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Title: Integrating genetic and otolith microchemistry data to understand population structure in the Patagonian Hoki (*Macruronus magellanicus*).

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Abstract

Information from genetic (microsatellites and mtDNA Control Region) and previously collected otolith (trace element fingerprinting of otolith core and edge) markers was jointly interpreted to describe dispersal and gene flow in the Patagonian hoki (*Macruronus magellanicus*), an intensively harvested marine fish with seasonal migrations between spawning and feeding grounds. Spawning adults from a Chilean (Pacific) spawning site and three feeding ground samples (one from Chile and two temporal samples from the Falkland Islands (Atlantic)) were analysed. The data indicated a high level of Atlantic/Pacific connectivity by means of non-natal homing of individuals to spawning aggregations. Against this background of regional connectivity however, genetic data support the existence of a reproductively isolated population within the overwintering stock. Otolith core results are compatible with reproductive isolation being effected by natal homing to an Atlantic spawning site and/or local adaptation. The discordance between geopolitically defined Atlantic and Pacific management stocks and underlying biocomplexity, and implications for sustainability, are discussed. The study highlights the importance of intraspecific variation in homing behaviours in shaping population structure and the merit of employing complementary analytical approaches.

Keywords: population genetics; homing; fisheries; food security; biodiversity; conservation; management

1. Introduction

The Patagonian hoki, *Macruronus magellanicus* (hereafter hoki), is a migratory pelagic species inhabiting water depths of 60-600 m throughout its range from 33^0S in the Southwest Atlantic, and 29^0S in the Southeast Pacific, to 57^0S around Cape Horn (Wöhler and Giussi, 2001). From austral spring to autumn, adult hoki are dispersed throughout their feeding grounds south of 48^0S on the Patagonian shelf (Atlantic) and southern Chile (Pacific) (Wöhler and Giussi, 2001). In austral winter part of the stock migrates to more northern spawning areas, but a substantial proportion of adults remain on feeding grounds and skip spawning (Rideout et al., 2005). Large spawning aggregations have been reported around Guamin Island, Chile, between 43^0S and 48^0S (Galleguillos et al., 1996; Paya et al., 2002), while in the southwest Atlantic smaller aggregations of spawners and juveniles have been reported in the Gulf of San Matias (42^0S) and Gulf of San Jorge (46^0S) in Argentina (Wöhler and Giussi, 2001).

Exploitation of hoki intensified in the late 1980s as an alternative to the overfished common hake *Merluccius hubbsi* (Wöhler et al., 1999), with peak annual catches of 473,900 t reported in 1999 (FAO, 2008). The species is currently managed as two separate geographical stocks in Pacific and Atlantic waters. Pacific stocks have declined in recent years (Chong et al., 2007) while abundance in the Atlantic has increased (Wöhler et al., 2007), interpreted by some as supporting the Pacific / Atlantic stock distinction. However, it seems that known San Matias and San Jorge (Atlantic) spawning aggregations cannot sustain the observed biomass in the Atlantic region, implicating connectivity between the regions and/or additional high seas spawning in the southwest Atlantic (Wöhler and Giussi, 2001). There are also uncertainties regarding stock structuring on finer geographical scales within regions with spatial and temporal patterns in the distribution of juvenile and mature or post-spawned fish suggesting complex demographic stock heterogeneity (Giussi, 1996; Perier and Di Giacomo, 1999). Spawning site fidelity in hoki, as suggested for the closely related New Zealand hoki (*M. novaezelandiae*, Hicks et al., 2003), could restrict gene flow. Initial population genetic studies have suggested subtle genetic differentiation in hoki between Pacific and Atlantic stocks (Machado-Schiaffino and Garcia-Vazquez, 2011) and within Atlantic waters (D'Amato, 2006), however robust inferences on stock structure are prevented for a number of reasons. Firstly, reported levels of genetic differentiation are so low that their biological significance could be questioned (Hedrick, 1999). Secondly, for species with spatially/temporally partitioned spawning and feeding periods the nature of sampling (i.e. spawning vs. non-spawning individuals) may be vital to the resolution of population structure (Hauser and Ward, 1998). None of the genetic studies of hoki to date have included samples from spawning populations and in such cases mechanical admixture, as opposed to hybridisation (Nielsen et al., 2003), may compromise estimates of population structure. The

relevance of this for hoki is emphasised by the findings of Schuchert et al. (2010) who reported extensive admixture of Atlantic and Pacific spawned individuals, determined by trace element analyses of otoliths, in both areas.

Dispersal and gene flow, due to their respective influences on population structuring, are key processes affecting both short-term population dynamics and long-term evolutionary change. Dispersal mediates the abundance and exchange of individuals among subpopulations and the extent to which local populations may fluctuate independently. Gene flow, through dispersal and consequent interbreeding, determines how populations are bound together as evolutionarily cohesive units. Spectrometric trace element analysis of otoliths permits elucidation of ontogenetic movements of individuals between habitats with different water chemistry (Campana, 1999) and has been used to study stock structure in Southwest Atlantic fish such as southern blue whiting *Micromesistius australis* (Arkhipkin et al., 2009). Genetic markers, which may also be applied to study 'real-time' dispersal (Castric & Bernatchez 2004), are the only tools that can describe effective dispersal across generations (i.e. interbreeding). Combining genetic and otolith trace element approaches may confer synergistic insights into population structure (Svendäng et al., 2010). In this study a primary objective was to compare patterns of genetic variation among hoki samples collected from Pacific and Atlantic waters. While the spatial arrangement of samples was similar to that of Machado-Schiaffino and Garcia-Vazquez, (2011) an important distinction is that in this study both spawning and overwintering aggregations were analysed. As the genotyped individuals were collected along with those used in the otolith trace element study by Schuchert et al. (2010) (i.e. identical sampling time and location) an implicit additional objective was to combine both types of information towards a more informed description of dispersal and gene flow in the species.

2. Materials and methods

2.1 Sample collection and molecular analyses

Samples of adult hoki were obtained from commercial and research trawl catches. Hoki were collected from a known spawning site in Chile during the spawning period in austral winter (July 2007 - CSG1), and from two geographically distant feeding grounds in the southeast Pacific (southern Chile - CFG1) and in the southwest Atlantic (Patagonian Shelf southwest of the Falkland Islands - FFG1) during austral spring (October 2007) (Fig.1 and Table 1). As a temporal comparison, a second sample, of overwintering fish, was collected on the northern Falkland Islands feeding grounds (FFG2) in late austral summer 2008. For each fish pre-anal length, weight, sex and maturity stage was recorded, and samples taken for otolith chemistry (detailed in Schuchert et al. 2010) and genetic analysis (muscle fixed in 95% ethanol).

Total DNA was extracted using a standard CTAB-chlorofrom/isoamylalcohol method (Winnepenninckx et al., 1993). Nuclear genetic variation was assessed at two tetranucleotide (*Mm 5-4* and *Mm 14-1T4*) and four dinucleotide (*Mm 9-2*, *Mm 18-1*, *Mm 110-8*, *Mm 110-13*) microsatellite loci described by D'Amato et al. (1999). Hansen et al. (2001) demonstrated that misclassification of 4% of genotypes could produce an apparent F_{ST} of 0.001 to 0.003 when true $F_{ST} = 0$. Given that low F_{ST} might be expected between the hoki samples a number of steps were taken to maximise accuracy of genotyping: (i) PCR products of four individuals with known genotypes were run for every locus in every gel; (ii) all genotyping was performed independently by two experienced individuals with any mismatching genotypes being included in the repeat analysis (step iii); (iii) ~20% of all individuals were re-assayed (i.e. PCR, electrophoresis and genotyping) to assess rates of genotyping error.

Previous population genetic studies of hoki mtDNA variation have assayed variation in coding genes by either RFLP (ND5/6 - D'Amato and Carvalho, 2005) or direct sequencing (COI - Machado-Schiaffino and Vazquez, 2011) and have reported low variation and star-shaped genealogies with a single ancestral haplotype being found in the majority of individuals. The mtDNA Control Region does not code for a functional gene and therefore is under fewer functional and structural constraints, leading to a high average substitution rate (Saccone et al., 1987). As it is usually the fastest evolving region in the mtDNA of vertebrates, and therefore potentially more sensitive to fine scale population structuring, this region was targeted in this study. Predicting that hoki adhered to the ancestral mtDNA gene order of gadoids wherein the control region is flanked by the cytochrome b and 12S genes (Roques et al., 2006), GenBank sequences for Patagonian hoki and *M. novaezelandiae* were used to design primers rooted in the cytochrome B (HokiCR-F 5'-CAGCCTTTTCATCTGTTGTCC-3') and 12S (Hoki CR-R5'-GGCGACGGTGGTATATAAGC-3') genes to PCR amplify a fragment containing the entire Control Region. PCR reactions were performed in a total volume of 30ul, containing ~100ng of template DNA, 1 μM of each primer, 1X PCR Buffer, 2.0MM MgCl2 and 0.5U Taq DNA polymerase (Bioline UK). The PCR thermoprofile was 3min at 95 ^{o}C, followed by 35 cycles of 30s at 95 ^{o}C, 30s at 55 ^{o}C and 45 s at 72 ^{o}C; followed by a final 5 min extension at 72 ^{o}C. PCR products were purified using ExoSAP IT and sequenced from both ends with the PCR primers on an ABI 3130 DNA sequencer. Sequence chromatograms were examined and edited in CHROMAS. Initially a small number of samples were sequenced, then following confirmation using BLAST that the Control Region was being sequenced internal primers (HokiCR-F2 5'-AGAGCACCAGCCTTGTAAG-3' and HokiCR-R2 5'-GGGGTTTTCTAGGTCCCATC-3') were designed to amplify and sequence (from both ends), using the same conditions as the initial primers, the Control Region in a larger number

of individuals. Sequence alignment was performed using the CLUSTAL W (Thompson et al., 1994) program executed in BIOEDIT (Hall, 1999) with adjustments made by eye where necessary.

2.2 Statistical analysis of microsatellite data

Numbers of alleles (N_A), allelic richness (A_R; El Mousadik and Petit, 1996), observed heterozygosity (H_O), and expected heterozygosity (H_E), were calculated using FSTAT 2.9.3.2 (Goudet, 1995). Genotype frequency conformance at individual loci to Hardy-Weinberg equilibrium (HWE) expectations and genotypic linkage equilibrium between pairs of loci were tested using exact with default parameters in GENEPOP 3.3 (Raymond and Rousset, 1995). Multilocus values of significance for HWE tests were obtained using Fisher's method (Sokal and Rohlf, 1995) to combine probabilities of exact tests. Locus-by-sample combinations were tested for the presence of null alleles using MICROCHECKER (van Oosterhout et al. 2004) with significant effects adjusted for using the van Oosterhout algorithm. Genetic structuring was assessed using a number of approaches. Single- and multi-locus values of the unbiased F_{ST} estimator, θ (Weir and Cockerham, 1984), were calculated using FSTAT, with the significance of estimates tested by 10 000 permutations of genotypes among samples (Goudet et al., 1996). Genotypic differentiation was tested using the log likelihood (G) based exact test, and genic differentiation by Fishers exact test, both implemented in GENEPOP (with default settings). Genetic structure was also investigated without *a priori* sample information included using two clustering methods. Firstly, the Bayesian clustering analysis implemented in the program STRUCTURE (Pritchard et al., 2000) was used to identify the number of clusters, *K* (from a range of 1-4), with the highest posterior probability. Both the 'no admixture model' (as recommended for low F_{ST}; Pritchard et al., 2000) and 'admixture model with correlated allele frequencies' were employed. Each

MCMC run consisted of a burn in of 10^6 steps followed by 5 X 10^6 steps. Three replicates were conducted for each *K* to assess consistency. The *K* value best fitting the data set was estimated by the log probability of data [Pr(X/K]. The second clustering method used was the discriminant analysis of principal components (DAPC) implemented in ADEGENET (Jombart et al. 2010). Whereas STRUCTURE assigns cluster memberships by minimising Hardy-Weinberg and linkage disequilibria within clusters DAPC has less assumptions and simply maximises differences between groups while minimising differences within groups. The optimal model (i.e. number of genetic clusters) was identified by the lowest associated Bayesian information criterion (BIC) after 10^6 iterations for models of $K = 1$ to 5.

2.3 Statistical analysis of mtDNA data

All analysis was performed using ARLEQUIN 3.1 (Excoffier et al., 2005) unless stated otherwise. Genetic variation was described using indices of haplotype and nucleotide diversity (*h* and π respectively; Nei and Tajima, 1981; Nei, 1987) and their variances. A minimum spanning network was constructed in NETWORK (www.fluxus-engineering.com/sharenet.htm). Fu's *Fs* (Fu, 1997) and Tajima's *D* (Tajima, 1989) tests were used to test for deviations from mutation-drift equilibrium that could be attributed to selection and/or population size changes. Mismatch distributions (Harpending, 1994), the frequency distribution of numbers of pairwise differences between haplotypes within a sample, and simulated distributions under a model of demographic expansion were compared with the sum of squared deviations (SSD) between observed and expected distributions (significance assessed after 10 000 bootstrap replicates) used as a test statistic, and the expansion parameter τ estimated. Rough dates of population expansion were estimated with the formula $T = \tau/2u$ (Rogers and Harpending, 1992) assuming a mutation rate of 11% per million years (Bargelloni et al. 2003) and an average generation time of 3.8 years (Argentinean hoki

(*Macruronis magellanicus*) Fishery assessment report 2011). The partitioning of variation was analysed using AMOVA (Excoffier et al., 1992) derived estimates of various Φ-statistics (and their variance components), the significance of which were assessed by 10 000 permutations. Differentiation between pairs of samples was further tested by exact tests of haplotype frequency homogeneity and pairwise Φ_{ST} (significance assessed by permutation).

2.4 Estimation of Type I and Type II error rates

For both the microsatellite and mtDNA markers the sample size-dependent probability of Type I and Type II errors was estimated using the simulation method in POWSIM (Ryman and Palm, 2006). For microsatellite markers the observed global allele frequencies were used as representative of the ancestral population in the analysis. As the detected number of mtDNA haplotypes (n =75) exceeded the maximum number of alleles (n = 50) permitted in POWSIM the analysis for mtDNA was performed assuming 50 alleles at equal frequencies (0.02 - the observed average global haplotype frequency was 0.013).

3. Results

Levels of single- and multi-locus microsatellite variability were similar across samples (Supplmentary Table 1). Number of alleles per locus ranged from 3 to 21 (average = 13), and of 78 alleles resolved 13 were private alleles (CSG1 = 3; CFG1 = 3; FFG1 = 4; FFG2 = 1) with an average intra-sample frequency of 0.013 (range 0.006 – 0.031). No significant deviations from random associations of genotypes between loci were detected, either across all samples (data pooled) or in any single sample, indicating that the loci are independent. Tests for conformity to Hardy Weinberg equilibrium expectations revealed a number of deviations, in all cases due to deficits of heterozygotes. With the exception of *Mm* 18 and *Mm* 110-8, all loci exhibited significant global deviations from HWE. Significant multi-locus

heterozygote deficits were also detected for each of the samples, however the number of individual loci exhibiting such deficits was lower for the putative spawning site sample (CSG1 – only *Mm*9-2) than for the 3 feeding aggregation samples (4 loci in CFG1 – *Mm 5-4, Mm 9-2, Mm 14-1T4, Mm 110-13*; 3 loci in FFG1 – *Mm 5-4, Mm 9-2, Mm 14-1T4, Mm 110-8*; 3 loci in FFG2 – *Mm 5-4, Mm 9-2, Mm 110-13*). Locus *Mm*9-2 exhibited a significant heterozygote deficit in all samples. Microchecker identified underlying null alleles for all cases of locus/sample heterozygore deficits.

Both the STRUCTURE and DAPC clustering analyses reported no evidence for more than one genetic cluster within the data. However, all global tests of population structure among samples yielded significant outcomes : F_{ST} = 0.005 (P = 0.001), and exact tests for genic (P < 0.0001) and genotypic (P = 0.0004) differentiation. Analysis of pairwise tests between samples (Table 2) identified the main contribution to the global structuring to be the differentiation of the FFG2 sample, which was significantly differentiated from all other samples according to F_{ST} and tests for genic and genotypic differences. Similar results were obtained after correcting for null alleles: significant global differentiation (F_{ST} = 0.005, P = 0.001) and pairwise differentiation of FFG2 from all samples with nonsignificant results for all other pairwise comparisons (Table 2).

Pruning of mtDNA sequences permitted comparison of 1125 sites across 101 individuals. The sequenced region was AT rich (A= 33.82%, T = 33.11%) and contained 81 polymorphic sites (51 transitions, 21 transversions, 11 indels) defining 75 haplotypes (GenBank accession numbers x to x). Sixty-five haplotypes were found within only single samples (private haplotypes), with 62 (unique haplotypes) being represented by single individuals (Table 3, Fig. 3). Overall haplotype diversity was 0.9846 (SD = 0.0065) and nucleotide diversity was 0.0040 (SD = 0.0022), with levels of variability similar among the four samples (Table 3).

Adjacent haplotypes in the network were separated by an average of 1.86 mutations (maximum 6 mutations) and there was no obvious phylogeographic structure in their distribution among samples (Fig. 2). Tajima's *D* and Fu's *Fs* statistics were significantly negative for each sample (Table 3) and for global analyses (global D = -2.327, P = 0.002; global Fs = -25.683, P < 0.001). Mismatch distributions were compatible with a model of rapid population expansion with similar values of τ for each sample (Table 3). The global τ was 4.409 resulting in an estimated expansion occurring 17,823.86 years ago. AMOVA reported nearly all the variation (99.3%) to be contained within samples, with a non-significant amount partitioned among samples (Φ_{ST} = 0.006, P = 0.06). Pairwise Φ_{ST} and exact tests of haplotype frequency homogeneity were non-significant in all cases (Table 2).

POWSIM analysis indicated that the microsatellite data (average sample size = 74) had a low Type I error (Fisher P = 0.03) and a high probability (Fisher P = 0.998) for detecting differentiation at F_{ST} = 0.010. The employed sample sizes for mtDNA (average NmtDNA = 26) conferred a low Type I error probability (Fisher P = 0.03) but also a low power (Fisher P = 0.62) to detect differentiation at F_{ST} = 0.010.

4. Discussion

The genetic data reported here adds to the number of studies indicating that Patagonian Hoki around southern South America do not belong to a single panmictic unit (D'Amato, 2006, Machado-Schiaffino and Garcia-Vazquez, 2011). However, combining genetic data with associated information on individual fish natal area and adult movements derived from otolith trace element analyses (Schuchert et al., 2010) provides new insights into the biological significance of, and underlying mechanisms driving, this population structure.

MtDNA control region polymorphism was among the highest reported for a marine species (McMillen-Jackson and Bert, 2004) highlighting the potential utility of the control region as an informative marker in future hoki studies. However, simulation analysis indicated that the large number of low frequency haplotypes conferred a high Type II error probability for pairwise tests with the sample sizes employed here. A salient feature of the nuclear data was the significant differentiation of the austral summer Falkland Islands feeding sample (FFG2) , hereafter referred to as the overwintering sample, from all other samples. Statistical differentiation of FFG2 was supported by all pairwise tests employed with power analysis indicating a low probability of Type I error. This differentiation was not revealed by the clustering analysis, although such analyses have been shown to lack resolution at low levels of interpopulation divergence (Latch et al., 2006).

While the relationship between statistical and biological significance is complicated (e.g. Jorde and Ryman, 1996) a number of features support the biological significance of the differentiation for FFG2. Firstly, sampling of adults rather than younger individuals reduces the probability that the differentiation is linked to non-random sampling within sites due to family aggregations (Hansen et al., 1997). Secondly, although FFG2 was sampled at a later time than the other samples the intervening period would be insufficient to introduce intergenerational noise. Furthermore, identical results were obtained when pairwise tests among feeding ground samples were performed including only 2 year old fish (the most abundant cohort, Supplmentary figure 1). Therefore, the differentiation of FFG2 from the other feeding ground samples can not be attributed to temporal genetic changes within a single population. Thirdly, mtDNA variation revealed evidence of demographic fluctuations concordant with those suggested by D'Amato and Carvalho (2005). The mismatch distributions, high haplotype diversity and shallow phylogenetic structure support a post-last

glacial maximum (LGM = 20KYA) population expansion, with neutrality tests indicating non equilibrium signatures in the genetic diversity. This demography has implications for the detection of population differentiation using genetic markers. In the case of hoki, where there is no evidence of historical divergence, loci that are not at migration-drift equilibrium may retain signatures of historical gene flow and underestimate contemporary population isolation. Fourthly, F_{ST} reflects the proportion and not absolute number of migrants. Therefore, when populations are large, even very low F_{ST} values may reflect contemporary migration rates that are so low that populations may be reciprocally autorecruiting on time scales of relevance to fishery management (Palumbi, 2003; Hauser & Carvalho 2008). Finally, the sample was composed of overwintering fish and revealed a distinctive pattern of otolith core and edge trace element concentrations (Schuchert et al. 2010) indicating that the genetic differences for this sample are associated with life history differences.

The data therefore reveal a population within the overwintering stock that exhibits a degree of reproductive isolation. The companion otolith core results indicate that this population is largely composed of Atlantic spawned individuals. These features are compatible with the findings of D'Amato (2006) who reported evidence of four genetic groups occurring in Atlantic waters with the most divergent samples postulated to belong to an overwintering stock. Other features of the data indicate that the differentiation of the overwintering population is seemingly maintained against a background of high Atlantic/Pacific connectivity. Otolith core fingerprints revealed most CSG individuals to be Atlantic spawned (63.3%; Schubert et al., 2010) demonstrating a high level of dispersal from Atlantic to Pacific spawning sites. Concordant with this was the lack of genetic differentiation between the FFG1 sample and both Pacific samples. Although both the genetic and ontogenetic patterns could be generated by mechanical mixing without interbreeding this must be considered unlikely: the

CSG sample was collected at spawning time, suggesting that the presence of Atlantic spawned individuals does reflect reproductive dispersal. Furthermore, there was no evidence of cryptic admixture of genetically distinct units revealed by the clustering analysis or tests of Hardy-Weinberg equilibrium. The high degree of adult mediated dispersal and presumed gene flow from Atlantic to Pacific spawning grounds reported here is concordant with patterns of parasite diversity between both regions (Mackenzie et al. 2013) but contrasts with the Atlantic/Pacific differentiation suggested by Machado-Schiaffino & Garcia-Vasquez (2011). However, based on the results of this study it seems likely that within Atlantic structuring may have confounded estimates of interregional divergence by Machado-Schiaffino & Garcia-Vasquez (2011).

A central discussion in marine population structure is the relative roles of physical structuring and behaviour (Heath et al., 2008). The high levels of mixing of Atlantic and Pacific spawned adults at feeding grounds, however, emphasises the potential importance of homing behaviours in shaping population connectivity. Here, the distinction between homing and natal homing is important. Homing, where adults return to spawning grounds irrespective of whether they were hatched there has been widely reported in a number of species (Lundy et al., 2000). Natal homing, where fish return to spawn at their natal site, though more difficult to demonstrate, has also been reported (Svendäng et al., 2010). The distinction is vital as homing may not result in genetic differentiation, and may actually effect gene flow where there is non-natal recruitment of individuals to spawning aggregations. The identification of large numbers of Atlantic-spawned individuals spawning at the Pacific spawning site reveals a high level of non-natal homing recruitment of individuals, presumably through social learning of spawning behaviour within feeding assemblages (McQuinn 1997). In contrast, the genetic differentiation reported for the overwintering sample indicates restricted allo-

recruitment. The high proportion of Atlantic spawned individuals within the overwintering sample would be compatible with natal homing as a mechanism maintaining reproductive isolation through spatial/temporal isolation of spawning. Selection against member-vagrant hybrids (Sinclair 1988) could also act as a postzygotic reproductive isolating mechanism.

In conclusion, otolith chemistry and genetic marker analyses provided complementary insights into population structure in Patagonian hoki, and in agreement with both Machado-Schiaffino & Garcia-Vazquez (2011) and Mackenzie et al. (2013) confirmed that current management policy based on separate national regulations (Chile/Argentina/Falkland Islands) is discordant with underlying species biocomplexity. The data indicate a high level of connective Atlantic / Pacific gene flow within a system of non-natal spawning site homing. Although such a system is expected to buffer populations against stochastic demographic change (McQuinn 1997) an important consideration in light of reported declines in the Pacific hoki population is the possibility that the predominance of Atlantic individuals at the CSG sample may reflect a reduction in Pacific self recruitment. The reproductively isolated and potentially locally adapted population within the Atlantic overwintering stock may be particularly susceptible to population declines, due to cryptic overfishing within the mixed stock fishery. Improved understanding of species ecology, and additional genetic and ontogenetic marker analysis of short interval time-series samples of spawning and feeding grounds will be needed to confidently match hoki recruitment dynamics to an appropriate management strategy. Advances in molecular techniques allowing genome wide analysis (Moen et al., 2008) and genotyping of markers under directional selection may prove to be particularly insightful.

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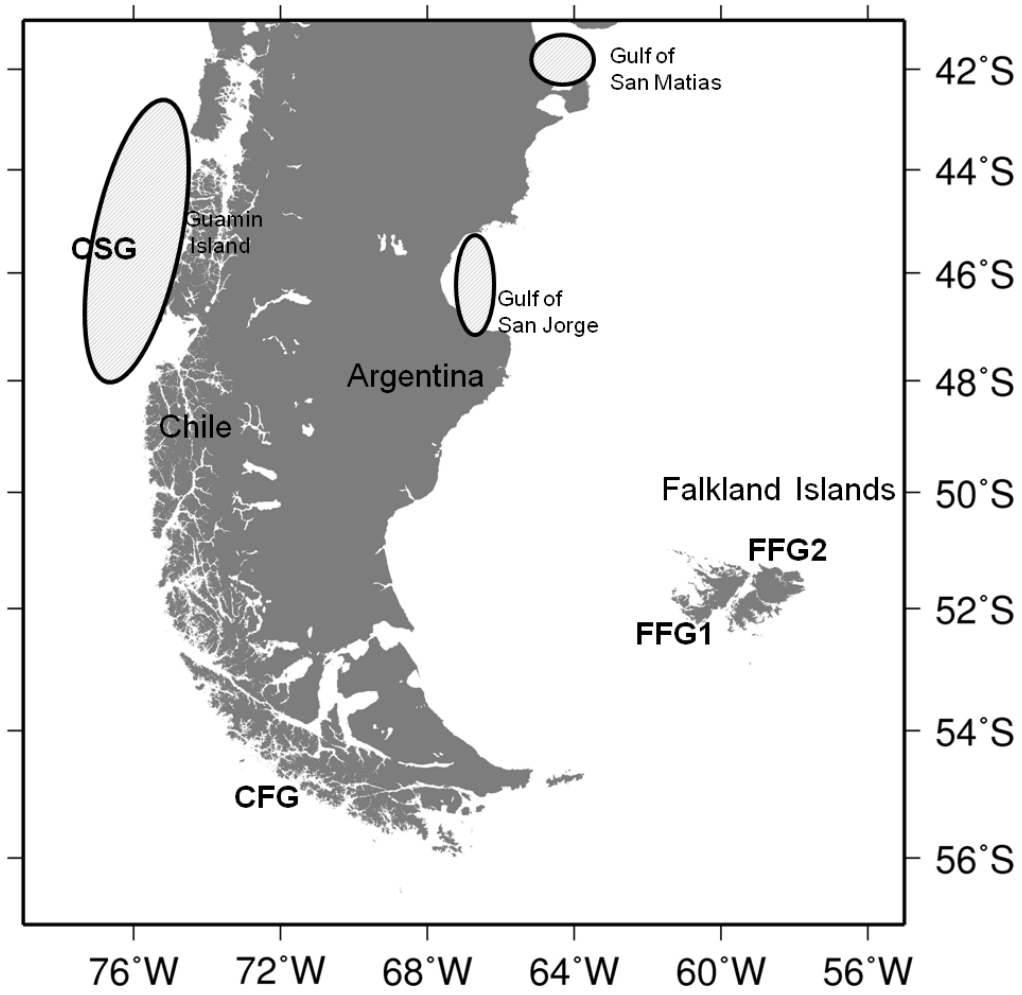


Figure 1. Sampling locations (CSG, CFG, FFG1 and FFG2) around the South Atlantic and Pacific, confirmed spawning grounds in Chile and the Gulfs of San Matias and San Jorge in Argentina indicated by light grey shaded areas.

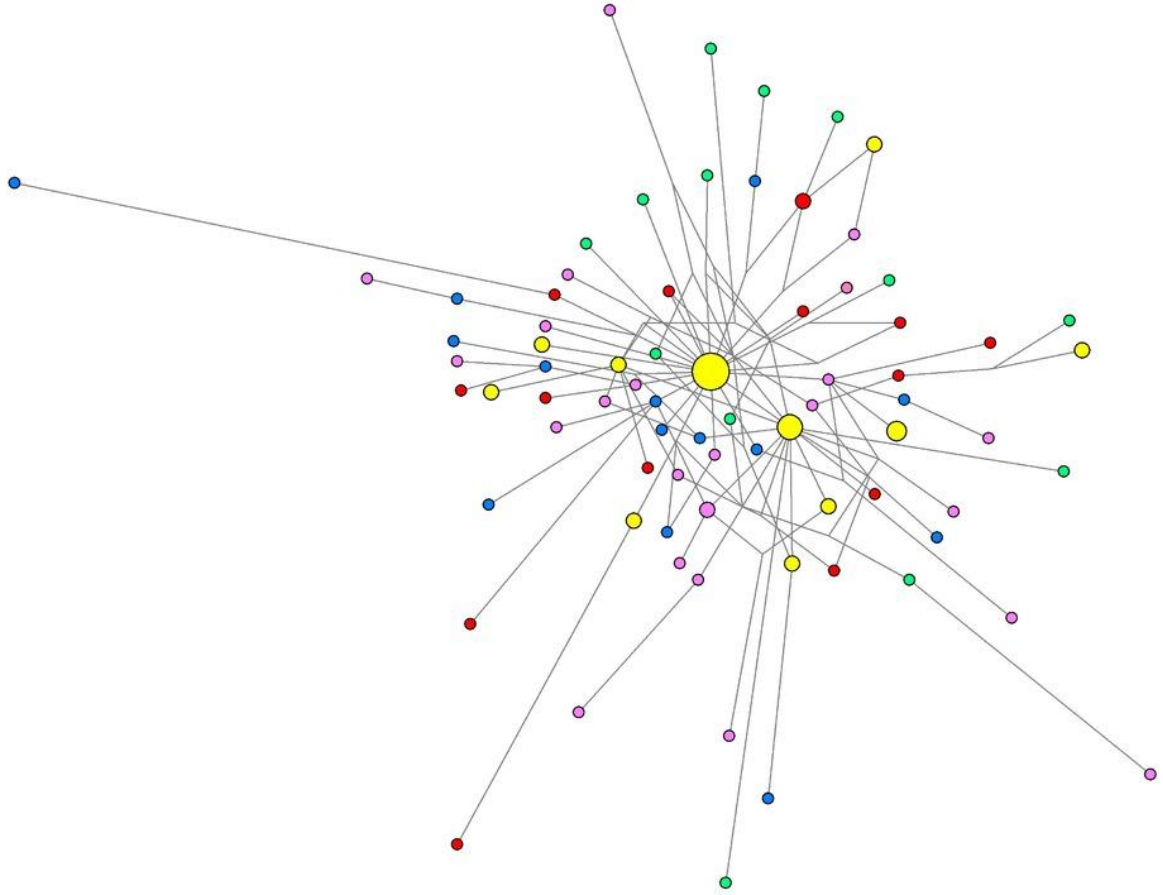


Figure 2. Median joining haplotype network, with private haplotypes marked as pink (CSG), blue (CFG), red (FFG1) and green (FFG2) and haplotypes detected in more than one sample as yellow. Disc sizes are proportional to overall frequency. Median vectors are not shown.

Table 1. Details of *M. magellanicus* samples included in this study, including sample names corresponding to locations indicated in Figure 1, time of sampling, sample sizes for microsatellite (*N*) and sub-sample sizes for mtDNA (*N*mtDNA) analyses. Also presented are admixture proportions of Atlantic and Pacific spawned individuals derived from otolith core analysis reported in Schuchert et al. (2010).

Sample	Date	*N* (*N*mtDNA)	Atlantic:Pacific admixture
CSG	Jun-07	49(31)	63.3:36.7
CFG	Oct-07	60(21)	52.8:47.2
FFG1	Oct-07	91(22)	77.6:22.4
FFG2	Feb-08	95(27)	80.9:19.1

Table 2. Pairwise tests of differentiation in allele/haplotype (Exact) and genotype (G test) frequencies, and estimates of F_{ST} for which significance (F_{ST} *P*) was tested by 10 000 permutations. NAC denotes test results with correction for null alleles. Significant values in bold.

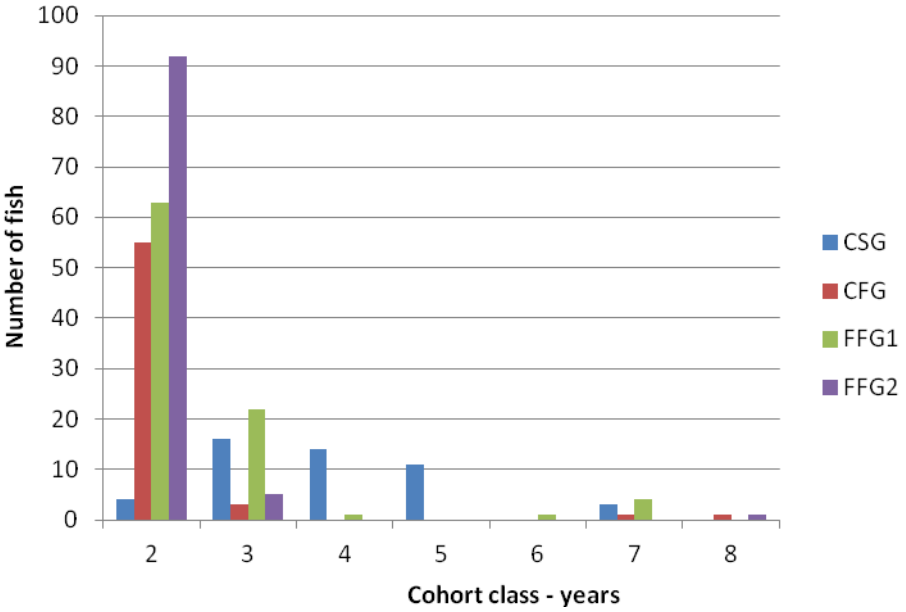
	Microsatellite				MtDNA		
	Exact	G test	F_{ST} (NAC)	F_{ST} *P* (NAC)	Exact	Φ_{ST}	Φ_{ST} *P*
CSG v CFG	0.136	0.325	0.0003(0.0036)	0.243(0.150)	0.755	0.015	0.068
CSG v FFG1	**0.031**	0.132	0.0012(0.0002)	0.113(0.183)	0.429	0.017	0.056
CSG v FFG2	**0.002**	**0.009**	**0.0071(0.0082)**	**0.001(0.025)**	0.755	-0.005	0.679
CFG v FFG1	0.059	0.232	0.0029(0.0042)	0.110(0.075)	0.435	-0.003	0.584
CFG v FFG2	**0.009**	**0.023**	**0.0049(0.0062)**	**0.031(0.008)**	0.479	0.0067	0.396
FFG1 vFFG2	**0.0004**	**0.005**	**0.0094(0.0075)**	**0.007(0.031)**	0.065	-0.002	0.527

Table 3. Descriptive statistics for the samples analysed for mtDNA variation including the numbers of private singleton haplotypes and occurrence of non-private haplotypes identified. Haplotye (*h*) and nucleotide (π) diversities and associated standard deviations, and results of demographic tests (mismatch distribution, Fu's Fs, Tajima's D), all obtained using ARLEQUIN 3.1 (Excoffier et al. 2005). *P*(SSD) denotes the probability that the empirical distribution of mismatches was significantly different than the distribution simulated under a demographic expansion model. Probabilities for Fu's Fs and Tajima's D estimated following 10 000 bootstrap replicates.

	CSG	CFG	FFG1	FFG2
Singleton private haplotypes	22	14	14	14
Hap_3	1		2	
Hap_6	2			
Hap_10	1	1		
Hap_16	1	2		2
Hap_17		3	2	6
Hap_25		1	1	
Hap_31	1			1
Hap_35	1			1
Hap_36	1			1
Hap_40	1		1	
Hap_41			1	1
Hap_43			2	
h (SD)	0.9978 (0.0089)	0.9810 (0.0225)	0.9870 (0.0175)	0.9516 (0.0320)
π (SD)	0.004475 (0.002486)	0.003545 (0.002061)	0.003924 (0.002246)	0.003748 (0.002139)
Fu Fs (P)	-25.53307 (>0.001)	-13.97864 (>0.001)	-14.42352 (>0.001)	-13.11913 (>0.001)
Tajima's D (P)	-2.07731 (0.007)	-2.18863 (0.002)	-1.77490 (0.023)	-2.21836 (0.001)
P(SSD)	0.4501	0.3435	0.3912	0.3302
τ	4.984	3.582	4.445	3.76

Supplementary table 1. Summary statistics of microsatellite intrasample diversity, including allele number (Na), allelic richness (Ar), observed (H_O) and expected (H_E) heterozygosity and P- values for tests of Hardy-Weinberg equilibrium (*P*HW) for which significant deviations are indicated by values in bold.

Locus	Index	CSG	CFG	FFG1	FFG2	Overall
Mm 5-4	Na	11	11	12	11	14
	Ar	10.96	10.66	11.01	9.68	10.784
	H_E	0.854	0.832	0.822	0.808	0.857
	H_O	0.783	0.623	0.667	0.697	0.692
	*P*HW	0.296	**0.0099**	**0.0049**	**0.0443**	**0.0004**
Mm 9-2	Na	14	11	12	9	15
	Ar	13.78	10.57	10.89	8.87	10.89
	H_E	0.818	0.831	0.82	0.783	0.728
	H_O	0.617	0.714	0.662	0.623	0.654
	*P*HW	**0.0061**	**0.0007**	**0.0084**	**0.0047**	**<0.0001**
Mm 14-1T4	Na	7	9	7	6	10
	Ar	6.93	8.27	6.76	5.725	6.81
	H_E	0.545	0.474	0.521	0.597	0.821
	H_O	0.563	0.456	0.42	0.628	0.517
	*P*HW	0.8255	**0.0366**	**0.0008**	0.054	**0.0007**
Mm 18-1	Na	1	1	2	2	3
	Ar	1	1	1.75	1.87	1.69
	H_E	0	0	0.022	0.033	0.665
	H_O	0	0	0.022	0.033	0.0138
	*P*HW	-	-	1	1	1
Mm 110-8	Na	12	10	13	11	15
	Ar	11.68	9.3	10.43	9.52	10.123
	H_E	0.652	0.573	0.655	0.547	0.504
	H_O	0.646	0.667	0.637	0.603	0.638
	*P*HW	0.2522	0.2146	**0.0221**	0.8531	0.0879
Mm 110-13	Na	13	15	16	15	21
	Ar	12.42	15	11.64	11.75	12.75
	H_E	0.543	0.639	0.571	0.657	0.456
	H_O	0.49	0.444	0.518	0.561	0.503
	*P*HW	0.1459	**0.0025**	0.0641	**0.0037**	**0.0001**



Supplementary Figure 1. Cohort composition of the samples included in genetic analysis.