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Robert W. Parker · Ken N. Paige · Arthur L. DeVries

Genetic variation among populations of the Antarctic toothfish: evolutionary insights and implications for conservation

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Abstract Commercial fishing is having an increasingly negative impact on marine biodiversity, with over 70% of the world's fish stocks being fully exploited and, in many cases, overexploited. On top of this, the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) has granted commercial fishing permits in the most remote marine environment on earth, the high-latitude Southern Ocean. The primary target of these new commercial fishing ventures is the large pelagic piscivorous predator, the Antarctic toothfish (*Dissostichus mawsoni*). Unfortunately, little information is available on the demography, genetics, or life history of this large fish. Without such information we have little idea as to the effects of commercial fishing on the population structure and survival of this species. In this study, we focus on patterns of genetic diversity within and between geographically disparate populations of the Antarctic toothfish, using randomly amplified polymorphic DNA markers. Results of our study showed high levels of genetic similarity within and between populations. Despite high levels of genetic similarity, genetic analyses detected significant population structure, including fixed differences among populations, a significant fixation index (Fst) and between-population differentiation via a Mantel test. From a conservation perspective, low levels of genetic diversity may be indicative of relatively small populations that would not be able to withstand heavy commercial fishing pressures. Given that there is evidence for significant genetic structure, it will be important to manage these fisheries in a manner that will help prevent the loss of unique genetic variation from regional overfishing.

R.W. Parker · K.N. Paige (✉) · A.L. DeVries
Department of Animal Biology,
University of Illinois, 515 Morrill Hall,
505 S. Goodwin Avenue, Urbana, IL 61801, USA
E-mail: k-paige@uiuc.edu
Tel.: +1-217-2446606
Fax: +1-217-2444565

R.W. Parker
Department of Natural Resources and
Environmental Sciences, University of Illinois,
W-503 Turner Hall, 1102 S. Goodwin Avenue,
Urbana, IL 61801, USA

Introduction

Even though there have been numerous national and international efforts, in the form of conventions and protocols, directed towards the implementation of ocean management policies, there is still growing concern that marine biodiversity is rapidly decreasing (Milewski 1995). One of the main reasons for this decline has been the exponential growth of commercial fishing efforts, including the most remote marine environment on earth, the high-latitude Southern Ocean surrounding Antarctica.

The waters surrounding the Antarctic continent are inhabited by over 270 species of fish (Eastman 1993; Moyle and Cech 2000). The dominant suborder among these fish is the perciform suborder Notothenioidei, representing roughly 50% of the fish fauna on the continental shelf of Antarctica. Notothenioids show a large degree of ecological diversity and occupy several distinct ecological niches. The majority of these fish are bottom dwellers that are small and sedentary (Eastman 1993), with average sizes ranging from 15 to 30 cm. The most prominent fish present is the Antarctic toothfish (*Dissostichus mawsoni*), also commonly referred to as the Antarctic cod. The Antarctic toothfish is unique among Antarctic fishes due to its size, its ability to maintain neutral buoyancy, its piscivorous feeding habit, and its pelagic lifestyle (Eastman and DeVries 1982, 2000). The Antarctic toothfish dwarfs all other Antarctic fish species and can reach lengths of more than 170 cm and maximum weights of 100–110 kg (A.L. DeVries, unpublished data). The physiological adaptations of the Antarctic toothfish to this harsh environment have been extensively studied for the past 25 years (e.g., DeVries 1980; Chen et al. 1997) but little information is available concerning its life history. From ongoing mark and

recapture studies (DeVries 1980), it has been established that these fish grow slowly, only gaining up to 1 kg in weight and 2.5 cm in length per year after reaching sexual maturity.

Until the late 1960s/early 1970s, the only vessels trawling the high-latitude Southern Ocean were research vessels investigating the marine fauna; prior to this time, commercial fishing in the Antarctic had been basically non-existent (Koch 1992, 1994). Commercial fishing interest had been low because these waters are remote and difficult to fish due to drifting pack ice. For a long period of time there was also a general belief that these waters did not contain fish species of any great commercial value (Hureau and Slosarczyk 1990). As scientific exploration and exploratory fishing continued, commercially valuable species were found and targeted for global markets. By the 1970s, the Atlantic sector of the high-latitude Southern Ocean, in particular, was being intensively fished (Koch 1992).

The Patagonian toothfish (*D. eleginoides*), which is a close relative of the Antarctic toothfish, has recently become a valuable targeted resource. This fish has a more sub-Antarctic range, occurring north of the Antarctic Front, than the Antarctic toothfish and is mainly fished off the southern coasts of South America and sub-Antarctic islands north of the Antarctic Front. The size of the fish, similar to that of the Antarctic toothfish, and its high value made fishing efforts exceptionally profitable. The high profit margins have increased the exploitation of this species and, in turn, caused fishing vessels to push further and further south where they discovered overlap with *D. mawsoni* and eventually grounds where the catches were only *D. mawsoni*.

With the approval of the Commission for Conservation of Antarctic Marine Living Resources (CCAMLR), commercial vessels have begun exploratory fishing in the Pacific sector of the Southern Ocean, the Ross Sea and adjacent Antarctic peninsula (Eastman and DeVries 2000). The fishing areas appear to yield only the Antarctic toothfish and some skates. Since the catch is predominantly the Antarctic toothfish, the main concern is that there is little information available on the population size, structure, range, migration patterns, or genetic diversity of this fish. Such information is essential for the proper management of any commercial fishery.

In this study, we focus on patterns of genetic diversity within and between geographically disparate populations of the Antarctic toothfish. Gaining an understanding of genetic diversity is important for at least three reasons. First, as populations are reduced in size, loss of genetic variation can lead to an increased probability of extinction through a decline in fecundity and viability, due to factors such as inbreeding depression (Franklin 1980; Frankham 1995). Second, the loss of genetic diversity may reduce opportunities for adaptive evolutionary change (Lande and Barrowclough 1987; Cheverud et al. 1994). Third, understanding genetic structure is useful for identifying evolutionarily significant units for conservation, i.e., genetically distinct populations of particular management concern (Moritz 1994).

Although, in general, marine fishes show less genetic differentiation among local populations than do freshwater and anadromous fishes, given that marine environments are less fragmented than freshwater environments (Carvalho 1993; Ward et al. 1994), population genetic data are available for only a limited number of Antarctic fish species (Williams et al. 1994; Reilly and Ward 1999; Smith and McVeagh 2000), and virtually nothing is known about the population genetic structure of the Antarctic toothfish [genetic studies on the Antarctic toothfish have thus far focused only on the development of molecular tools (Gaffney 2000; Smith et al. 2001) that have been used for interspecific phylogenetic comparisons (Bargelloni et al. 1994, 2000)]. It is possible that Antarctic fish populations experience a high degree of gene flow and exhibit little genetic differentiation among disparate locations, given that the major currents south of the Polar Front are circumpolar. Whether there is substantial genetic structure or not is of particular importance in the management of this fishery; i.e., without genetic information we risk losing unique, and ecologically and evolutionarily important diversity.

Randomly amplified polymorphic DNA (RAPD) markers were used to assess the level of genetic diversity within and between the two Antarctic toothfish populations. RAPD markers have proven useful in the evaluation of population genetic structure (e.g., Bardakci and Skibinski 1994; Bielawski and Pumo 1997; Mamuris et al. 1999) and in determining levels of genetic variation (Bielawski and Pumo 1997; Maki and Horie 1999).

Materials and methods

Sampling

Antarctic toothfish (*D. mawsoni*) samples were collected from two sites located approximately 3,000 miles apart. Collection sites included McMurdo Sound (77°52.79′S; 166°34.37′E), the southernmost embayment of the Ross Sea, and the shallow waters west of Brabant Island (63°25′S; 62°16′W) adjacent to the Antarctic Peninsula bordering the Bellingshausen Sea. Each sampling area was located over the continental shelf.

At McMurdo Sound, fish samples were obtained by drilling a large hole in the ice and lowering a 1,000-lb test line, with baited hooks spaced every 5 m at depths of 450–500 m. Toothfish were bled, dissected and tissue samples flash frozen in liquid nitrogen. Tissue samples were transported to the laboratory at the University of Illinois in liquid nitrogen. Individuals from the Antarctic Peninsula were obtained by otter trawling from the RV *Polar Duke*. After the catch had been brought aboard, it was sorted by species and each toothfish specimen was flash frozen in liquid nitrogen. Dry ice was used to keep the samples frozen while they were transported to Illinois. All samples were stored at –80°C upon arrival at the University of Illinois.

DNA extraction and RAPD amplification

A total of 21 individuals were sampled from each site for this study. Heart tissues, from the McMurdo samples, and muscle tissues,

from the Peninsula samples, were used for the DNA extraction. The heart and muscle tissues from McMurdo and Peninsula, respectively, were ground into a fine powder using a mortar and pestle. All samples were ground in liquid nitrogen to minimize DNA degradation.

Tissues were digested in 500 µl extraction buffer containing 0.01 M Tris-HCL, 0.1 M EDTA, 0.5% SDS and 1.5 µl proteinase K (20 mg/ml) at 50°C for 2 h. Following digestion, 3 µl RNaseA was added and the samples were incubated at room temperature for 4 h. The DNA was extracted with an equal volume (600 µl) of phenol followed by several extractions with chloroform-Isoamyl alcohol (24:1). After the extraction, sodium acetate (0.25 M final concentration) and 2 volumes of ethanol (95%) were added. DNAs were precipitated overnight at –20°C. After precipitation, DNAs were centrifuged for 30 min to form a pellet. The DNA pellet was washed once with ethanol (70%) and allowed to air dry. Dried pellets were then re-dissolved in 30 µl TE [10 mM Tris (pH 8), 1 mM EDTA]. DNAs were quantified using a Hoefer TKO 100 DNA fluorometer and diluted to a final concentration of 20 ng/µl for RAPD PCR analysis.

Each RAPD reaction contained 20 ng DNA, 1×PCR Buffer (20 mM Tris-HCL, 50 mM KCL), 0.2 µM of an Operon 10-base primer (from kits A and C; Operon Technologies), 1.5 mM $MgCl_2$, 0.1 mM each deoxyribonucleotide triphosphate (dNTP), 0.5 units of *Taq* polymerase, and sterile water (Sigma supplies) to a final volume of 25 µl. The PCR buffer, primer, $MgCl_2$, DNA, and water were combined to a volume of 15 µl. This mixture was then placed in a polycarbonate v-bottom microplate (MJ Research) and capped with 10 µl liquid wax and subjected to a "hot start" (Chou et al. 1992) at 85°C. Following the hot start, *Taq* polymerase, dNTPs, and water were added to each sample bringing the final volume to 25 µl. The samples were then subjected to the following RAPD PCR profile: (1) 3 min at 94°C; (2) 30 s at 94°C; (3) 30 s at 36°C; (4) 1.5 min at 72°C; (5) 10 min at 75°C (Williams et al. 1990). Steps 2–4 were repeated 44 times. Samples were run on an MJ Research PTC 100 thermal cycler. Each reaction was then run on a 1% agarose gel for 1.5 h at 60 V. Gels were stained with ethidium bromide, visualized, and photographed under UV light for scoring. Negative control reactions that contained no DNA were also used with every run in order to check for possible contamination or primer dimers.

Primers were chosen based on a screening process using 40 Operon primers from kits A and C. Each primer was assessed using two randomly selected individuals from each site. From these 40 primers, 13 produced clear, bright bands and were used to assess patterns of genetic variation within and among Antarctic toothfish populations. The 13 primers included A02, A03, A04, A06, A07, A09, A10, A14, C4, C6, C7, C11, and C12. Repeatability between PCR runs was also assessed, comparing the same DNA samples used to screen RAPD primers with those used in runs containing all DNA samples. Results showed that RAPD patterns were identical between runs.

Genetic analysis

RAPD polymorphisms were analyzed under the following assumptions: (1) bands from different loci do not comigrate; (2) each locus is a two-allele system in which only one allele is amplifiable; (3) alleles arise from identical mutations among individuals (Black 1993; Lynch and Milligan 1994; Apostol et al. 1996). Levels of within- and between-population genetic variation were assessed by calculating the number of unique bands, mean percent band sharing, and levels of genetic differentiation. A band was considered unique only if it was detected within a single population. Percent band sharing was calculated using the following equation

$$\text{Percent Band Sharing} = 2N_{AB}/(N_A + N_B) \times 100$$

where N_{ab} represents the number of bands that individuals A and B have in common. N_a and N_b are the total number of bands scored for each individual, respectively (Wetton et al. 1987). Percent band-sharing data were analyzed using an analysis of variance (ANOVA). Differences between means were evaluated using a Least Significant Difference (LSD) test.

Genetic differentiation between the McMurdo and Peninsula sites was assessed using MANTEL-STRUCT (Miller 1999). This program calculates interobservational similarity/distance measures and tests the hypothesis that observations within groups are more similar than observations between by using the Mantel test. This test can be thought of as a method for assessing the correlation between two distance matrices (Miller 1999). This test was chosen because of the nature of RAPDs. The RAPD markers are dominant, so allelic proportions are unknown. The MANTEL-STRUCT program starts by calculating the interobservational genetic similarity, or distance values. In this study, Dice's (1945) similarity index was used. MANTEL-STRUCT then creates a matrix of all pairwise combinations between individuals. The distances of observations within groups form triangular submatrices along the diagonal of the matrix, while observations between groups form rectangular off-diagonal submatrices. Then a congruent binary matrix is formed containing 1s in locations corresponding to between-group distances and 0s in locations corresponding to within-group distances. A correlation coefficient (r) of the two matrices is then calculated and this provides a measure of the amount of genetic differentiation between populations. Finally, MANTEL-STRUCT determines levels of significance between populations using a Monte Carlo procedure, where the 1s and 0s of the constructed binary matrix are redistributed randomly 1,000 times.

In addition, Fst (the fixation index, which is the reduction in heterozygosity of a subpopulation due to random genetic drift) was calculated from 68 RAPD fragments (loci) using the method described by Lynch and Milligan (1994) for RAPD data using the program RAPDFST 4.0.1 (Apostol et al. 1996). A contingency chi-square value was estimated to test $Fst = 0$. Fst was used in the following equation to estimate the effective migration rate: $Nm = (1–Fst)/(4Fst)$.

Results

Among the 13 RAPD primers used in this study, 12 were polymorphic and 1 was monomorphic, with an average of 5 bands generated per primer (ranging from 1 to 7 bands per primer). Levels of within- and between-population similarity, as measured by % band sharing, were high. Within the McMurdo population, on average, $85.4 \pm 0.6\%$ of all bands were shared among individuals while $88.6 \pm 0.6\%$ of all bands were shared among individuals within the Peninsula site. On average, $82.0 \pm 0.9\%$ of all bands were shared between the McMurdo population and the Peninsula population (Fig. 1).

Despite high levels of genetic similarity within and among individuals from these two sites, significant population structure was still detected. Individuals within the Peninsula site were significantly more similar to each other than to individuals within the McMurdo site ($P < 0.05$, Fig. 1). Individuals within these two populations were also found to be significantly more similar than individuals between these two populations via a Mantel test ($P = 0.001$), indicating substantial differentiation of these two populations. *F*-statistics also indicated significant population structure ($Fst = 0.297 \pm 0.082$, $\chi^2 = 24.9$, $df = 1$, $P < 0.0001$), with an Nm of less than 1 ($Nm = 0.6$) migrant per generation. Among 68 fragments generated from 13 primers, 3 fixed differences

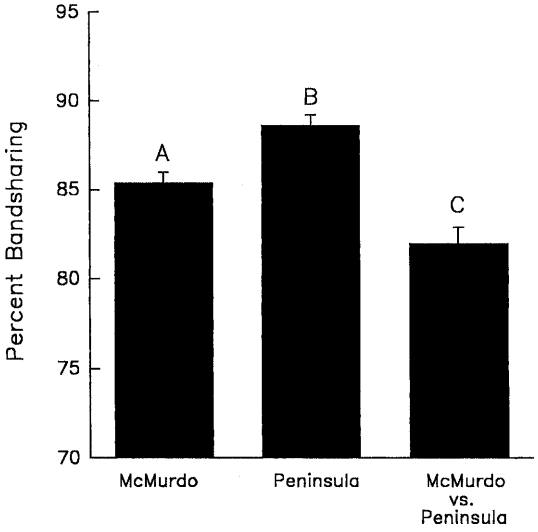

Fig. 1 Percent band sharing within and between the McMurdo and Peninsula populations. Means ± 1 SE ($F = 20.4$, $df = 2,60$, $P < 0.0001$). Means with different *letters* indicate significant differences at the 0.05 level, least significant difference multiple range test

were found between these 2 populations (all 3 present in all individuals in the McMurdo population and all 3 absent from all individuals in the Peninsula population), again suggesting significant differentiation.

Discussion

Percent band-sharing data showed exceptionally high levels of genetic similarity (i.e., low genetic variation) within and between populations of the Antarctic toothfish. Such results may be indicative of small effective populations, or historic bottleneck events that purged much of the genetic variation, and subsequent founder events (Wright 1931, 1969; Nei et al. 1975). Unfortunately, no information is available on the demographic history or population sizes of the Antarctic toothfish, or for that matter, any other species of toothfish (Eastman and DeVries 2000). Although there are few data with which to compare, high levels of genetic similarity from RAPD band-sharing data within and between populations of the freshwater tilapia, *Oreochromis niloticus*, have been directly attributed to known founder events from populations of small size (Bardakci and Skibinski 1994).

Another possible explanation for the low levels of genetic variation and high levels of genetic similarity within and between populations of the Antarctic toothfish is that severe environmental conditions (i.e., excessively cold waters) have resulted in low rates of microevolutionary change. Metabolic rates of Antarctic fish are known to be considerably lower than those of temperate-zone fish (Eastman 1993; Bargelloni et al. 1994). If a link were to be established between metabolic rates and the rate of molecular evolution, one might expect evolutionary rates of change in notothenioids to be much slower (Bargelloni et al. 1994). Recent empirical evidence in vertebrates indicates a strong positive correlation between the rate of metabolism and DNA substitution rate, for both nuclear and mitochondrial DNA (Avise et al. 1992). Functionally, such a relationship may be due to oxidative damage by radicals, which are generated as by-products of aerobic metabolism (Richter et al. 1988). In addition to slow rates of change, the Antarctic environment may also act as a strong "canalizing" agent, restricting the amount of genetic variation by way of natural selection.

Although there are high levels of genetic similarity (i.e., percent band sharing) within and between populations of the Antarctic toothfish, genetic analyses detected significant population structure. In particular, the estimated migration rate indicates that there is less than one migrant per generation being exchanged between these two populations ($Nm = 0.6$). Theoretically, less than one migrant per generation can lead to fixed differences among alleles (Mills and Allendorf 1996), particularly in numerically small populations. In fact, three fixed differences were found between the McMurdo and Peninsula sites, supporting this prediction. Of course, it is important to point out that populations that currently have no gene flow at all, but have a shared ancestry can create an Fst value less than 1 that could erroneously imply gene flow (Templeton et al. 1995). The point here is that effective gene flow is either quite low or nonexistent, which has led to fixed differences between the two populations.

These results are also similar to those found for the Patagonian toothfish (Smith and McVeagh 2000). Using microsatellite data, comparisons of Patagonian toothfish populations from the Atlantic, Pacific, and Indian Ocean sectors of the Southern Ocean showed significant genetic structure (significant Fst and Rst values) but no alleles unique to any of the basins. As in this study, patterns of genetic variation suggest that there is restricted gene flow through the Southern Ocean and that different fishing grounds support independent stocks (Smith and McVeagh 2000).

Although there are no known physical or environmental barriers, coastal currents may play a substantial role in limiting gene flow between populations of the Antarctic toothfish. In the major embayments of the Ross and Bellingshausen Seas, where these populations are located and where these fish spend much of their lives, Antarctic coastal currents form clockwise gyres that may act in localizing fish populations (Eastman 1993). In addition, if there were site fidelity to natal coastal grounds for spawning, such behavior would also act to limit gene flow. It is thought that *D. mawsoni* spawns in coastal waters (Yukhov 1982).

The results of our genetic studies are of particular importance from a conservation perspective in light of the fact that the Commission for the Conservation of Antarctic Marine Living Resources has recently granted commercial fishing permits in Antarctic waters.

Althoughonly suggestive, low levels of genetic diversity may be indicative of relatively small populations that would not be able to withstand heavy commercial fishing pressures. Independent of population size, *D. mawsoni* would be less capable of rebounding from heavy fishing pressures given their slow growth rates and the amount of time it takes to reach sexual maturity (approximately 8 years; Burchett et al. 1984).

Furthermore, since there is evidence for significant genetic structure, it will be important to manage fisheries in a manner that will help prevent the loss of unique genetic variation from potential regional overfishing. This will require additional studies to genetically map the entire Southern Ocean.

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