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ORIGINAL ARTICLE

Genetic differentiation in the ice-dependent fish *Pleuragramma antarctica* along the Antarctic Peninsula

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ABSTRACT

Aim *Pleuragramma antarctica* is an Antarctic notothenioid fish with a pelagic life-cycle. It plays a major trophic role in coastal Antarctic waters as a predator of krill and as prey for penguins and seals. A previous study, using mtDNA to investigate *Pleuragramma* population structure at multiple Antarctic sites was unable to discriminate between hypotheses of panmixia, with occasional fluctuations of allelic frequencies, and population structure. The aim of the present study was to investigate the population structure of *P. antarctica* along the Antarctic Peninsula (AP) shelf by microsatellite genotyping.

Location Four areas along the AP shelf: Charcot Island, Marguerite Bay, Joinville Island and the Larsen Ice Shelf.

Methods A total of 562 individuals from nine population samples were genotyped at 16 expressed sequence tag (EST)-linked microsatellites. Genetic variability, Hardy–Weinberg probabilities and *F*-statistics were calculated, and a hierarchical analysis of molecular variance was carried out. A Bayesian method was applied to estimate the migration rates between geographical localities.

Results We found one genetically homogeneous population with no interannual variability in the south-western AP and one off the northern tip of the AP. Significant differences were recorded between the two geographical regions, which also differed from the eastern AP. The extent of differentiation changed between years and significant genetic differentiation was found between clusters of individuals of different length modes collected at Joinville Island in 2010. Bayesian analysis suggested weak gene flow along the western AP, with a prevailing direction from north to south, following the anticlockwise-flowing Coastal Current.

Main conclusions Population genetic structure can be detected in AP shelf samples of *P. antarctica*. Thus, even in this area characterized by strong oceanographic currents, genetic mixing is not complete. Temporal fluctuations of allelic frequencies and genetic differentiation between individuals of different length collected at the same location suggest the importance of genetic drift in this highly abundant species.

Keywords
Antarctic Peninsula, Antarctic silverfish, EST-linked microsatellites, genetic drift, genetic patchiness, Notothenioidei, population genetic structure, Weddell Sea.

INTRODUCTION

Life history, ecology and large-scale ocean circulation all influence the population genetic structure of marine fish by forming complex interactions that shape gene flow (Hauser & Carvalho, 2008). In the Southern Ocean, most Antarctic notothenioids are characterized by a benthic, sedentary habit as adults, potentially limiting their dispersal ability. Yet those same species often show long larval and early juvenile pelagic stages that would promote connectivity between populations

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via passive transport by two strong currents (Matschiner *et al.*, 2009): the clockwise-flowing Antarctic Circumpolar Current (ACC), which transports more water than any other ocean current (Klinck & Nowlin, 2001), and, closer to the continent, the anticlockwise Coastal Current (CC). The interaction between the two currents forms clockwise gyres in the Weddell Sea, Ross Sea and Bellingshausen Sea (Mintenbeck *et al.*, 2012). The ACC is believed to be the main current that sustains connectivity, and it determines a prevailing eastward direction of dispersal. Early studies indicated that young krill (*Euphausia superba*) entrained in fronts are advected along the ACC from the western Antarctic Peninsula (AP) to South Georgia (Fach & Klinck, 2006), and simulations of the large-scale circulation predicted that potential transport pathways may connect disparate areas of the Southern Ocean (Thorpe *et al.*, 2007).

In Antarctic notothenioid fish, empirical studies using otolith chemistry (Ashford *et al.*, 2006, 2008, 2010a) found evidence consistent with population structuring and connectivity related to the large-scale circulation of the Southern Ocean. For instance, Antarctic currents and fronts influenced the population structure of the Patagonian toothfish (*Dissostichus eleginoides*), and otolith chemistry revealed significant differences between samples from South America and Antarctica, consistent with the hydrogeographical barrier formed by the polar front of the ACC. A cluster analysis also suggested the presence of three groups in the Antarctic sector, mixing as a result of advective transport along the ACC (Ashford *et al.*, 2008).

Analyses using genetic approaches, which can directly address gene flow, have thus far shown considerable variability in population structuring among notothenioid species. Matschiner *et al.* (2009) pointed out that the connectivity generated by oceanographic currents may promote gene flow around Antarctica, precluding genetic differentiation. Their study, using mtDNA and microsatellite data, indicated genetic homogeneity in samples of *Gobionotothen gibberifrons* collected in the Scotia Sea and around the north-western AP. Unidirectional eastward gene flow was inferred between sampling sites, consistent with the direction of the ACC (Matschiner *et al.*, 2009). The authors suggested that the lack of population genetic structure could be a general pattern in Antarctic fish due to the combined effect of ACC transport and long-lasting larval stages. Similarly, population differences were low or absent, and high levels of gene flow were found in several species inhabiting the southern Scotia Arc that were characterized by distinct life-history traits and larval durations (Damerau *et al.*, 2012; Papetti *et al.*, 2012). In the same geographical area, however, genetic structuring was found among population samples of the icefish *Chaenocephalus aceratus*. Microsatellite markers showed significant differentiation both geographically and over time. Although the prevailing direction of gene flow followed the ACC, a signal of countercurrent migration was also detected (Papetti *et al.*, 2009, 2012). Evidence of population structuring has also been reported in other Antarctic notothenioids (Patarnello *et al.*, 2011; Damerau *et al.*, 2014).

In this study, we examine genetic structure and gene flow in the Antarctic silverfish, *Pleuragramma antarctica* Boulenger, 1902 (family Nototheniidae; see Eschmeyer, 2014 for species designation), which has a circumpolar distribution and, unusually for an Antarctic notothenioid, is characterized by a pelagic life-cycle that includes a cryopelagic egg stage. It is an important species in the trophic web of the Antarctic marine ecosystem: as one of the principal consumers of zooplankton, it occupies a similar ecological role in the food web to that of Antarctic krill in the seasonal sea-ice zone, in that all of its developmental stages are among the main food sources for a large number of predators from other fish to seals and penguins (Mintenbeck *et al.*, 2012). Silverfish are found in most shelf areas around the Antarctic continent, and are the dominant fish in terms of both abundance and biomass (La Mesa & Eastman, 2012). They are found at depths of 0–700 m (DeWitt *et al.*, 1990), and their various developmental stages display stratification by depth (La Mesa *et al.*, 2010). Eggs are found in coastal waters adherent to the platelet ice layer under fast ice. The early larvae are typically epipelagic and distributed at depths of 0–100 m, whereas postlarvae and juveniles live in deeper waters, down to 400 m. Larger fish are generally found deeper than 400 m and migrate vertically in the presence of seasonal light (Lancraft *et al.*, 2004).

In contrast to its pelagic distribution after hatching and consequent exposure to the physical circulation, substantial evidence indicates that the egg stage of *Pleuragramma* is ice-dependent, which may constrain connectivity. The reproductive migration of thousands of individuals to coastal areas with sea-ice cover has been observed from June to October, suggesting that spawning aggregations form under sea ice in winter (Daniels & Lipps, 1982). Large numbers of eggs with embryos and newly hatched larvae were found under fast ice in Terra Nova Bay, in the Ross Sea (Vacchi *et al.*, 2004). Vacchi *et al.* (2012) also provided evidence that the early life-cycle of *P. antarctica* begins in association with the coastal sea ice, and that eggs and newly hatched larvae constitute a major component of the cryopelagic community. Its early life stages appear to be well adapted to the ice environment: the egg chorion protects developing embryos from freezing (Cziko *et al.*, 2006), and early larvae, which have no significant antifreeze capacity, display underdeveloped gill structures that reduce exposure to ice nuclei (Bottaro *et al.*, 2009). Recent evidence points toward a trophic role for sea ice in the early life-cycle. The underside of sea ice and the platelet ice layer are sites of enhanced primary production that constitute the primary habitats for many invertebrate grazing species that use sea ice as a major source of food and refuge from predation (Bluhm *et al.*, 2010; Caron & Gast, 2010). Granata *et al.* (2009) showed that the diet of postlarvae of *P. antarctica* is mainly composed of grazers associated with sea ice. Moreover, the structure of irregularly disc-shaped ice platelets may also protect eggs and early larvae from predation (La Mesa *et al.*, 2010). As a result, the available evidence points towards a strong interaction

between the sea-ice environment and life history that may structure the spatial distributions of silverfish through the availability of suitable spawning and nursery habitats.

Despite its obvious importance to the Antarctic marine ecosystem, the population genetic structure of *P. antarctica* has not yet been fully clarified. Partial sequencing of the mitochondrial control region, in samples collected at four locations around the Antarctic (Weddell Sea, Ross Sea, Elephant Island and King George Island) in different years, showed small but significant differences over time and space (Zane *et al.*, 2006). Genetic differentiation was detected between samples taken 2 years apart in the Weddell Sea and between one of the Weddell Sea samples and a population sample collected in the Ross Sea. Nonetheless, genetic homogeneity was found in most pairwise comparisons, even between samples from opposite sides of the continent. Two hypotheses were proposed to explain this pattern. First, silverfish might have significant but so far unresolved population structure. Second, a general context of panmixia might exist, with sporadic and weak differences between populations as a consequence of local fluctuations of allelic frequencies (Zane *et al.*, 2006).

In the present study, we used a different set of markers, consisting of a panel of 16 expressed sequence tag (EST)-linked microsatellite loci, and extended the genetic analyses to a previously unexamined geographical area along the western Antarctic Peninsula, where the southern boundary of the ACC follows the continental slope. As a result, shelf areas are bounded on the seaward side by the ACC and on the landward side by the CC. We tested for genetic structuring and gene flow following the large-scale circulation by examining (1) genetic differentiation among silverfish sampled along the AP, (2) genetic stability over time, and (3) eastward gene flow following the ACC, or westward gene flow along the CC.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 562 individuals from nine population samples, representative of four geographical areas, were included (Fig. 1, Table 1). Samples from Marguerite Bay in 2001 and 2002 were collected as reported in Donnelly & Torres (2008). The 2010 sampling was performed as described by Ashford *et al.* (2010b); fishing activity was conducted at six sites along the western AP shelf off Charcot Island, in Marguerite Bay, south of Joinville Island, south of Renaud Island, over the shelf west and south of Anvers Island, and in Croker Passage (Fig. 1). In the last three sites, the species was not detected, except for a single specimen collected in Croker Passage. In 2011, sampling was conducted based on the Palmer Long-Term Ecological Research (LTER) regional grid (900 km × 200 km, west of the AP) from Anvers Island to Charcot Island (Waters & Smith, 1992), with successful collection at Marguerite Bay (Table 1). The sampling locations along the western AP during 2010 and 2011 were based on the position of known

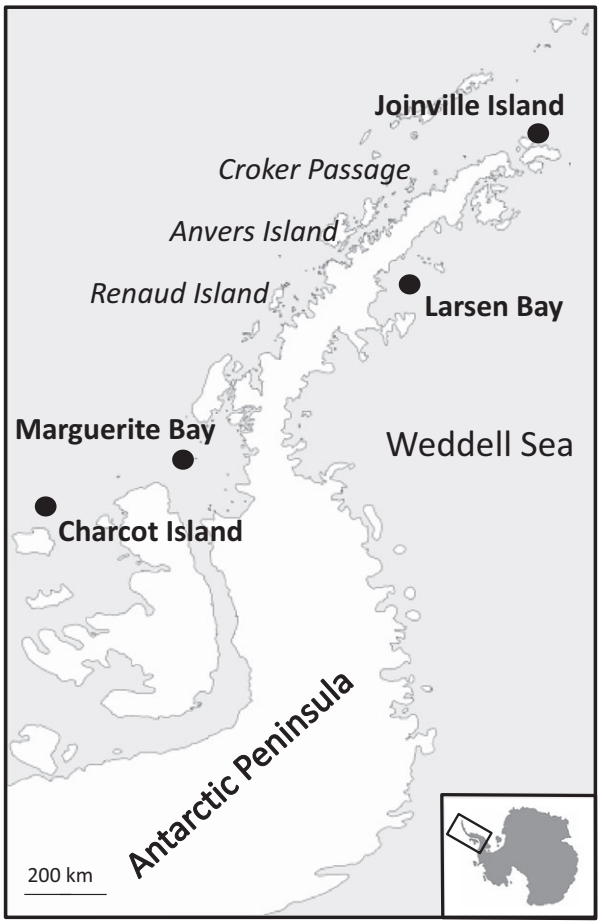

Figure 1 Approximate sampling locations of *Pleuragramma antarctica* along the Antarctic Peninsula shelf (circles). Localities where the species was not detected in 2010 and 2011 are indicated in italics.

rookeries of Adélie penguins (*Pygoscelis adeliae*; major predators of *Pleuragramma antarctica*), changes in Adélie penguin diet from north to south, and anecdotal reports of the disappearance of *P. antarctica*. Three additional samples were included in the study; one from the Larsen Ice Shelf in the north-western Weddell Sea (hereafter, Larsen Bay 2007) and two from Joinville Island (2007 and 2012) caught during two Antarctic expeditions aboard the research vessel (R/V) *Polarstern* of the Alfred Wegener Institute (Table 1).

Standard length (SL) was recorded for all specimens, and a small piece of muscle tissue was collected and preserved in RNAlater (absolute ethanol for 2001 and 2002 samples) at −80 °C until the molecular analyses. Genomic DNA was extracted from 10–100 mg of muscle tissue following a standard salting-out protocol. DNA solutions were stored at −20 °C before amplification by polymerase chain reaction (PCR).

DNA amplification and microsatellite genotyping

Population samples were analysed with 16 published EST-linked microsatellites (Molecular Ecology Resources Primer

Table 1 *Pleuragramma antarctica* population samples collected between 2001 and 2012 at four different locations off the Antarctic Peninsula. Collection site, sampling year, sample acronym, sampling campaign, coordinates and number of individuals (n) analysed at 16 EST-linked microsatellites are indicated.

Site	Year	Acronym	Campaign	Coordinates	n
Charcot Island	2010	CI10	NBP 10-02[a]	70°07′ S, 76°02′ W	60
Marguerite Bay	2001	MB01	SO GLOBEC-Cruise 1[b]	67°57′ S, 68°21′ W	28
	2002	MB02	SO GLOBEC-Cruise 3[c]	68°08′ S, 68°01′ W	49
	2010	MB10	NBP 10-02[a]	67°49′ S, 68°09′ W	60
	2011	MB11	LMG Cruise 11-01, Palmer LTER[d]	67°39′ S, 70°04′ W	83
Joinville Island	2007	JI07	ANT-XXIII/8[e]	62°35′ S, 54°45′ W	34
	2010	JI10	NBP 10-02[a]	63°30′ S, 56°40′ W	148
	2012	JI12	ANT-XXVIII/4[e]	62°14′ S, 55°18′ W	54
Larsen Bay	2007	LB07	ANT-XXIII/8[e]	65°30′ S, 61°40′ W	46

[a]Cruise conducted during late March–April 2010 on board the Research Vessel (R/V) *Nathaniel B. Palmer*.
[b]Cruise conducted during 2001 austral autumn aboard the Antarctic Research Support Vessel (ARSV) *Laurence M. Gould*, as part of the Southern Ocean Global Ocean Ecosystems Dynamics (SO GLOBEC) programme.
[c]Cruise conducted during 2002 austral autumn aboard the ARSV *Laurence M. Gould*, as part of the SO GLOBEC programme.
[d]Cruise conducted during January 2011 aboard the ARSV *Laurence M. Gould*, as part of the Palmer Long-Term Ecological Research (LTER) programme.
[e]Aboard the R/V *Polarstern*, Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven, Germany.

Development Consortium *et al.*, 2011, 2013). All loci were mined from a database of about 24,000 contigs obtained by high-throughput sequencing of a normalized cDNA library from *Chionodraco hamatus* skeletal muscle (Coppe *et al.*, 2013). The 16 loci were multiplexed in two PCR reactions, specifically optimized for cross-amplification in *P. antarctica* (see Appendix S1 in Supporting Information for primer sequences and final conditions for all loci). Multiplexed PCRs were performed in a total volume of 10 μL, containing 1× QIAGEN Multiplex PCR Master mix (HotStarTaq DNA Polymerase, Multiplex PCR Buffer, dNTP Mix; Qiagen, Hilden, Germany), 0.2 μM primer mix and 100 ng of template DNA. PCR conditions were as follows: (1) initial activation step of 15 min at 95 °C; (2) 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 90 s and extension at 72 °C for 60 s; and (3) a final extension of 30 min at 60 °C.

Fragment analysis was performed on an ABI 3730xl automated sequencer (Applied Biosystems, Waltham, MA, USA) and microsatellite analysis was carried out using PEAK SCANNER 1.0 (Applied Biosystems). In order to minimize the negative consequences of poor allele calling, binning was automated with FLEXIBIN 2 (Amos *et al.*, 2007), and the final scoring was manually checked to ensure the accuracy of the process.

Data analysis

The genetic variability within each sample was assessed by computing the observed heterozygosity (H_O) and unbiased expected heterozygosity (H_E) with GENETIX 4.05.2, and the number of alleles (N_A) and allelic richness (A_R) were calculated using FSTAT 2.9.3 (Goudet, 2001). Deviations from Hardy–Weinberg equilibrium and genotypic linkage equilibrium were tested with GENEPOP online (Rousset, 2008). The significance of all tests was estimated by the Markov chain Monte Carlo (MCMC) method (10,000 dememorization steps, 500 batches of 10,000 iterations); the significance threshold ($\alpha = 0.05$) was adjusted using a standard Bonferroni correction.

LOSITAN 1.0.0 (Antao *et al.*, 2008), which implements an F_{ST} outlier detection approach, was used to identify loci potentially under selection. The program uses coalescent simulations based on observed data to generate the expected distribution of F_{ST} versus H_E with neutral markers. This distribution is used to detect outlier loci with significantly higher or lower F_{ST} values than neutral expectations. In our analysis, 1,000,000 simulations were run assuming a stepwise mutation model (SMM).

Population structure was studied by calculating pairwise F-statistics and hierarchical analysis of molecular variance (AMOVA) with ARLEQUIN 3.5.1.3 (Excoffier & Lischer, 2010), and significance was assessed by 10,000 permutation tests; the significance threshold ($\alpha = 0.05$) was adjusted using a correction for multiple tests as implemented in SGoF+ (Carvajal-Rodriguez & de Uña-Alvarez, 2011) when required.

Patterns of gene flow between geographical localities (Charcot Island + Marguerite Bay versus Joinville Island) were investigated by the Bayesian coalescent approach implemented in the program MIGRATE-N 3.6 (Beerli & Palczewski, 2010), using only individuals collected in 2010. MIGRATE-N was run using a Bayesian model with distinct populations and independent migration rates, with the following parameters: microsatellites Brownian model, uniform priors for θ (the mutation-scaled effective population size) ranging from 0 to 500, and for M (the ratio of immigration rate to mutation rate) ranging from 0 to 1500. A static heating scheme was used, with four chains and temperatures set at 1, 1.5, 3 and 10^6, ordered from cold to boiling, and two replicated runs were performed. For each run, after discarding the first

6.4×10^6 steps as burn-in, 80,000 genealogies were recorded with a sampling increment of 800 (giving a total length of the run after burn-in 6.4×10^7). Convergence was assessed by inspecting the posterior distributions of the parameters and comparing the results of two replicated runs. Percentages of immigrants and effective population sizes were calculated assuming a microsatellite mutation rate of 5×10^{-4} per generation (Estoup & Angers, 1998).

To examine whether the level of gene flow estimated by Migrate-n was compatible with the significant differentiation observed among samples, a forward-time simulation was performed using Easypop 2.0.1 (Balloux, 2001). Two populations were simulated with the same number of loci, the approximate level of variability of 2010 samples, and effective population sizes corresponding to the Migrate-n estimates. The populations were allowed to exchange individuals at a rate equal to the maximum value estimated by Migrate-n (0.77%, see Results), and mutations were allowed to occur following a stepwise mutation model with the previously used mutation rate. The simulation was run for 40,000 generations to attain an approximate equilibrium. In the final generation, two samples of 134 individuals (corresponding to the average sample size of 2010 collections at Charcot Island + Marguerite Bay and at Joinville Island) were taken from the two populations and used for F_{ST} and significance estimation. In particular, we performed 100 replicates and recorded the number of times that a significant F_{ST} value ($P < 0.05$) was observed.

RESULTS

Sixteen microsatellites were successfully amplified and genotyped in all 562 individuals, with no missing genotypes. This high performance of amplification indicates a good transferability of microsatellite loci isolated from *Chionodraco hamatus* to *P. antarctica* despite the relatively large phylogenetic distance between the two species (time to most recent common ancestor *c.* 20 Myr; Near *et al.*, 2012).

All population samples showed relatively high levels of genetic variation at the 16 microsatellite loci (Table 2, Appendix S2) and no significant difference in N_A, A_R, H_O and H_E was found across samples using one-way ANOVA ($P > 0.05$). No significant genotypic linkage disequilibrium was observed between any pair of loci, and all loci were in Hardy–Weinberg equilibrium in all population samples after Bonferroni correction for multiple tests (Appendix S2). No outlier loci were identified using Lositan: none of the 16 microsatellites showed significantly higher or lower F_{ST} values than neutral expectations.

Population pairwise F_{ST} (Table 3) showed no genetic differentiation among replicated samples collected in the same area, i.e. at Marguerite Bay and Joinville Island. This suggests genetic stability over time at the two locations. Genetic homogeneity was also found between samples collected off Charcot Island and in Marguerite Bay, indicating a single genetic population across this area. On the other hand, the comparison of Joinville Island samples with those from the south-western AP revealed a less obvious pattern. Samples collected at Joinville Island in 2010 and 2012 were both significantly different from Charcot Island and, to some extent, to the four replicates of Marguerite Bay (two and three significant values out of four, respectively, after correction for multiple tests), whereas samples from Joinville Island 2007 showed no significant differences. Finally, the comparisons involving the sample from Larsen Bay were all characterized by positive F_{ST} values, although significant differentiation was found only for the comparisons with MB11 and JI12.

A much simpler picture emerged when pooling replicates collected in different years (Table 4), for which the sample size was larger for several comparisons. Significant pairwise F_{ST} values were recorded for all comparisons except that involving the pooled Marguerite Bay sample and the Charcot Island sample, which supported a single population as previously observed (Table 4). Accordingly, the hierarchical AMOVA showed significant genetic differentiation among three geographical groups, corresponding to (1) Marguerite Bay + Charcot Island, (2) Joinville Island and (3) Larsen Bay ($F_{ST} = 0.0043$, $P < 0.0001$; $F_{SC} = 0.0001$, $P = 0.4645$; $F_{CT} = 0.0042$, $P = 0.0034$).

For 2010, the Joinville Island sample showed considerable variability in the distribution of lengths, whereas a single

Table 2 Genetic variability at 16 microsatellite loci of *Pleuragramma antarctica* population samples collected along the Antarctic Peninsula shelf. Number of alleles (N_A), allelic richness (A_R), observed heterozygosity (H_O) and unbiased expected heterozygosity (H_E) are shown; allelic richness is calculated based on a minimum sample size of 28 diploid individuals. Standard deviation (± SD) is given in parentheses. Sample acronyms are as in Table 1.

Population sample	$N_A \pm$ SD	$A_R \pm$ SD	$H_O \pm$ SD	$H_E \pm$ SD
CI10	8.31 (4.85)	7.01 (4.08)	0.5948 (0.2071)	0.6311 (0.2151)
MB01	7.50 (4.55)	7.50 (4.55)	0.6183 (0.2684)	0.6375 (0.2433)
MB02	8.13 (4.15)	7.01 (3.77)	0.6327 (0.2410)	0.6310 (0.2338)
MB10	8.69 (4.96)	7.33 (3.92)	0.6646 (0.2096)	0.6588 (0.2079)
MB11	9.13 (5.24)	7.07 (3.91)	0.6333 (0.2218)	0.6314 (0.2293)
JI07	7.56 (4.59)	7.20 (4.23)	0.6526 (0.2261)	0.6462 (0.2170)
JI10	10.88 (5.58)	7.39 (3.57)	0.6503 (0.1927)	0.6653 (0.1944)
JI12	7.94 (3.92)	6.84 (3.49)	0.6123 (0.2208)	0.6380 (0.2123)
LB07	7.69 (4.19)	6.95 (3.72)	0.6196 (0.2360)	0.6335 (0.2247)

Table 3 Genetic differentiation among population samples of *Pleuragramma antarctica* from the Antarctic Peninsula based on microsatellite data. Pairwise F_{ST} estimates (above the diagonal) and corresponding *P*-values (below the diagonal) are shown; bold indicates values with uncorrected probability below 0.05. Sample acronyms are as in Table 1.

	CI10	MB01	MB02	MB10	MB11	JI07	JI10	JI12	LB07
CI10	—	−0.0011	−0.0008	0.0007	−0.0001	−0.0009	0.0065	0.0102	0.0061
MB01	0.7027	—	−0.0015	−0.0026	−0.0015	0.0017	0.0057	0.0086	0.0063
MB02	0.6216	0.7207	—	0.0005	0.0009	0.0007	0.0037	0.0093	0.0035
MB10	0.3333	0.8018	0.4054	—	0.0004	−0.0028	0.0017	0.0062	0.0029
MB11	0.5496	0.6757	0.2342	0.2793	—	−0.0004	0.0041	0.0060	0.0089
JI07	0.7838	0.2973	0.3874	0.8559	0.5315	—	−0.0015	0.0008	0.0001
JI10	**< 0.0001***	**0.0180**	**< 0.0001***	0.0541	**< 0.0001***	0.8108	—	0.0020	0.0020
JI12	**< 0.0001***	**0.0090***	**< 0.0001***	**0.0270**	**< 0.0001***	0.3874	0.0991	—	0.0057
LB07	**0.0180**	0.0991	0.0811	**0.0451**	**< 0.0001***	0.4955	0.0991	**0.0090***	—

*Significant after correction for multiple tests as implemented in SGoF+ (Carvajal-Rodriguez & de Uña-Alvarez, 2011).

Table 4 Genetic differentiation among *Pleuragramma antarctica* samples from the Antarctic Peninsula, pooling replicates from Marguerite Bay and Joinville Island. Pairwise F_{ST} estimates (above the diagonal) and corresponding *P*-values (below the diagonal) are shown; bold indicates values with uncorrected probability below 0.05. Sample and pool acronyms are: CI, Charcot Island; MB, Marguerite Bay; JI, Joinville Island; LB, Larsen Bay. Sample sizes are given in parentheses.

	CI (60)	MB (221)	JI (236)	LB (46)
CI	—	−0.0001	0.0060	0.0061
MB	0.5433	—	0.0037	0.0057
JI	**< 0.0001***	**< 0.0001***	—	0.0023
LB	**0.0180***	**0.0010***	**0.0487***	—

*Significant after correction for multiple tests as implemented in SGoF+ (Carvajal-Rodriguez & de Uña-Alvarez, 2011).

length mode was found in both the Charcot Island and Marguerite Bay samples. For this reason, we tested for genetic differentiation between clusters of individuals from Joinville Island 2010 with different length modes. Two slightly but significantly differentiated groups were detected (F_{ST} = 0.0032, P = 0.0227) when individuals were separated based on a SL of 10 cm, which corresponds to the transition from cohort 3+ to cohort 4+ (Hubold & Tomo, 1989). In pairwise comparisons with other population samples, the 'Joinville Island 2010 small' cluster (n = 68 individuals; SL < 10 cm; seven significant pairwise comparisons out of eight) showed greater genetic differentiation than the 'Joinville Island 2010 large' fish cluster (n = 72; SL > 10 cm; three significant pairwise comparisons out of eight) (Table 5). No difference in the level of genetic variation was detected between the two clusters (one-way ANOVA, P > 0.05) and both were in Hardy–Weinberg equilibrium (P > 0.05).

Despite the evidence of geographical heterogeneity, Migrate-n indicated the existence of genetic exchange between the two locations. Consistent with a prevailing gene flow along the anticlockwise CC, migration rates suggested a higher flow of migrants from Joinville Island to Charcot Island + Marguerite Bay ($m_{JI \rightarrow CI+MB}$ = 0.77%) than in the opposite direction ($m_{CI+MB \rightarrow JI}$ = 0.28%); however, the 95%

Table 5 Genetic differentiation between the two clusters of *Pleuragramma antarctica* ('JI10 small' and 'JI10 large') within the Joinville Island 2010 (JI) population sample based on a standard length (SL) of 10 cm, and other population samples. Pairwise F_{ST} estimates and corresponding *P*-values (in parentheses) are indicated; bold indicates values with uncorrected probability below 0.05. Sample acronyms are as in Table 1.

	JI10 small (SL < 10 cm)	JI10 large (SL ≥ 10 cm)
CI10	**0.0083** (< 0.0001*)	**0.0067** (< 0.0001*)
MB01	**0.0082** (< 0.0001*)	**0.0054** (0.0270*)
MB02	**0.0057** (< 0.0001*)	0.0032 (0.0721)
MB10	**0.0038** (0.0451*)	0.0011 (0.2883)
MB11	**0.0060** (< 0.0001*)	**0.0045** (< 0.0001*)
JI07	0.0022 (0.1441)	−0.0036 (0.9820)
JI12	**0.0034** (0.0270*)	0.0026 (0.0991)
LB07	**0.0050** (0.0180*)	0.0001 (0.4505)

*Significant after correction for multiple tests as implemented in SGoF+ (Carvajal-Rodriguez & de Uña-Alvarez, 2011).

confidence intervals (95% CI) overlapped considerably ($m_{JI \rightarrow CI+MB}$, 5.00×10^{-8} – 0.0195; $m_{CI+MB \rightarrow JI}$, 5.00×10^{-8} – 0.0150). Estimates of effective population size were 6484 (95% CI, 0–12,333) for Charcot Island–Marguerite Bay and 10,924 (95% CI, 0–15,000) for Joinville Island. Forward-time simulations indicated that this level of migration can still be associated with significant population differentiation, in that 86 of 100 simulations resulted in statistically significant (P < 0.05) F_{ST} values (average F_{ST} 0.0016, ranging from −0.0001 to 0.0032).

DISCUSSION

The pattern of migration and differentiation found in this study is quite different from the genetic homogeneity predicted for Antarctic notothenioids by Matschiner *et al.* (2009). Testing between previous hypotheses of panmixia and genetic structuring in *P. antarctica*, using a panel of 16 EST-linked polymorphic microsatellites in fish caught along the AP shelf, revealed local genetic homogeneity over time and differentiation among geographical regions. Samples

from Charcot Island (2010) and Marguerite Bay (2001, 2002, 2010 and 2011) composed a single, stable over time, genetic population in the south-western AP. Similarly, off Joinville Island, no genetic difference was detected among replicates from 2007, 2010 and 2012. In contrast, pairwise F_{ST} and AMOVA between geographical pools (Charcot Island + Marguerite Bay, Joinville Island and Larsen Bay) showed significant differentiation that falsified the hypothesis of panmixia and instead suggested population genetic structuring between the south-western AP, its northern tip and the western Weddell Sea.

Despite the pelagic life history of silverfish, migration rates were relatively low, with less than 1% of individuals exchanged among populations per generation. Moreover, rather than unidirectional eastward gene flow mediated by the ACC (Matschiner *et al.*, 2009), the analyses using Migrate-n suggested a higher flow of migrants from Joinville Island towards Charcot Island and Marguerite Bay, implying that the migration of silverfish is influenced more by the CC along the western AP shelf than by the flow of the southern ACC along the shelf slope. Taking into account effective population sizes in the order of thousands of individuals, as obtained by Migrate-n, these rates indicate a level of migration higher than the threshold (commonly considered to be $Nm = 1$) above which migration effects are more important than genetic drift for an island model with symmetrical gene flow (Waples & Gaggiotti, 2006). This result suggests that such levels of migration, in a model allowing different population sizes and asymmetrical gene flow, can still be associated with statistically significant departures from panmixia, as indicated by our forward-time simulations. Consequently, our results add to the increasing number of studies (Papetti *et al.*, 2012; Damerau *et al.*, 2014) that demonstrate that genetic homogeneity is not a generalized pattern in Antarctic notothenioids, despite large-scale circumpolar current flow.

A more nuanced interpretation may be appropriate, however. The general pattern of geographical differentiation and stability over time in silverfish becomes more complicated when considering pairwise comparisons among all the available samples. The population samples collected at Joinville Island in 2010 and 2012 were significantly different from Charcot Island and, partly, from Marguerite Bay samples, whereas samples taken at this site in 2007 showed no differences in any comparison. In addition, the sample from Larsen Bay 2007 showed significant differences only from Marguerite Bay 2011 and Joinville Island 2012. Separating individuals from Joinville Island 2010 based on a standard length of 10 cm, two slightly but significantly differentiated groups were detected, in which the group with smaller fish ($\leq$ 10 cm) was differentiated genetically from seven of the eight other population samples in the study, whereas the group with larger fish ($>$ 10 cm) showed only three significantly different pairwise comparisons.

Aspects of the study design may partly explain the variability over time. The small sample sizes of JI07 and MB01 ($n = 34$ and $n = 28$, respectively; Table 1) could help account for the lack of differentiation found between JI07 and the other samples. Likewise, small sample sizes may be responsible for the homogeneity observed between MB01 and JI10. Alternatively, highly differentiated populations outside our study area could have contributed immigrants, causing fluctuations in allele frequencies that generated differences among samples (Papetti *et al.*, 2009). This explanation is inconsistent with the general weakness of differentiation found for the target species by Zane *et al.* (2006), however. In that study, the pattern of mtDNA differentiation in *P. antarctica* over a wider area that included the Weddell Sea, Ross Sea, Elephant Island and King George Island showed weak and sporadic differences between samples, with no stability over time and space. Instead, this kind of ephemeral structure has been described by Johnson & Black (1982), who used the term 'chaotic genetic patchiness' in a study on the pulmonate gastropod *Siphonaria jeanae*. Larson & Julian (1999) observed that, in many cases, populations separated by less than 10–100 km could be as genetically different as populations separated by 100–1000 km. Many factors can contribute to chaotic genetic patchiness (Johnson & Black, 1982), with divergence renewed each generation without accumulating (Broquet *et al.*, 2013; Aglieri *et al.*, 2014). The transitory patterns of genetic structure may be generated by variation in the larval pool and recruitment, which are counteracted in the long term by dispersal and gene flow (Iacchei *et al.*, 2013).

We hypothesize that, for silverfish, a complex dispersal pattern is most likely to interact with high recruitment variability and genetic drift (Broquet *et al.*, 2013) to shape the observed genetic pattern. We suggest that geographical differentiation and genetic patchiness are both important features of silverfish population structure, and that physical and biological processes may interact to produce the pattern of variability observed by Zane *et al.* (2006) and in this study. Thus, the pelagic life-cycle of silverfish may facilitate dispersal and connectivity at development stages from larvae to adults, but is constrained prior to hatching by the availability of sea-ice habitat suitable for adhesion of eggs to the sub-ice platelet layer. The developmental stages are subsequently stratified by depth in the water column (La Mesa *et al.*, 2010); in particular, the early larvae are distributed closer to the continent, at depths of 0–100 m characterized by seasonally warmer and well-stratified Antarctic Surface Water (Hubold, 1984; White & Piatkowski, 1993; Morales-Nin *et al.*, 1998; La Mesa & Eastman, 2012). At this stage, larvae are likely to be transported by local currents over the continental shelves (La Mesa *et al.*, 2010; La Mesa & Eastman, 2012). Subsequently, postlarvae and juveniles are transported to progressively deeper offshore waters, contributing to the species' dispersal, often in close proximity to the slopes surrounding banks and near the shelf break (Hubold, 1984; White & Piatkowski, 1993; La Mesa *et al.*, 2010; Ferguson, 2012; La Mesa & Eastman, 2012). Adult silverfish may further influence the observed genetic structure through

reproductive migrations to seek spawning grounds in suitable ice-habitats (Daniels & Lipps, 1982; Vacchi *et al.*, 2004, 2012).

Nevertheless, dispersal may lead to interaction with other features of shelf circulation that affect spatial distribution and mixing. Lagrangian particle tracking simulations showed that the circulation of the south-western AP shelf, close to Charcot Island and Marguerite Bay, is influenced by several clockwise gyres, the main ones of which are in Crystal Sound, in the Laubeuf Fjord, and off Alexander Island in the Bellingshausen Sea (Piñones *et al.*, 2011). These gyres, which have been shown to favour the local retention of pelagic and planktonic species (Piñones *et al.*, 2011), might alter connectivity, especially for the larvae and juveniles of Antarctic silverfish (Ferguson, 2012). As a result, the relationships between population structure, transport and retention may account for the low levels of gene flow between the north-western and the south-western AP, in accordance with the geographical genetic differences found between population samples in this study.

Differentiation could also be generated in other ways. Notably, the lack of silverfish in the diet of Adélie penguins at Palmer Station and penguins' precipitous decline (Emslie *et al.*, 1998; Moline *et al.*, 2008; Ducklow *et al.*, 2012) may mirror a discontinuity in silverfish distributions between the northern and the southern regions of the western AP. This is supported by a reduction of *P. antarctica* larval catches reported in the central-western AP (Moline *et al.*, 2008; Ducklow *et al.*, 2012) and the lack of fish caught during sampling in 2010 and 2011 at Anvers Island, Renaud Island and Croker Passage (Parker, 2012), where large populations of silverfish were present through the 1970s and 1980s (Kellermann, 1996; Lancraft *et al.*, 2004). High recruitment variability and unstable differences in reproductive success between genetically differentiated populations along the AP could then provide the conditions for increased pressure of genetic drift. Moreover, if not counterbalanced by gene flow, genetic drift combined with constraints to dispersal can lead to reduced population resilience to environmental changes (Underwood *et al.*, 2012).

Variation in the availability of suitable spawning and nursery habitat may also be important. Substantial recent evidence suggests that there have been rapid environmental changes along the western AP, including marked reductions in seasonal sea-ice production (Vaughan *et al.*, 2003; Stammerjohn *et al.*, 2008) and large increases (6 °C) in midwinter temperatures. Sea-ice declines have been particularly pronounced in the mid-Peninsula region from Anvers Island to northern Adelaide Island (Stammerjohn *et al.*, 2008), coinciding with the gap in distribution of *Pleuragramma* (Parker, 2012). Results from the present study suggest that a substantial fraction of local *Pleuragramma* populations are the result of local reproductive events, with spatially variable contributions by immigrants from other source populations. Declines in potential spawning and nursery habitat along the western AP may further reduce connectivity.

CONCLUSIONS

We have shown that even on the western AP, a region characterized by strong oceanographic currents, a species with a pelagic life history can show incomplete genetic mixing. Fluctuations over time of allelic frequencies and genetic differentiation between individuals of different length collected at the same location indicate the importance of genetic drift in this highly abundant species. Genetic drift and limited immigration from other source populations may lead to an increased risk of local extinction, which may be further exacerbated by declines in potential habitat of this ice-dependent species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Amplification conditions and descriptive statistics for the 16 EST-linked microsatellite loci used in this study.

Appendix S2 Number of alleles, allelic richness, observed and unbiased expected heterozygosity, and Hardy–Weinberg equilibrium probability for each locus across population samples.

BIOSKETCH

The research team brings together an international group of scientists with complementary suites of skills to address the state of *Pleuragramma antarctica* on the west Antarctic Peninsula shelf, under the funding of the NSF award 0741348 and the National Program for Antarctic Research (PNRA). The ongoing research aims to evaluate the importance of local reproduction versus larval advection, and the extent to which populations in the target subregions are genetically distinct, via analysis of population structure, otolith microchemistry and molecular genetics of fish.

Author contributions: J.J.T., J.R.A. and T.P. conceived the ideas; C.A., J.J.T. and J.R.A. collected the data; C.A., C.P. and L.Z. analysed the data; J.J.T., J.R.A. and T.P. contributed to data analysis and results interpretation. C.A., C.P., J.R.A., J.J.T. and L.Z. wrote the manuscript. All authors contributed to the final version of the manuscript.

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