. Springer-Verlag, Berlin, p 75–96
- Weber ME, Clark PU, Ricken W, Mitrovica JX, Hostetler SW, Kuhn G (2011) Interhemispheric ice-sheet synchronicity during the Last Glacial Maximum. *Science* 334: 1265–1269
- Wilson NG, Schrödl M, Halanych KM (2009) Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelensis* (Mollusca, Nudibranchia). *Mol Ecol* 18:965–984
- Young EF, Rock J, Meredith MP, Belchier M, Murphy EJ, Carvalho GR (2012) Physical and behavioural influences on larval fish retention: contrasting patterns in two Antarctic fishes. *Mar Ecol Prog Ser* 465:201–215
- Zane L, Ostellari L, Maccatrozzo L, Bargelloni L, Battaglia B, Patarnello T (1998) Molecular evidence for genetic subdivision of Antarctic krill (*Euphausia superba* Dana) populations. *Proc R Soc Lond B Biol Sci* 265:2387–2391
- Zane L, Marcato S, Bargelloni L, Bortolotto E and others (2006) Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum*. *Mol Ecol* 15:4499–4511

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