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ABSTRACT

The blue shark (*Prionace glauca*) is globally distributed, occupying the pelagic, open ocean in temperate and tropical waters. It is the most abundant shark in the north Pacific and is exploited in several fisheries. Catch size and sex composition in fisheries and research data, along with tagging and distribution patterns, have been used to infer the existence of distinct north and south Pacific stocks. The present study consisted of a comprehensive survey of nuclear genetic diversity at microsatellite loci within and among six regions encompassing the north Pacific blue shark distribution to rationalize current management practice which assumes a single stock. We tested microsatellite diversity at 14 loci in 786 tissue samples for east–west population differentiation, examined regional samples for evidence of population mixture, and estimated historical and contemporary effective population sizes (N_e). The results strongly supported the existence of a single population of blue shark in the north Pacific. Historical and contemporary N_e values between 4500 and 5500 and the resultant low ratio of effective to census size (N_c) does not reflect recent population perturbation. However, it does highlight that the population may be more vulnerable to reduced reproductive success arising from natural or fishing mortality coupled with environmental change than total abundance would imply.

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1. Introduction

The blue shark (*Prionace glauca*) is a large, open-ocean shark found worldwide in temperate and tropical waters. It is considered to be the most abundant pelagic shark, with extensive information available on its biology (Nakano and Seki, 2003), and to a lesser extent, on its ecology (Nakano and Stevens, 2008). Its abundance reflects a generalist lifestyle and demographic resilience arising from large litter size relative to other elasmobranchs and early age-at-maturation, leading to a generation time estimated at seven years (Nakano, 1994; Cortes, 2002). Blue sharks are vagile and highly migratory (Kohler and Turner, 2008), with seasonal distributions corresponding to both prey availability and the reproductive cycle (Nakano and Stevens, 2008). In addition, blue sharks segregate by sex and size soon after birth, with the genders likely fully integrated again only at breeding (Strasburg, 1958; Nakano, 1994; Nakano and Seki, 2003).

Little is known of blue shark population structure, on either global or local scales, beyond the tagging results indicating equatorial migratory restriction (Kohler et al., 1998; Kohler and Turner, 2008). Emerging genetic information corroborates the tagging depiction of a weakly-structured species due to its flexible habitat requirements, great abundance and high mobility. Unlike some vagile shark species that may occupy pelagic regions but rely on coastal breeding grounds, blue shark do not exhibit mitochondrial DNA variation reflecting fine scale reproductive subdivision and female philopatry (Blower et al., 2012; Keeney et al., 2005; Ahonen et al., 2009; Taguchi et al., 2015). These results are consistent with a model of north Pacific gender-based blue shark distribution and reproduction constructed from research survey catch data (Nakano, 1994). Both mating and pupping grounds are located in the sub-Arctic boundary. Females may give birth annually. Mating occurs during early summer months in waters between 20 and 30°N. Over the course of a year, pregnant females migrate northward to parturition grounds (35–45°N) and birth occurs in early summer. The nursery grounds for juvenile females include the pupping grounds and the region just to the north and into the Gulf of Alaska; whereas nursery grounds for juvenile males include the pupping grounds,

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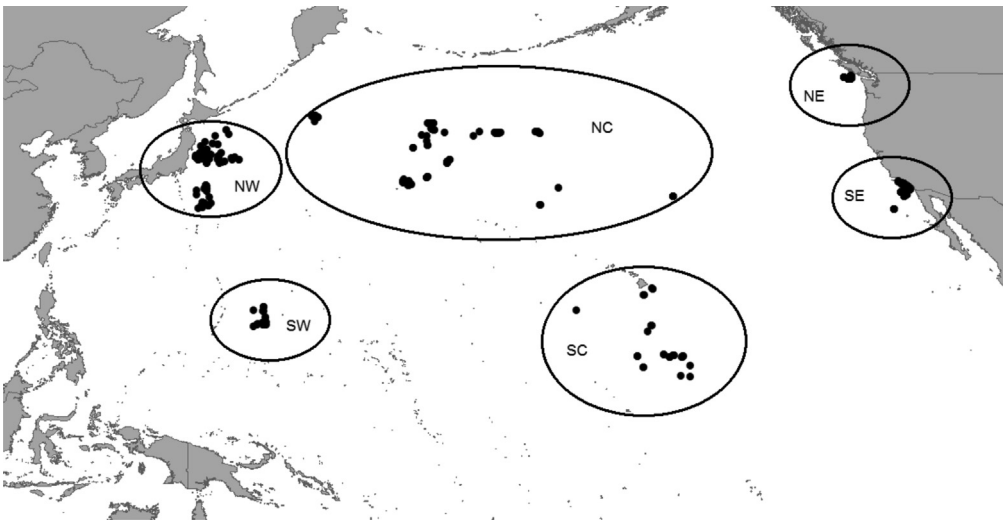

Fig. 1. Location of blue shark samples (n = 812) in the north Pacific, with aggregates representing geographic regions used in population structure analyses: NW—northwest; SW—southwest; NC—north central; SC—south central; NE—northeast; SE—southeast.

but also the region just to the south. Adults range southward of the nursery grounds to equatorial waters.

Existing genetic data for blue shark are limited, but have indicated that the species is weakly structured over broad geographic scales (Ovenden et al., 2009; Taguchi et al., 2015). There is a paucity of information on the effective population size (N_e) in blue shark, but Taguchi et al. (2015) suggested that a lack of genetic differentiation over the widespread distribution of the species, combined with the biological attributes of large litter size, early maturation, high vagility and high abundance, lead to the expectation of large genetically effective population size. N_e is a standardized measure of size of the successful reproductive cohort in a population that can be measured over historical times frames (HN_e) or for the contemporary population (CN_e). Historical estimates reflect the cumulative effects of gene flow and demographic events since, and possibly preceding, the establishment of a population. Since HN_e is the harmonic, rather than arithmetic, mean of N_e in each contributing generation, its value will be strongly influenced by low numbers associated with population bottlenecks, recent or ancient (Frankham et al., 2010).

Blue shark in the north Pacific ocean may therefore comprise a commercially exploited species that is abundant and both demographically and genetically resilient, although this premise has not been subjected to rigorous evaluation based on extensive genetic sampling. The basis of the genetic resilience is a postulated existence of a single, large population with a correspondingly large effective size that provides the population with a strong adaptive capability to respond to environmental fluctuations and harvest pressure. The present study assesses diversity and population structure of north Pacific blue shark at nuclear microsatellite loci with a regional sample set characterized by large sample size and extensive geographical coverage. The study is intended to provide results to justify or cause re-examination of the treatment of blue shark in the north Pacific as a single population (stock) in assessment efforts that provide direction for management actions (Kleiber et al., 2009; ISC, 2014). We use the microsatellite data set to test a hypothesis of genetic differentiation between blue sharks

Table 1
Number of blue shark sampled (N) from each geographic region in the north Pacific (NW—northwest; SW—southwest; NC—north central; SC—south central; NE—northeast; SE—southeast). Precaudal length (cm) and sex were recorded for a sub-set (n) of the total sharks sampled.

			Precaudal length (cm)		Sex ratio	
Location	Year	N	Median	n	Female (%)	n
NW	2003	1	135	1	0	1
	2005	41	129	41	36.6	41
	2009	18	139	18	38.9	18
	2010	38	128	38	71.1	38
SW	2011	18	163	18	100	18
NC	2010	12	122	12	16.7	12
	2011	65	153	65	33.8	65
SC	2007	1	136	1	0	1
	2008	32	–	0	–	0
	2011	23	–	0	–	0
NE	2007	93	103	92	83.9	92
	2010	229	149	229	86.5	229
	2011	2	–	0	–	0
SE	2007	100	77	47	51.0	89
	2010	81	67	31	49.4	78
	2011	90	150	57	49.1	55
Total		844	131	650	63.5	737

of the eastern and western Pacific and to provide the first estimates of HN_e, CN_e and CN_e:N_C for blue shark.

2. Materials and methods

2.1. Sample collection

Blue shark muscle tissue samples were collected opportunistically from fisheries and research surveys in the north Pacific from 2003 to 2011. Samples were preserved in 95% undenatured ethanol. The location (latitude and longitude) was recorded for each tissue sample, and all samples were designated into one of six geographic regions of the north Pacific (Fig. 1, Table 1). For some specimens, sex and length (cm) were recorded. Lengths were recorded as precaudal (PCL), forklength (FL) or total length (TL). Lengths were converted to PCL based on conversion in Nakano and Seki (2003).

2.2. DNA extraction and amplification of microsatellite loci

DNA for polymerase chain reaction (PCR) amplification of microsatellite loci was extracted using Qiagen (Toronto, Ontario, Canada) DNeasy® 96 Blood and Tissue kits according to the manufacturer's protocol. PCR amplification was conducted for 14 microsatellite loci (Table 2) with primer sequences described by Fitzpatrick et al. (2011) and Mendonça et al. (2012). In general, amplification was conducted using a DNA Engine Tetrad2 thermal cycler (BioRad, Hercules, California, United States) in 5 μL volumes consisting of 0.14 units of HotStar Taq DNA polymerase (Qiagen), 1 μL of undiluted DNeasy extracted DNA, 1× PCR buffer, 84 μM of each nucleotide, 0.50 μM of fluorescently labelled M13 forward primer (TGTAAAACGACGGCCAGT), 0.13 μM of M13 tailed forward primer, 0.50 μM of reverse primer with 5′-GTTT consensus sequence, and deionized water. The thermal cycling profile involved one cycle of Taq activation for 15 min at 95 °C followed by cycles of denaturation for 30 s at 94 °C, annealing for 30 s, and extension for 30 s at 72 °C; and a final extension for 10 min at 72 °C. Annealing temperatures and cycle number varied among loci (Table 2). Microsatellite alleles were size fractionated on an ABI 3730 capillary DNA sequencer, and genotypes were scored with GeneMapper software v 4.0 (Life Technologies, Burlington, Ontario, Canada) using an internal lane sizing standard.

2.3. Data analyses

2.3.1. Genetic diversity and population structure

Blue shark samples were pooled across collection years for each geographic region and multilocus genotypes successfully scored at 10 or more of the 14 loci were included in the data analysis. Microsatellite diversity within regional samples was assessed using allelic richness (A_R) standardized to a sample size of 17 individuals, and expected (H_E) and observed (H_O) heterozygosities calculated using FSTAT (Goudet, 2001). Deviations from Hardy Weinberg equilibrium were examined by sample region and locus.

Population subdivision of blue shark within the north Pacific ocean was initially explored without *a priori* assignment of samples to particular regions using the Bayesian approach in Structure version 2.3.3 (Pritchard et al., 2000). All samples with complete genotypes (659) were included. Posterior probabilities for the existence of up to six blue shark subpopulations were evaluated with independent runs of $K = 1$–6. All runs were performed with ten iterations of a burn-in of 100,000 cycles and 200,000 MCMC repetitions with no prior information. Allele frequencies were assumed to be correlated and admixture was allowed. A likelihood ratio test was used to determine the most probable number of subpopulations and chain convergence was assessed by degree of stability in alpha values after the burn-in period.

In subsequent analyses samples were assigned to their regional groupings. Pairwise F_{ST} values (Weir and Cockerham, 1984) were estimated between regions in FSTAT (Goudet, 2001). Population structure was further explored with analysis of molecular variance (AMOVA) using a hierarchical approach in Arlequin (v. 3.5.1.3) (Excoffier, 2005). We tested a postulated trans-Pacific restriction of gene flow by separating the eastern and western samples into two groups. Samples from the two central regions were included in the eastern group by virtue of the low to absent F_{ST} differentiation between them and in all pairwise comparisons with the eastern regions. In addition, AMOVA was used to estimate temporal genetic variation across the sampling period, with samples pooled into two groups: early (2003–2008) and late (2010–2011). We did not analyse allele frequencies based on latitude since the sampling design did not allow for the extrication of confounded effects of uncontrolled variables such as dispersal routes, gender composition, age, longitude, season or year of sampling. Historical gene flow between geographic regions was estimated on a pairwise basis in Arlequin as the absolute number of migrants (M) exchanged between regions per generation. The latter is estimated using a derivation of F_{ST} under several assumptions: mutation rate is negligible compared to the migration rate, population(s) are migration-genetic drift equilibrium, and regions in the pairwise comparisons only exchange migrants with one another and no other region.

2.3.2. Effective population size

Both historical (HN_e) and contemporary (CN_e) effective population estimates were generated for blue shark in the entire north Pacific. HN_e was also estimated using an assumed mutation rate for microsatellite loci of 10^{-4}, following the rationale of Karl et al. (2011). HN_e was estimated without assuming mutation-drift equilibrium by Bayesian coalescent modelling to estimate θ (the value of $4N_e\mu$, where μ is the per gene, per generation mutation rate) and the scaled migration rate M (the migration rate between samples/mutation rate) in the program Migrate (Beerli and Palczewski, 2010). Forty individuals with complete 14-locus genotypes were included from all sampling regions except that of the SW region (too few samples). The program was run with uniform priors, a Brownian mutation model and initial values of θ generated from F_{ST} values. We performed 10 replicate runs, each with a burn in of 10^6 steps and a chain of 2.5×10^6 steps. Effective sample size (ESS) estimates were used to assess convergence. Values of θ for all six blue shark regional samples were also estimated under the assumption of mutation-drift equilibrium (θ_H) from expected heterozygosity levels using Arlequin, using both the single step and infinite allele mutation models.

A CN_e value for blue shark of the north Pacific was estimated from linkage disequilibrium (LD) between loci in the microsatellite data using NEEstimator 2.0 (Do et al., 2014). The LD method provides less precise values for large ($CN_e > 1000$) than small populations, and the use of rare alleles (those alleles present in a sample at less than the desired frequency) in small samples may produce upward bias of CN_e estimates (Waples and Do, 2008). As a result, for large populations, a finite upper confidence limit for CN_e is often not defined. Large sample sizes (>200 individuals) improve precision and enable the use of rare alleles in CN_e estimation with little increase in bias (Waples and Do, 2008). In NEEstimator software, rare alleles can be screened out with a P_{CRIT} parameter. We therefore combined all blue shark genotypes into a single sample and performed CN_e estimation with $P_{CRIT} = 0.01$.

To estimate the ratio of N_e to current census size (N_C), we converted the two most recent estimates of north Pacific blue shark biomass (ISC, 2014) to number of adults. We applied the length-weight conversion for both sexes reported in Rice et al. (2013) to a length-at-50% maturity estimate of 150 cm PCL (Nakano and Seki, 2003) to estimate weight-at-50% maturity as 32.61 kg and

Table 2
Repeat motif, number of alleles, allele size range, polymerase chain reaction annealing temperature (T_m) and number of amplification cycles for blue shark microsatellite loci.

Locus	Repeat motif	Alleles	Range (bp)	T_m	Cycles
Pgla-01	(TCC) 7-(TCC) 3 TCG(TCC) 5	8	220–253	56	35
Pgla-02	(TCC) 5TCG(TCC) 2 (TCG) 2	11	143–176	52	35
Pgla-03	(GGA) 3AAA(GGA) 4TGA(GGA) 2	6	198–213	52	35
Pgla-04	(TCT) 4(TCC) 6	6	230–245	57	35
Pgla-05	(GT) 27(GA) 19	45	186–294	59	40
Pgla-06	(CA) 2(GA) 10CA	8	140–162	48	40
Pgla-07	(TCC) 14	10	197–236	52	30
Pgla-08	(TCC) 7(TCC) 5	8	184–205	52	35
Pgla-09	(GGA) 6(GAA) 5	7	137–155	53	35
Pgla-10	(GA) 2CA(GA) 7 (GACA) 3CAGACA (GA) 13	13	135–161	57	35
TB01	(GA) 9	14	140–168	50	35
TB02	(GT) 14	35	220–290	50	35
TB04	(CT) 8	6	148–158	50	35
TB13	(TG) 6	4	174–180	52	35

convert biomass to number of adults. The estimated weight-at-50% maturity was applied to the female adult biomass estimate of 435,351 tonnes from an age-structured catch-at-length statistical model (ISC, 2014) and we then doubled the number to account for males. That model also provided estimates both of female adults and of exploitable biomass; the total spawner biomass estimate was 16% of the exploitable biomass estimate. Since the other model, a Bayesian surplus production model (ISC, 2014) provided only an estimate of exploitable biomass (622,000 tonnes comprised of juveniles and adults of both sexes), we estimated the total adult biomass as 16% of 622,000 tonnes and then applied the estimated weight-at-50% maturity.

3. Results

A total of 844 tissue samples from 2003 to 2011 were collected for analyses (Table 1). Not all years were sampled in each geographic region. Samples included a mixture of juvenile (<150 cm PCL i.e., age-at-50% maturity based on Nakano and Seki, 2003) and adult individuals, and were dominated by immature sharks except for the small sample of sharks collected in southwest (SW) region (Table 1).

3.1. Genetic diversity

Genotypes at 10 or more loci were obtained for 789 or 93.5% of shark samples. Three individuals, including one from NE and two from SE, were removed from the data because they displayed genotypes identical at all loci to another sample from the same location and year. Given the high exclusion power provided by the 14 loci, it was assumed that each duplicated genotype resulted from the inadvertent double sampling of an individual. A total of 786 samples were used for subsequent analyses.

The total number of alleles observed ranged from 4 to 45 among the fourteen microsatellite loci (Table 2) and the average number of alleles observed at individual loci, averaged over geographic regions, ranged from 3.8 for Pgla04 to 31.3 for Pgla05 (Table 3 , means not shown). Expected heterozygosity by locus ranged from 0.1571 to 0.9635 (Table 3). Alleles at all loci except Pgla06 tended to conform to Hardy Weinberg equilibrium in most sampling regions (Table 3). The consistent deficit of observed heterozygotes relative to those expected at Pgla06 ($P < 0.05$ after correction for multiple samples) indicated the likely presence of one or more non-amplifying alleles at this locus. Pgla06 was excluded from subsequent analyses. Four other loci, Pgla01, Pgla04, Pgla09 and TB13, displayed heterozygote deficiencies in some geographic regions but were not consistently out of Hardy Weinberg equilibrium. No statistically significant linkage disequilibrium within regions was detected in pairwise testing of loci. Across geographic regions the level of genetic variation was uniform. Regional values of H_E and A_R averaged over loci ranged from 0.60 to 0.63 and 6.5 to 6.9 among regions, respectively (Table 3).

3.2. Population structure

The Bayesian analysis of STRUCTURE, in which genotypes were clustered into one to six subpopulations ($K = 1–6$) irrespective of regional sampling location indicated that blue shark of the north Pacific ocean constituted a single population. Blue shark genotypic data from all regions combined were best explained by the analysis of the analysis of $K = 1$, which had the highest likelihood and lowest variance associated with it. Chain convergence was poor, with alpha values failing to stabilize after the ten iterations (mean of 7.6, range from 0 to 10), indicating no population substructure in the data. Likelihood values associated with other possible subpopulation structures tested ($K = 2–6$) decreased with each proposed additional subpopulation and individual genotypes were partitioned equally to all K subpopulations in each run. The absence of an apparent Wahlund effect (shortage of observed heterozygotes relative to those expected across all loci) in regional genotypic frequencies also indicated that the apparent lack of population structure across the north Pacific ocean was not due to a mixing of genotypes from multiple distinct subpopulations in all regional shark collections.

Pairwise F_{ST} values between regions were <0.008; only F_{ST} values involving other samples paired with the poorly sampled SW region exceeded 0.004. All comparisons of allele frequencies between regions were nonsignificant (all $P > 0.05$) when corrected for multiple comparison. The AMOVA with geographic regions structured into western and eastern 'groups' (NW and SW versus NC, SC, NE, and SE) did not support the proposed existence of longitudinally-based blue shark subpopulation structure ($F_{SC} = 0.004$; $P = 0.205$). Virtually all the genetic variation (99.93%) was accounted for by intra-regional and intra-individual variation. Thus, variation between the east–west groups and among regions within groups combined accounted for much <1% of total genetic variation. Moreover, AMOVA of samples collected in the 'early' and 'late' time periods indicated that no significant genetic variation was attributable to differences between samples in the final two years of the study compared with those collected in earlier years ($F_{SC} = 0.0007$; $P = 0.110$). Based on AMOVA results, we assumed a single panmictic population in the subsequent analyses for the effective population size.

3.3. Gene flow and effective population size

The estimated number of migrants exchanged between some regions was infinite (Table 3). Pairwise unidirectional estimates of θ and the scaled migration rate M, the number of migrants over

Table 3
For each microsatellite locus, number of genotyped individuals (n), number of alleles (A), allelic richness (A_R), expected (H_E) and observed (H_O) levels of heterozygosity; and departure from Hardy Weinberg equilibrium of genotypic frequencies (inbreeding coefficient; F_{IS}) are shown for blue shark samples by geographic region. Means are given by geographic region. Asterisks indicate statistically significant departures from Hardy Weinberg equilibrium after sequential Bonferroni correction. Sample region abbreviations are those in Table 1.

		NW	SW	NC	SC	NE	SE
Pgla01	n	91	18	71	54	318	219
	A	4.0	2.0	4.0	7.0	6.0	5.0
	A_R	2.7	2.0	3.0	3.8	3.5	3.6
	H_E	0.1743	0.1571	0.1965	0.2953	0.2685	0.2930
	H_O	0.1758	0.1667	0.1831	0.2222	0.2862	0.2603
	F_{IS}	−0.0088	−0.0625	0.0686	0.2491	−0.0660	0.1120
Pgla02	n	95	17	72	52	317	217
	A	9.0	6.0	10.0	8.0	10.0	11.0
	A_R	7.3	6.0	7.5	7.4	6.8	6.9
	H_E	0.7870	0.7184	0.7833	0.8015	0.7634	0.7478
	H_O	0.8105	0.6471	0.7778	0.8269	0.7760	0.7327
	F_{IS}	−0.0300	0.1020	0.0071	−0.0320	−0.0166	0.0202
Pgla03	n	94	17	72	52	320	217
	A	4.0	3.0	4.0	4.0	5.0	5.0
	A_R	3.6	3.0	3.7	3.7	3.7	3.8
	H_E	0.5804	0.5847	0.5663	0.5207	0.5302	0.5671
	H_O	0.5638	0.7059	0.5000	0.5577	0.5188	0.5207
	F_{IS}	0.0287	−0.2152	0.1179	−0.0717	0.0217	0.0820
Pgla04	n	91	18	67	50	315	205
	A	3.0	4.0	4.0	5.0	4.0	3.0
	A_R	3.0	3.9	3.3	3.9	3.1	3.0
	H_E	0.6382	0.6873	0.6127	0.6442	0.6331	0.6230
	H_O	0.6813	0.5556	0.6119	0.6600	0.6063	0.6439
	F_{IS}	−0.0680	0.1962	0.0013	−0.0247	0.0424	−0.0337
Pgla05	n	95	18	73	54	320	215
	A	32.0	19.0	30.0	31.0	41.0	35.0
	A_R	17.7	18.6	16.9	17.6	17.1	17.8
	H_E	0.9465	0.9635	0.9393	0.9439	0.9429	0.9472
	H_O	0.9158	0.8889	0.9452	0.9444	0.9438	0.9535
	F_{IS}	0.0326	0.0795	−0.0063	−0.0006	−0.0009	−0.0067
Pgla06	n	96	18	74	54	318	220
	A	7.0	4.0	8.0	6.0	8.0	8.0
	A_R	4.8	3.8	4.9	4.6	5.5	5.2
	H_E	0.3418	0.1619	0.2845	0.3304	0.4210	0.3536
	H_O	0.2500	0.1667	0.2297	0.2963	0.2579	0.2364
	F_{IS}	0.2697	−0.0303	0.1936	0.1041	**0.3879***	**0.3320***
Pgla07	n	95	18	69	53	318	204
	A	7.0	6.0	9.0	6.0	10.0	8.0
	A_R	5.0	5.9	5.4	4.5	5.0	5.4
	H_E	0.5298	0.5444	0.4785	0.5508	0.4796	0.5767
	H_O	0.5895	0.5000	0.4928	0.5849	0.4654	0.6078
	F_{IS}	−0.1134	0.0838	−0.0301	−0.0626	0.0296	−0.0542
Pgla08	n	95	17	74	53	317	219
	A	5.0	4.0	7.0	7.0	8.0	7.0
	A_R	4.5	4.0	5.3	5.5	4.8	4.8
	H_E	0.6663	0.5348	0.6722	0.6706	0.6669	0.6828
	H_O	0.6211	0.4706	0.6892	0.6981	0.6814	0.6484
	F_{IS}	0.0683	0.1233	−0.0255	−0.0414	−0.0218	0.0505
Pgla09	n	96	18	74	54	319	213
	A	6.0	5.0	6.0	5.0	7.0	7.0
	A_R	3.8	4.9	4.0	3.6	3.6	4.1
	H_E	0.4440	0.3889	0.4064	0.3480	0.4075	0.4755
	H_O	0.4063	0.3889	0.3514	0.2593	0.3699	0.4883
	F_{IS}	0.0855	0.0000	0.1363	0.2569	0.0923	−0.0268
Pgla10	n	94	18	73	53	320	219
	A	9.0	9.0	12.0	10.0	12.0	10.0
	A_R	7.5	8.8	8.7	8.4	7.4	7.7
	H_E	0.7892	0.7921	0.8161	0.7729	0.7518	0.7575
	H_O	0.8191	0.7778	0.7808	0.7358	0.7375	0.6849
	F_{IS}	−0.0381	0.0186	0.0435	0.0483	0.0190	0.0960
TB01	n	96	18	74	53	320	219
	A	10.0	8.0	11.0	9.0	13.0	12.0
	A_R	8.8	7.9	7.9	8.0	8.6	8.4
	H_E	0.8677	0.8714	0.8428	0.8404	0.8624	0.8537
	H_O	0.8542	0.9444	0.8514	0.9057	0.8750	0.8447
	F_{IS}	0.0157	−0.0865	−0.0102	−0.0784	−0.0146	0.0105

Table 3 (*Continued*).

		NW	SW	NC	SC	NE	SE
TB02	*n*	94	18	74	52	318	221
	A	27.0	17.0	27.0	26.0	33.0	28.0
	A_R	15.6	16.6	16.6	17.6	16.1	15.8
	H_E	0.9128	0.9460	0.9315	0.9492	0.9247	0.9217
	H_O	0.8936	0.9444	0.9189	0.8462	0.8962	0.9095
	F_{IS}	0.0212	0.0017	0.0136	0.1095	0.0308	0.0133
TB04	*n*	95	18	71	53	321	221
	A	4.0	5.0	5.0	5.0	5.0	4.0
	A_R	3.5	4.9	3.9	4.3	3.9	3.6
	H_E	0.5071	0.6032	0.5416	0.6022	0.5693	0.5455
	H_O	0.5053	0.5556	0.6197	0.5472	0.5545	0.5656
	F_{IS}	0.0036	0.0811	−0.1454	0.0921	0.0260	−0.0370
TB13	*n*	96	18	74	52	319	222
	A	4.0	4.0	4.0	4.0	4.0	4.0
	A_R	3.4	3.9	3.5	3.2	3.4	3.2
	H_E	0.4970	0.5032	0.4892	0.4098	0.4655	0.4615
	H_O	0.5417	0.4444	0.5000	0.2692	0.4514	0.4505
	F_{IS}	−0.0904	0.1197	−0.0221	0.3453	0.0303	0.0240
Mean	*n*	95	18	72	53	319	217
	A	9.4	6.9	10.1	9.5	11.9	10.5
	A_R	6.5	6.7	6.8	6.9	6.6	6.7
	H_E	0.6202	0.6041	0.6115	0.6200	0.6205	0.6290
	H_O	0.6163	0.5826	0.6037	0.5967	0.6014	0.6105
	F_{IS}	0.0063	0.0365	0.0128	0.0379	0.0307	0.0295

Table 4
Pairwise estimates of the absolute number of migrants (M) exchanged between blue shark regions (Inf—infinite). Sample region abbreviations are given in Table 1.

	NW	SW	NC	SC	NE	SE
NW						
SW	411					
NC	inf	110				
SC	195	97	inf			
NE	inf	95	inf	871		
SE	inf	55	1486	326	443	

Table 5
Effective population size (N_e) estimates, with confidence intervals (CI) and mean θ for blue shark sampled from six regions of the north Pacific ocean. Estimates were derived from three methods applied to microsatellite genetic data: contemporary estimates (CN_e) were estimated from pairwise linkage disequilibrium between loci in the entire blue shark data set; historic estimates (HN_e) were estimated from Bayesian coalescence modelling; and from the expected heterozygosity at microsatellite loci under the assumption of drift-mutation equilibrium in the blue shark population.

Parameter	CN_e	Coalescent HN_e	Equilibrium HN_e
N_e	5468	4675	4461
95% CI	2802–52352	1425–7800	4365–4648
Mean θ (range)	–	1.87 (1.40–2.92)	1.78 (1.59–1.64)

the mutation rate, from the Bayesian analysis of population structure in which migration was permitted between all sample regions also indicated significant bidirectional gene flow throughout the north Pacific ocean. In the Bayesian analysis, estimates of θ ranged from 1.4 to 2.9 (Table 5) with ESS values ranging between 17,000 and 37,000, indicating good convergence. Pairwise unidirectional *M* values ranged from 3.7 to 15.7 for the 20 potential exchanges of migrants between the five regions (SW excluded) with ESS values ranging from 1600 to 4700. By generation, the pairwise estimates of migrant individuals between regions (θM) ranged from 7 to 30. By region, estimated total immigrants ranged from 57 to 81 per generation, consistent with the observed lack of regional differentiation in allele frequencies.

Estimates of N_e for blue shark of the north Pacific, resulting from three different models incorporating different assumptions and covering different (historic and contemporary) time periods were consistent and less than 5500 (Table 5). The CN_e estimate based on pairwise LD between loci for all blue sharks sampled over the nine year time period (approximately equal to estimated seven year time generation interval) can be considered an estimate of the contemporary effective size of the north Pacific blue shark population (i.e., the size of the parental cohort that produced this single, recent generation of blue shark). In contrast, the HN_e estimate obtained through Bayesian coalescence modelling is a multigenerational average value over time dating back at least to establishment of the north Pacific blue shark population. This estimate is not based on the assumption that the population is in mutation-drift equilibrium, whereas the HN_e estimate derived from expected heterozygosity is also a historical average value but based on the assumption that the population has reached equilibrium and is stable in abundance. This assumption may be unfounded if population expansions and/or contractions have occurred (or are in progress) since colonization. Nevertheless, the values obtained by the three methods ranged tightly between 4461 and 5468 although the linkage-based CN_e confidence limits were wide (Table 4). We therefore used 5000 as an approximately estimate of N_e. We estimated total spawner abundance of blue shark in the north Pacific to be between 3.1×10^6 individuals (Bayesian surplus production model) and 26.7×10^6 individuals (age-structured catch-at-length statistical model). Using our estimated N_e of approximately 5000 therefore provides a N_e:N_C range from 2×10^{-3} to 10^{-4}.

4. Discussion

4.1. Genetic diversity and population structure

The comprehensive sampling of blue shark throughout the north Pacific ocean encompassed in this study provided a portrait of a highly mobile oceanic species with moderate genetic variability and a panmictic breeding structure. Blue shark is relatively fecund, fast-growing and early-maturing for a viviparous species. These characteristics may provide a demographic resilience that underlies its global distribution and great abundance, but that abundance and the tendency of blue shark to form migratory aggregations also makes it vulnerable to targeted exploitation. The results of this study supported current assessment practices in which blue

sharks of the north Pacific are treated as a single stock. Limited genetic analysis had furthermore indicated little genetic differentiation between blue shark of Indo-Pacific and north Pacific waters (Ovenden et al., 2009; Taguchi et al., 2015), but practical management constraints and prudence both dictate continued management of Pacific blue shark as independent northern and southern units. This consideration is supported by the probable existence of distinct breeding grounds in the south Pacific (Mejuto and García-Cortés 2005) and restriction of blue shark passage through equatorial waters.

In both the Pacific and Atlantic oceans, the existence of segregated northern and southern hemisphere populations has been postulated based on the occurrence of distinct reproductive cycles (Nakano and Seki, 2003; Nakano and Stevens, 2008; Pratt, 1979; Amorim, 1992). Blue shark density tends to be high in higher latitudes for both southern and northern hemispheres (Strasburg, 1958; Nakano, 1994) and low in equatorial waters, indicating that the equator may represent a temperature barrier to migration. Tagging data from the north Atlantic suggests that blue sharks constitute a single stock in that region (Kohler and Turner, 2008), although tagged blue sharks have exhibited some movement between the north and south Atlantic (Kohler et al., 1998). In the north Pacific, blue sharks from eastern waters carrying satellite-transmitting tags travelled extensively throughout the central and eastern north Pacific, but did not migrate below equatorial waters (Block et al., 2011). Differences in sex ratio and female reproductive stages between the southeast north Pacific and the northeast south Pacific have been used to suggest a discontinuity in sex segregation patterns between the two regions (Mejuto and García-Cortés 2005). The likely resultant subdivision into two distinctive Pacific blue shark populations is a prospect worthy of examination in a comprehensive genetic sampling of blue shark across the south Pacific ocean.

In both the Pacific and Atlantic oceans, the existence of segregated northern and southern hemisphere populations has been postulated based on the occurrence of distinct reproductive cycles (Nakano and Seki, 2003; Nakano and Stevens, 2008; Pratt, 1979; Amorim, 1992). Blue shark density tends to be high in higher latitudes for both southern and northern hemispheres (Strasburg, 1958; Nakano, 1994) and low in equatorial waters, indicating that the equator may represent a temperature barrier to migration. Tagging data from the north Atlantic suggests that blue sharks constitute a single stock in that region (Kohler and Turner, 2008), although tagged blue sharks have exhibited some movement between the north and south Atlantic (Kohler et al., 1998). In the north Pacific, blue sharks from eastern waters carrying satellite transmitting tags travelled extensively throughout the central and eastern north Pacific, but did not migrate below equatorial waters (Block et al., 2011). Differences in sex ratio and female reproductive stages between the southeast north Pacific and the northeast south Pacific have been used to suggest a discontinuity in sex segregation patterns between the two regions (Mejuto and García-Cortés, 2005).

The lack of genetic distinction among regions within the north Pacific, and of any indication that regional blue shark samples constituted mixtures of individuals from segregated spawning groups, is consistent with the postulated existence of a single, central, latitudinally-constricted mating region between 20 and 30°N (Nakano 1994). This study provided no indication that the age and gender-specific migration patterns typical of this species has resulted in assortative mating that might arise from nonrandom fraternization at breeding and lead to population subdivision. In the north Pacific ocean, parturition grounds are hypothesized to lie to the north of the breeding grounds (between 35 and 45°N), whereas juvenile females are found northward to 50°N, and in the Gulf of Alaska to 55°N, and juvenile males are found southward to 30°N. Although tagging data indicate that male blue shark may be more mobile than females, there is no evidence that females have greater geographic fidelity at breeding than males. However, we did not test this possibility explicitly with concurrent microsatellite and mtDNA analysis. Intensive sampling of blue shark in the north Atlantic also provided evidence of the importance of the mid-ocean regions to breeding and parturition (Mejuto and García-Cortés, 2005; Vandeperre et al., 2014). Breeding fidelity to restricted geographic location has been documented primarily for coastally-dependent shark species such as lemon shark (*Negaprion brevirostris*), blacktip reef shark (*Carcharhinus melanopterus*) and bull shark (*C. leucas*) (Karl et al., 2011; Mourier and Planes, 2013; DiBattista et al., 2008), for which riverine, estuarine or shallow coastal waters provide the breeding habitat.

4.2. Effective population size

The CN_e provides a size measure for the reproductive cohort that produced the extant generation of a population. Typically, CN_e values in the range of 500–1000 and higher are considered indicative of a population that is resilient to loss of diversity due to genetic drift in an evolutionary timeframe (Frankham et al., 2010; Franklin, 1980). N_e values for other vagile species, even those at lower abundances and reflecting more population substructure than blue shark, have been estimated in the tens and hundreds of thousands (Schultz et al., 2008; Chabot and Allen, 2009; Schmidt et al., 2009; Karl et al., 2011). Our estimates of contemporary and historical N_e for north Pacific blue shark obtained in this study were relatively low (4461–5468) for a pelagic, wide-ranging shark species characterized by high abundance, high vagility, apparently high reproductive capacity, and comprising a single breeding unit in the north Pacific ocean. A lower N_e value of 8200, more similar to our blue shark estimates, was obtained on the basis of mtDNA variation for basking shark (*Cetorhinus maximus*), sampled on a worldwide basis by Hoelzel et al. (2006). Those authors suggested that the value was low, and possibly the result of a recent (i.e., within the past 12,000 years) population bottleneck.

The concordance of the blue shark HN_e and CN_e values indicates that the low effective size does not result from a recent bottleneck, but is a condition that likely has persisted at least since colonization of the north Pacific ocean. Thus, it is not due to recently altered environmental conditions, nor recent levels of exploitation, both of which could influence the numbers of the breeding population. Phylogeographic analysis indicated that, in fact, blue shark across the Indo-Pacific region had undergone population expansion and may have been little impacted by recent glacial-interglacial alternations of the Pleistocene period (Taguchi et al., 2015). Those authors attributed the demographic resilience of the blue shark to its flexible habitat and dietary requirements, a lack of reliance on coastal habitats most affected by climate fluctuation and high vagility. Historical population expansion could contribute to low N_e in blue sharks, with the population of the north Pacific ocean not yet having reached a stable abundance and condition of mutation-drift equilibrium. If this is the case, a population expansion may have been underway prior to blue shark colonization of north Pacific waters. Alternately, other biological variables not yet understood, may contribute to more variable reproductive success (temporally or spatially) than is currently hypothesized from demographic and life history knowledge.

The CN_e of a population is generally less than the reproductive adult census size (N_C), but the ratio of effective to census size (CN_e:N_C) is variable among organisms. For many species, the ratio ranges from 0.1 to 0.5 (Hare et al., 2011). Marine teleosts, dominated by highly fecund broadcast spawners with 'sweepstakes' style reproductive success, often exhibit low ratios, ranging down to 10^{-3} even after minimization of effects that lead to N_e

underestimation in abundant, iteroparous species with overlapping generations (Hare et al., 2011). It has been postulated that the characteristic low fecundity and low variance in reproductive success in elasmobranchs might lead to CN_e:N_C values more closely aligned with typical values ranging upwards from 0.1 (Dudgeon et al., 2012). N_e estimates for shark species are variable, largely reflecting the degree of juvenile and adult vagility, and breeding location fidelity, indicated by tagging studies. Coastally oriented shark species may exhibit strong population subdivision associated with limited vagility and female philopatry. Such species are additionally vulnerable to overexploitation due to their coastal residence, and these factors combined often result in low CN_e values and CN_e: N_C ratios between 0.5 and 1.0 (Portnoy et al., 2009; Blower et al., 2012). In other coastal species that exhibit high vagility and/or low male philopatry, resultant higher levels of gene flow obscure differentiation at nuclear loci and lead to greater N_e (Schultz et al., 2008; Karl et al., 2011). Similarly, N_e in weakly structured, vagile pelagic shark species with oceanic breeding grounds, although little studied, may be high and range up to hundreds of thousands (Hoelzel et al., 2006; Schmidt et al., 2009). However, there have been few attempts to examine CN_e:N_C in these species, likely because the geographic scope and total abundance of the 'population' are often poorly known.

Our estimates of the ratio for N_e:N_C ranged from 2×10^{-3} to 10^{-4} which is similar to those observed for many widespread marine fishes (Hare et al., 2011). It is an unusually low value for a shark species, in which values greater than 10^{-1} are typical (Dudgeon et al., 2012). The low HN_e of ~4,500 estimated under the assumption that the microsatellite mutation rate in blue shark is 10^{-4}, which may not be the true rate. It is, however, in general use as an accepted rate for sharks (see discussion of Karl et al., 2011), and in this study provided HN_e estimates in good agreement with the estimated CN_e, which is independent of mutation rate. Expanding the possible range of mutation rates by a factor of ten in both directions provides possible HN_e values as high as 45,000 and as low as 450. The higher value would increase the N_e:N_C correspondingly, but even this ratio of 10^{-2} is low for a shark. A HN_e of 500 would place the blue shark population at risk of losing evolutionary adaptive capacity (Frankham et al., 2010).

4.3. Management and conservation

The genetic analyses of this study, and of Taguchi et al. (2015), support the current assessment processes in which blue shark of the north Pacific ocean are treated as a single population (ISC, 2014). The lack of genetic differentiation at nuclear loci in large samples of blue shark from throughout the north Pacific ocean is consistent with the postulated existence of a single breeding ground (Nakano, 1994) and indicates that the age- and gender-specific migrations undertaken in the species do not introduce non-random associations of individuals during breeding. MtDNA analysis has indicated that the high level of gene flow among blue shark aggregations may extend beyond the confines of the north Pacific (Taguchi et al., 2015), but this result awaits confirmation by extension of the survey of nuclear variation to blue shark of southern Pacific waters.

The existence of a single, likely central, breeding area for north Pacific blue shark alleviates concern about regional harvest operations possibly impacting small local groups to the extent that fine scale

variation and adaptation could be lost. However, it highlights the need for international management and cooperation to responsibly manage a common resource. In the north Pacific ocean, blue sharks are harvested in both targeted fisheries and as bycatch in some longline and gill net fisheries, particularly those that target tuna, swordfish and marlins (Camhi et al., 2008; Dalzell et al., 2008; Sosa-Nishizaki et al., 2008). Recently, two Regional Fisheries Management Organizations requested scientific advice on the stock status of blue sharks: the Western and Central Pacific Fisheries Commission (WCPFC) and the Inter-American Tropical Tuna Commission (IATTC). Two international science organizations provided collaborative stock assessment advice; the Secretariat of the Pacific Community (SPC) provided an age-structured model assessment and the Shark Working Group of the International Scientific Committee for Tuna and Tuna-like Species (ISC) provided a Bayesian surplus production model assessment (ISC, 2014). Both assessments assumed a single population for the north Pacific and used the same fisheries catch and biological data. While the assessment models differed in underlying assumptions, each used similar indices of abundance and base case scenarios (ISC, 2014). Both assessment approaches classified the north Pacific blue shark stock status as healthy and concluded that current harvest levels are likely appropriate. The widely varying range in biomass estimates between the two approaches indicate a continuing need for improved assessment data.

The surprisingly low N_e compared to other shark species and correspondingly low N_e:N_C estimated for north Pacific blue shark in this study corroborate the suggested requirement for a greater understanding of stock status. The lower N_e may result from biological complexity in the species not currently well understood, such as more variable reproductive success than currently assumed, and/or from historical population expansion from a bottleneck. Extension of genetic surveys to a global scale (including the north Atlantic and throughout the southern hemisphere) should elucidate the worldwide population structure of blue shark and clarify whether the lower N_e is a universal blue shark condition. If so, it may reflect cryptic biological factors that lead to much lower and more variable reproductive success than that currently proposed for the species on the basis of its relatively early age-of-maturation coupled with significant longevity, large litter size, high abundance and vagility. This would highlight a need to better elucidate, and perhaps decrease anthropogenic effects on, breeding location and activity in international waters.

The estimate CNe values for blue shark are in the range that suggest that north Pacific population can respond adaptively to natural selection in the face of environmental change (Frankham et al., 2010; Franklin, 1980) and that it currently not at risk of breaching a critically low level of N_e. However the low N_e:N_C value indicates the population may be vulnerable to reduced reproductive success arising from natural (environmentally-mediated) mortality or fishing mortality. The relatively short generation time of blue shark should enable rapid detection of changes in CN_e, and timely implementation of remedial management actions, if a modest level of genetic monitoring and improvements in our understanding the biology of the species are maintained.

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