Potential for sustainable expansion of the dogfish (*Squalus acanthias*) fishery in the northeast Pacific

Lorenz Hauser, James H Franks, Nicole Vega & Vincent Gallucci

School of Aquatic and Fishery Sciences, University of Washington, Seattle, USA

Grant Number: NA03NMF4270156

May 6th, 2007

ABSTRACT

Spiny dogfish are one of the most abundant shark species and support major commercial fisheries. Because of their slow life history, including the longest gestation time among vertebrates, they are expected to be vulnerable to overfishing and slow to recover from depletion. Indeed, dogfish in the Atlantic have been declared overfished, and a petition to list them under the CITES agreement is currently underway. In the Pacific, on the other hand, there may be scope for the expansion of the current dogfish fishery. Here, we provide scientific information on stock structure and demographic parameters of dogfish, together with an analysis of the sensitivity of dogfish to exploitation. Genetic data suggested a deep phylogeographic split between dogfish in Atlantic / South Pacific and North Pacific, coinciding with major differences in life history between the two groups. Within the North Pacific, there was little evidence for genetic differentiation, though essentially self recruiting populations may still exist. Statistical comparisons of region-specific growth curves for females indicated differences in growth between dogfish in the northern and southern regions of the latitudinal range and between Puget Sound and outside waters of the open coast. As expected, slower growing and later maturing populations were found to be susceptible to fishing, suggesting that while the fishery may be expanded, dogfish in the North Pacific may sustain only relatively low levels of fishing mortality. From the socioeconomic perspective, spiny dogfish catches dominate elasmobranch landings on the Pacific coast, averaging 2270 mt annually, approximately 4% of the global catch. In Puget Sound, catches decreased over the last twenty years from 3,900 mt of round weight to about 64 mt round weight (as of 2000). Over the same period prices have changed from about 08 cents to 19 cents per round weight pound (as of 2004). The potential CITES listing of dogfish would change the economic situation described herein considerably.

EXECUTIVE SUMMARY

Spiny dogfish are one of the most abundant shark species and support major commercial fisheries. Because of their slow life history, including the longest gestation time among vertebrates, they are expected to be vulnerable to overfishing and slow to recover from depletion. Indeed, dogfish in the Atlantic have been declared overfished, and a petition to list them under the CITES agreement is currently underway. Because of this experience there is concern whether Pacific dogfish can sustain a potential expansion of the fishery – Pacific dogfish may be even more susceptible to fishing than Atlantic, because they mature later and are more long lived. Here, we provide data on the genetic relationship between Atlantic and Pacific dogfish, information on Pacific dogfish population structure and data on geographic variation in growth rates within the Pacific.

Genetic samples were collected along the American Pacific coast (Alaska, British Columbia, Oregon, California) as well as from north (UK, USA) and south Atlantic (Argentina, South Africa) and the south Pacific (Chile, New Zealand). DNA sequences of the mitochondrial DNA D-loop as well as microsatellite analyses revealed a deep split between Atlantic / South Pacific and north Pacific dogfish, coinciding with the two life-history groups differing in growth, age at maturity and longevity. Our data showed considerable genetic differentiation between the northern and southern hemisphere in the Pacific but not the Atlantic, suggesting extensive migration across the equator or recent separation of populations in the Atlantic.

Smaller scale microsatellite analyses failed to reveal significant genetic differentiation within the northeast Pacific. Although a powerful genetic marker set with eight highly variable loci was used, the existence of essentially self-recruiting populations cannot be discounted, however, as low levels of gene flow or recent separation would suffice to maintain homogenous allele frequencies.

In contrast, we were able to observe significant demographic variation over a wide latitudinal gradient where no significant genetic variation could be observed at neutral markers. Dogfish from Washington and Canada showed slower growth and larger final sizes than their conspecifics from Oregon. While such differences in environmentally influenced traits cannot demonstrate genetic differentiation between populations, they show that dogfish are not randomly mixing along the coast. Furthermore, it has been noted that phenotypic differences in life history parameters among putative stocks, irrespective of genetic differences, provide a firm basis for separate management units and should be modeled separately for stock assessment purposes because of inherently related productivity differences.

Estimates of growth and natural mortality were used in a matrix population model to predict effects of fishing mortality in these two populations and in Atlantic dogfish. These models suggested that Pacific dogfish is less likely to be able to sustain high fishing pressure than Atlantic populations – a concerning results given the very pronounced effects of fishing in the Atlantic. Furthermore, our models indicated that northern dogfish may be more vulnerable than fish from Oregon. These results highlight the importance of estimating the vulnerability of species to exploitation before fishing commences.

The economic analysis of the fishery on the Pacific coast offers a different perspective from that of the Atlantic fishery. In Washington State, spiny dogfish fisheries were the second highest in value (after salmon) of non-treaty commercial fisheries in Puget Sound with an

average value of about 3.5 million dollars over 1985-2000. Management in Washington and Oregon takes place in the context of tribal treaty co-management regimes established for modern times by the Bolt and Rafeedie court decisions. As in the Atlantic fishery, an international border exists which fish pass over. However, in the Pacific fishery the Canadian – British Columbia border straddles the most productive fishing grounds for the species, complicating Puget Sound management. Reasonable estimates of abundance or catch if not to be arbitrary, would refer to ecological units, such as the Georgia – Puget Sound basin and thus include both the Canadian and U.S. fishing grounds. At present communication does exist but much remains to be done to carry out cooperative management.

Data obtained in this project were presented at a special symposium on dogfish, organized by Prof Gallucci in summer of 2005. This symposium brought together dogfish scientists from Atlantic and Pacific to discuss the biology and fisheries of dogfish. Like many other sharks, the life history characteristics of dogfish make them extremely vulnerable to overfishing, and available data suggest that a very cautionary approach to dogfish management in the Pacific is warranted. Many of the results of this research and of many other research projects concerned with the management of the spiny dogfish over the world will appear in a special publication of the American Fisheries Society in 2007.

1. PURPOSE

Sustainable harvests of wild fish populations require sound management based on good scientific information. Frequently such information is collected only after the stock assessment indicators suggest that the exploited populations are being over-harvested. The management measures taken to try to re-establish sustainable harvests often result in economic loss and hardship for the fishing industry and associated communities. It is therefore an unusual and valuable opportunity when it is possible to establish a firm scientific basis for management before a fishery develops or expands into new areas. The dogfish fishery in the Pacific Northwest presents such an opportunity, as the species is currently heavily exploited only in some local areas and apparently has potential for expansion.

Dogfish (*Squalus acanthias*) supports major fisheries in many areas of the world, and has been the basis of considerable economic return to a wide range of fishing communities. Nevertheless, due to slow growth, late maturity and low fecundity, the species is susceptible to overfishing, with a particularly slow rate of population recovery. Indeed, dogfish populations on the Atlantic coast of the US were declared overfished in 1998 (26th Northeast Regional Stock Assessment Workshop), and have since been subject to a fishery management plan (FMP). In the Pacific Northwest, on the other hand, some stocks may be at a critical level (e.g. in North Puget Sound) while in other areas, e.g., the Georgia Strait, an increase in fishing pressure may be sustainable. The dogfish fishery is co-managed (Jentoft *et al.*, 1998; Kaplan, 1998) under the Boldt decision between the State of Washington and the Tulalip and Lumi Tribes. Such uncertainties confirm the need for management attention in a coordinated effort between the U.S. and Canada since the Puget Sound basin includes both the Canadian Straits of Georgia and the U.S. Puget Sound. Our premise is that as fishing effort within the sheltered Puget Basin reaches sustainable levels and catches decrease, fishing effort will extend to other coastal areas, which are as yet essentially unexploited. The dominant work in coastal populations is from Canadian waters, with relatively little published work about populations off the U.S. coasts, although there are databases for dogfish captured in NMFS surveys. This situation has been recognized by the American Fisheries Society (Musick *et al.*, 2000) and by a call for research on the "distribution, stock structure and life history characteristics" in the Pacific (NMFS 2001).

Very little is presently known about demographic variation in the spiny dogfish in the Northeast Pacific (Jensen 1966, Ketchen 1986). If the life history of the spiny dogfish varies along the latitudinal gradient, this will have implications for region-specific fecundity and growth potential of this species, and this in turn will impact the sensitivity of the spiny dogfish to perturbation events. Patterns in life history variation may be used to inform the scale at which the population can be modeled in stock assessment. Growth in fish has been observed to be extensively correlated with other life history processes including fecundity, age at maturity, and natural mortality. In this study, we examine region-specific patterns of somatic growth to determine if life history in this species can be observed to vary along the latitudinal gradient.

Information on dogfish movement patterns is scarce, though some pertinent information has been obtained in long-term tagging studies. Tagging studies conducted on Pacific dogfish indicate that trans-oceanic migrations are possible; fish tagged in British Columbia and Washington have been recovered in waters off the coast of Japan and, more rarely, northern Mexico (McFarlane and King 2003). However, the majority of recaptures of tagged fish take place in the same region in which they were tagged (Holland 1957, Fujioka and DiDonato 1974,

McFarlane and King 2003). Furthermore, results of tagging studies indicate that dogfish can be found in the general region of first capture at the same time of year over subsequent years, and that schools of dogfish tend to remain together over time (Jensen 1966). It has been suggested that Pacific dogfish conduct a seasonal north-south migration along the coast as has been observed in dogfish in the Western Atlantic (Holland 1957); a winter migration into deeper offshore waters has also been suggested (Jensen 1966). Neither hypothesis on dogfish movement patterns can be summarily rejected based on currently available literature.

The extent of dogfish movement along the latitudinal gradient is an important consideration for management. Dogfish in regions that experience high rates of exchange of individuals are likely to be genetically and phenotypically similar, and extensive movement of dogfish between regions may provide opportunities for replenishment of local depletion events. If, as is the case for dogfish on the eastern US coast, these dogfish constitute a panmictic population, dogfish in all regions can be considered to belong to a unit stock for the purposes of stock assessment and conservation. If the dogfish from Alaska/Bering Sea to California/Mexico do not make up a single homogenous stock, it will be necessary to account for any observable variation in life history along the latitudinal gradient when constructing population models for this species.

Growth pattern by region is used as a metric to test for regional differentiation in this study. Growth is a complex phenotypic trait; the growth exhibited by an individual can be limited by genetic factors and controlled by the environmental conditions (temperature, feeding conditions, etc) experienced by that individual over the course of its lifetime. Degree of difference in observed growth patterns provides useful information on degree of disparity of putative stocks, and the interactions of age, size, and other life history traits provide the vital parameters used in stock assessment and are therefore of concern from a management perspective (Casselman et al 1981). Growth has been used as a metric in stock identification for other species (e.g DeVries and Grimes 1997); issues in use of growth as a metric for regional differentiation are reviewed in Begg (2005).

Conspecifics inhabiting different regions may show differences in growth patterns over the lifespan of individuals as a result of exposure to different conditions (phenotypic plasticity). It has been indicated that allocation of resources to growth may differ along a latitudinal gradient within a species (Castillo 1995) and that fecundity and mortality are correlated with rates of growth (Mollet and Calliet 2002). Environmental correlates for this trend have been proposed, including sea surface temperature (Ruttenberg et al 2005), variation in seasonal and day length (Conover and Present 1990), and regional variation in productivity and food availability (Ruttenberg et al 2005); final adult sizes can be expected to be correlated negatively with latitude or water temperature (Gunter 1950, Ruttenberg et al 2005). Significant differences in life history metrics such as growth can therefore be used to indicate broadly whether fish are occupying different environments over their lifespan (Ihssen et al. 1981).

In this project, we investigated the stock structure of dogfish along the latitudinal gradient from California to Alaska using both molecular genetic markers and demographic characteristics, and collected population parameters for the estimation of potential yields. The identification of self-recruiting populations is important information for the management of an expanded fishery along the coastal regions north and south of Washington State and Canada. In addition, a socio-cultural study of the fishers and the co-management plan is an important precursor to any socioeconomic study. The project was therefore particularly targeted at the priority areas *D.*

Optimum Utilization of Harvested Resources under Federal or State Management and *F. Fisheries Socioeconomics.*

Principal Aim:

To provide scientific data for the management of expanded dogfish fisheries in the coastal Pacific Northwest.

Specific Objectives:

- To identify self-recruiting stocks of dogfish along the North American west coast using genetic markers
- To compare population parameters along a latitudinal gradient
- To determine appropriate socio-economic indicators of the existing fishery

2. APPROACH

2.1. Molecular Genetics

2.1.1. Isolation of microsatellite markers

Initially, we planned to isolate microsatellites from dogfish ourselves, an undertaking that would have taken 6-12 months. Fortunately, this work had already been carried out in the laboratory of Dr Gaby Gerlach, Woods Hole. Dr Gerlach kindly provided us with her unpublished primers, which were later also published (McCauley *et al.*, 2004). The availability of microsatellite markers allowed us to spend more time and resources on experiments more intimately related to the specific objectives.

2.1.2. Broad scale population structure and phylogeography

The relationship between dogfish in the Pacific and their conspecifics in the Atlantic has been the subject of considerable interest, not least because experiences with the Atlantic dogfish fishery may be extremely valuable for the management in the Pacific. Pacific dogfish generally mature later, grow to a larger size and live longer than those in the Atlantic, and although these differences may reflect environmental factors, there is no information on the genetic relationship between the two populations. We therefore decided to carry out a broad scale study of dogfish population structure, including samples from throughout the range. Because sample sizes were small and because we were interested in the long-term phylogeographic history of dogfish, we decided to use about 1000 bp of the mitochondrial DNA D-loop for the analysis.

Dogfish tissues were obtained with the cooperation of several national agencies to sample throughout the species' entire range (Figure 1, Table I). Sample sizes ranged from n= 4 – 6 per geographic region; in total, 44 *S. acanthias* were sequenced. Tissue samples from one *Squalus mitsukurii* and one *Squalus megalops* (both from South Africa) were obtained for use as an outgroup to the *S. acanthias* sequences.

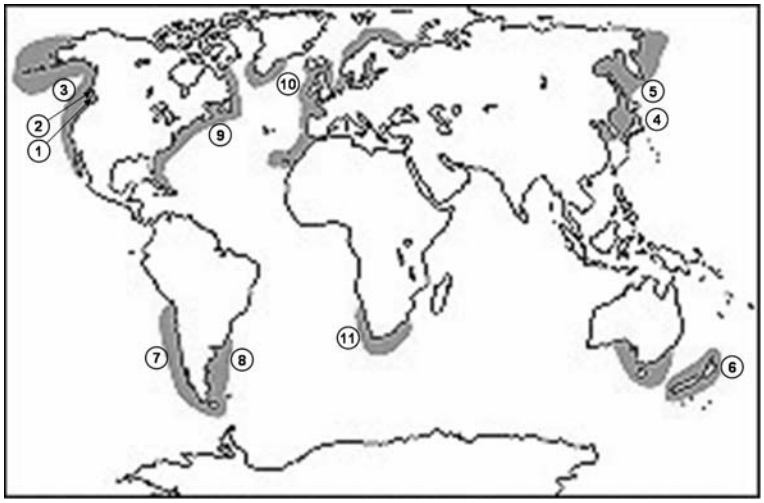


Figure 1: Distribution of *Squalus acanthias* (grey) and sampling locations (white circles). Numbers correspond to sample sizes in Table 1.

Tissue samples (fin clips or muscle tissue) were collected and preserved in ethanol. Genomic DNA was isolated using DNEasy column extraction kits (Qiagen Inc) following manufacturer's instructions and stored at 4°C until analysis.PCR amplification was performed on the mtDNA control region using ProL2 (5'-ctg ccc ttg gct ccc aaa gc-3') and PheCaCaH2 (5'-ctt agc atc ttc agt gcc at-3') primers (Pardini et al 2001) using 2.5 U of *Taq* polymerase (Promega, Inc.), 12.5 picomoles of each primer, 0.8mM dNTPs, 2.5mM $MgCl_2$, and 100 ng DNA in a 50 µl volume. The PCR consisted of an initial denaturing at 94°C for 5 min; thirty cycles of 90°C denaturing for 30s, 53°C annealing for 30s, and 72°C extension for 1 min; followed by a final extension step at 72°C for 10 min. Because of difficulties with directly sequencing the PCR product, the PCR fragment was ligated into a TOPO plasmid vector (Invitrogen Corp) and transformed into *E. coli*

Table I. *Squalus acanthias* sampling locations and sample sizes, and agencies that contributed to this project. Sample designations as in Figure 1 are shown in parentheses with the region. Total sample size *N*= 44

	Agency	Contact	Region	Larger Region	*n*
1	WA Dept. of Fish and Wildlife	Greg Bargmann	Puget Sound (PS)	Northeast Pacific	5
2	Fisheries and Oceans Canada	G. McFarlane	Strait of Georgia (CA)	Northeast Pacific	4
3	Univ. of Washington/NMFS	Vincent Gallucci	Gulf of Alaska (AK)	Northeast Pacific	3
4	Hokkaido Univ., Japan	Mitsuomi Shimazaki	Japan (JP)	Northwest Pacific	3
5	Russian Academy of Sciences	Dmitri Pitruk	Russia (RU)	Northwest Pacific	2
6	National Institute of Water and Atmos. Research, New Zealand	Peter Smith	Cook Strait, New Zealand (NZ)	Southwest Pacific	5
7	Universidad de Concepcion, Chile	Gustavo Aedo	Chile (CH)	Southeast Pacific	5
8	Centro Nacional Patagonico	Atila Gosztonyi	Argentina (ARG)	Southeast Atlantic	4
9	University of Maine	Irving Kornfield	Gulf of Maine (NW)	Northwest Atlantic	4
10	Centre for Env., Fisheries and Aquaculture Science	Jim Ellis	Irish Sea/Celtic Sea (UK)	Northeast Atlantic	5
11	S. Africa Dept of Env. Affairs				

competent cells according to manufacturer's protocol. Cells were plated onto X-Gal treated Luria agar plates with 50 μg/mL ampicillin and grown overnight at 37 ºC. Colonies containing inserts were picked and cultured overnight in LB media with 50 μg/mL ampicillin at 37 ºC. Plasmid DNA was isolated from these cultures using DNEasy Miniprep columns (Qiagen Inc). Presence of correct PCR product insert was confirmed by an EcoRI restriction digest on plasmid DNA and subsequent electrophoresis on a 1% agarose gel.

The complete control region insert (1146 bp) was sequenced using M13 forward and reverse priming sites on the TOPO plasmid. The sequencing reaction was performed on the plasmid vector containing the control region insert using fluorescently labeled dideoxynucleotide sequence terminators, then run on a 3730xl DNA Analyzer (Applied Biosystems, Inc.).

Groups of forward or reverse sequences were analyzed together to determine accuracy of base calls using Sequencher v4.5 (Gene Codes Corp.). Once base calls were verified, complementary forward and reverse sequences were combined into contigs, and the consensus sequence was exported to BioEdit v7.0.5 (Hall) for alignment. MEGA software v3.1 (Kumar et al. 2004) was used to analyze aligned sequences for sequence divergence, and to identify singleton mutations (substitutions which occurred in only one sequence) and parsimoniously-informative sites.

DnaSP 4.0 (Rozas *et al.*, 2003) was used to obtain summary statistics (number of polymorphic sites, number of parsimoniously informative sites, number of haplotypes, haplotype diversity h, and nucleotide diversity π). MODELTEST (Posada, Crandall, 1998) was run to determine the DNA mutational model that best approximated the sequence evolution of each dataset, and to calculate transition/transversion ratios. Neighbor joining phylogeographic trees were generated for each using PAUP 4.0 (Swofford, 2000) with 1000 bootstrap replicates.

2.1.3. Fine scale population structure in the north-east Pacific

To investigate the genetic population structure of dogfish on a smaller geographic scale (north-east Pacific), we employed microsatellite markers, which have been shown to reveal more genetic variability and thus allow greater power of statistical tests (Hauser *et al.*, 2001). Microsatellites are short DNA sequences of 2-6 base pairs in length that are tandemly repeated dozen or hundreds of times. High mutation rates during replication resulting in the change of repeat number result in high variability of the total microsatellite length that can easily be screened by PCR and electrophoresis. High variability and the availability of multiple loci make microsatellites the marker of choice for studies of small scale genetic differentiation (Selkoe, Toonen, 2006).

Adult spiny dogfish were captured with the cooperation of several agencies by research trawls or commercial hook-and-line fishing operations from six northeast Pacific sites (Gulf of Alaska, Bering Sea, California/Oregon coast, Puget Sound, Strait of Georgia, and the Washington coast), one south Pacific location (Isla Mocha, Chile), and one northwest Atlantic site (Gulf of Maine). Fin clips or muscle tissue were collected and preserved in 90% ethanol. Sample sizes ranged from n= 25-88; in total, 433 dogfish were collected (Table II).

Table II. *Squalus acanthias* sampling locations and sample sizes, and agencies that contributed to this project.

Region	**Agency**	***n***
Gulf of Alaska (AK)	University of Alaska, Fairbanks	47
Bering Sea (BS)	National Marine Fisheries Service	25
California/Oregon coast (CO)	National Marine Fisheries Service	71
Puget Sound (PS)	Washington Department of Fish and Wildlife	88
Strait of Georgia (SG)	Fisheries and Oceans, Canada	47
Washington coast (WA)	Arrowac Fisheries, Inc.	52
Chile (CH)	Universidad de Concepción	44
Northwest Atlantic (NA)	University of Maine	59
Total *N*		433

Genomic DNA was isolated using DNEasy column extraction kits (Qiagen Inc., Valencia, California) following manufacturer's instructions and stored at 4°C until analysis. Eight *S. acanthias*-specific microsatellite loci (McCauley *et al.*, 2004) were used in this study (Table III). The PCR was performed in a 10µl volume and consisted of ~100ng template DNA, 1µl 10X NH_4 buffer (Bioline USA Inc, Boston, MA), 1.5 mM $MgCl_2$ (2.0 mM $MgCl_2$ for locus *DF T289*,), 0.04 mM of each dNTP, 1 picomole each of forward and reverse primers, and 0.5U Biolase Taq Polymerase (Bioline USA Inc, Boston, Massachusetts). Forward primers were fluorescently labeled with NED (Applied Biosystems Inc., Foster City, CA), FAM, or HEX (Qiagen Inc, Valencia, CA). Touchdown PCR (Don *et al.*, 1991) was used for all but one locus (*DF U285*). Reactions were carried out in an MJ Research PTC-225 Tetrad thermal cycler (Bio-Rad Laboratories, Hercules, California) using profiles listed in Table III.

Fragment sizes of microsatellite DNA were estimated on a MegaBACE 1000 DNA Analysis System (GE Healthcare, Piscataway, NJ) using ET550-R fluorescently labeled size standard. Raw electropherogram data files were processed by Genetic Profiler v2.2 (GE Healthcare), and allele calls were scored manually to obtain individual genotypes. Micro-checker (Van Oosterhout *et al.*, 2004) was used to test for the presence of null alleles and scoring errors.

Table III: Thermal cycler PCR annealing temperatures and number of cycles for microsatellite loci used in this study. All profiles utilized a 5 min initial denaturation (94°C), 1 min denaturing (94°C), 1 min annealing (at T_A given), and 1 min extension (72°C), and a 10 min final extension (72°C). During the touchdown period of the PCR, the annealing temperature was lowered by 1°C in each cycle.

	Locus					
PCR step	*DF U273, DF V296, DF J451*	*DF T289*	*DF H429*	*DF J445*	*DF U285*	*DF H434*
Touchdown						
# cycles	3	3	4	4	n/a	4
Annealing T_A (°C)	54-52	52-50	63-60	62-59	n/a	63-60
Main PCR						
# cycles	23	31	31	23	31	26
Annealing T_A (°C)	53	52	60	59	53	61

Genotyping errors were estimated by re-screening 94 individuals at three loci (*DF T289, DF H429, DF J445*) (Hoffman, Amos, 2005). Allelic richness and observed and expected heterozygosity were calculated in FSTAT v2.9.3.2 (Goudet, 1995) and GENEPOP v3.3 (Raymond, Rousset, 1995), respectively. Deviations from Hardy-Weinberg equilibrium (HWE) at each locus within each sample was determined by a two-tailed exact test using the Markov chain algorithm (Guo, Thompson, 1992) implemented in GENEPOP v3.3 (dememorization number 1000, 100 batches, with 1000 iterations per batch) (Raymond, Rousset, 1995). Tests for deviations from linkage disequilibrium between loci were also carried out using GENEPOP v3.3 software.

Genetic differentiation among populations was determined by calculating pairwise F_{ST} and R_{ST} values in FSTAT v2.9.3.2 (Goudet, 1995); pairwise F_{ST} values were tested for significance after Bonferroni correction for multiple tests (Rice, 1989). To evaluate if the pattern of genetic differentiation observed was consistent with isolation by distance (IBD), a Mantel test using F_{ST} (Mantel, 1967) was carried out using the ISOLDE program within GENEPOP v3.3.

A locus-by-locus AMOVA in ARLEQUIN v2.0 was performed between the two clades determined by mtDNA analysis (north Pacific, and south Pacific/Atlantic). Multidimensional scaling plots (MDS) were used to display genetic distances among samples using SPSS v11.0 (SPSS Inc.). Cavalli-Sforza chord distances (Cavalli-Sforza, Edwards, 1967) were calculated from microsatellite allele frequency data in GENETIX v4.0.5.2 (Belkhir K., 2004). For comparison, D_{XY} distances (Nei, 1987) between populations were determined from mitochondrial DNA control region sequence data (Franks *et al.* submitted) by DNASP v4.0 (Rozas *et al.*, 2003). Finally, individuals were assigned to populations of origin using Bayesian algorithms (Rannala, Mountain, 1997) in GENECLASS v2.0.g (Piry *et al.*, 2004).

2.2. Demographic parameters

2.2.1. Geographic differentiation in growth rates

Samples of female dogfish (*Squalus acanthias*) were collected from a variety of sources. Most samples were obtained from research cruises conducted by the National Marine Fisheries Service (NMFS), by the Washington Department of Fish and Game (WDFW), and by the Shark Research Lab at the University of Washington in collaboration with WDFW; these cruises occurred in 2002-2005. Some samples from the North and South Puget Sound and Washington State were taken from commercial catch; these samples were taken in 2002-2003 and 2005. Total length (extended) and maturity stage was recorded for all samples, and the second dorsal spine was taken for age estimation. A subsample of weights was also taken. When females were found to be mature, numbers of developed ova in each ovary and total number of developed ova were recorded; if the female was pregnant, number of pups in each uterus, total number of pups, and total length (extended) of all pups were recorded.

Table IV Sample statistics by region.

	N	Min Age	Mean Age	Max Age	Min Size	Mean Size	Max Size
NPS	341	8	36.3	84.0	38.7	86.2	116.5
SPS	64	1	21.9	44.0	29.0	63.3	100.7
Washington	234	3	34.4	74.6	35.0	80.4	115.9
Oregon	135	0	14.3	79.0	22.5	48.2	106.0
Monterey	46	3	18.2	56.4	31.0	53.2	74.0
Conception	66	1	16.9	65.0	30.0	49.3	97.0

Data were taken from eight regions (Gulf of Alaska, North Puget Sound, South Puget Sound, Bering Sea, Washington State, Oregon, Monterey, Conception) (Table IV). As very few samples were available from the Bering Sea (n<25) and data quality for the Gulf of Alaska was poor, these data were not included in statistical analyses.

Growth in the spiny dogfish is obtained from repeated age at length measurements taken within a population. Age estimates are obtained from reading of annual ring counts on the second dorsal spine. This method was validated by Beamish and McFarlane (1987).

In unworn spines, the count of annuli is considered to represent the total age of the animal. In older animals, the tip of the spine has frequently been worn away, and it is necessary to estimate the number of missing annuli. Ketchen (1975) gives a procedure for calculating the number of missing annuli based on the diameter of the spine at the wear point. Estimated ages from conception are obtained for spines showing no discernable wear, and a functional relationship between spine base diameter (in mm) and age is estimated as

$$Y=aX^b,$$

where Y is the age in years from conception and X is the spine base diameter (Ketchen 1975). Number of missing annuli is estimated using this relationship, where Y is the number of missing annuli and diameter in mm at the wear point is X. This procedure almost certainly introduces some error into the aging process, but it is at present the best way to make maximum use of spines from large animals.

In order to prepare the dorsal spine of dogfish for ageing, the muscle tissue and tough sharkskin attached at the base of the spine must be removed. Once spines are cleaned and dried, annulus counts (where an annulus is as defined in the second section) may be taken. Spines are read under a dissecting microscope at low magnification (this lab used 8X; Ketchen 1975 used 7X). Lighting is from above, with oblique white light used to bring out ridges in the enamel more clearly. Total annulus counts are recorded along with base and wear point spine diameters (procedure from Ketchen 1975).

A minimum of two readings was done on each spine. No spine was re-read in the same two-day period to ensure that individual reads were blinded with respect to prior measurements. Where the first two reads were in agreement, the obtained count was accepted. Where the first two reads differed by one year, counts were accepted alternately. Where the difference was two years or greater, a third read was performed. It was assumed that any one read could have been inaccurate due to reader fatigue or other error, and any count consensus with the third read within one annulus was accepted. If no consensus could be reached after three reads, all previous reads were discarded and the spine was read again. Ketchen (1975) used the mean of measurements as the consensus count where the difference in annulus counts was two annuli or more. Since the statistical consequences of using the mean of annulus counts are unexplored, we deviated from this procedure and sought to reach an actual consensus count for all spines. For those spines that showed no apparent wear, final annulus count was accepted as an estimator of age. For worn spines, the missing annulus count was determined as in Ketchen (1975). Damaged or broken spines were considered to be unreadable.

Bias correction was performed on consensus annulus counts as read by N. Vega (primary author). Based on the results of an aging validation project performed in 2005-2006 (Rice et al, in prep), it was determined that the annulus counts produced in this lab contained some

percentage of "double aging" in larger spines, where split annuli are counted as two separate annuli. As small spines generally show good separation of annual rings, "double counting" does not occur. A bias correction factor of 0.8 was applied to all spines where more than 12 annuli were counted. This bias correction factor was shown to bring the results obtained by this lab in line with median results obtained from other labs in the aging validation study.

The von Bertalanffy growth function (VBGF) is widely used to describe the age-length relationship in fish. A number of parameterizations of the VBGF have been shown to have better behavior than the traditional parameterization. One such commonly used reparameterization was proposed by Gallucci and Quinn (1979):

$$\ell(t) = \frac{\omega}{K}\left(1 - e^{-K(t-t_0)}\right)$$

Here, the new parameter $\omega = L_\infty K$ corresponds biologically to the instantaneous rate of growth in the neighborhood of $\ell(t_0)$. The parameter is a true rate (units length/time). Both L_∞ and K are biologically meaningful parameters; if parameter-based statistical tests are to be used to make comparisons of growth curves, it is generally desirable to make comparisons based on both of these parameters, but conflicting results may be obtained. This reparameterization allows the effects of these two parameters to be considered simultaneously. It is possible to construct the estimator ω and its estimated variance directly from parameter values and the variance-covariance matrix obtained by performing regression on the general form or by performing regression on the reparameterized equation directly. A regression on the general form should be performed in any case, as it is often desirable to have an estimated value for L_∞.

Once an appropriate growth model was fitted to data from several regions, comparisons of growth between regions were conducted. We used the nonparametric likelihood ratio test (LRT) proposed by Cerrato (1990) in making comparisons of growth curves to avoid assumptions about the distributions of the parameters and to avoid the necessity of selecting a parameter or parameters on which to base the hypothesis test. Of the available multivariate tests, it was found that the LRT was most reliable (Cerrato 1990), though small sample sizes and unequal variances did undermine in some degree the reliability of this test.

A series of demographic models were created using information on growth, fecundity, and maturity obtained from field data. This information was obtained during field sampling 2002-2005. Background information for the terminology and notation used in this chapter and for the elasticity analyses and randomization procedures is summarized in Appendix A. Bootstrap simulations were performed using 10000 repetitions, bias was calculated using the formula from Efron and Tibshirani (1993) and α=0.025, and confidence intervals (uncorrected and bias-corrected) were created for all simulations.

2.2.2. Life History analysis and matrix population models for north-east Pacific dogfish

The estimation of mortality is central to demographic analysis, but this parameter is notoriously difficult to estimate in elasmobranchs. Mortality estimation from other life history parameters is possible, though these estimates are uncertain at best, and rest on assumptions that cannot be confirmed. Mollet and Calliet (2002), Peterson and Wroblowski (1984), Hoenig 1983, Pauly (1980), Frisk et al. (2001), Wood et al. (1979) each published a method of estimating M, though none of these estimators are entirely satisfactory. In lieu of relying on any single

indicator, we used the range of values provided by the available estimation methods to explore possible values for *M*. The available formulae presented above were used to generate estimators for *M* for each region. Median value of the range of estimators for *M* was used as the value for natural mortality in creating the matrix model, and the same value for *M* was applied to all ages within the model. Bootstrap values for *M* were drawn at random from a triangular probability distribution covering the full range of estimated mortality values for each population (Cortes 1999). The distribution used is continuous, nonuniform, and symmetric, with maximum probability of 0.5 assigned to the median value of the range and probability of 0 assigned to the maximum and minimum values in the range.

Maturity information was obtained from biological observations of female dogfish captured during sampling. A female was considered to be mature if demonstrating either pregnancy or large developing ova. Percent maturity was described as a function of total length. Fecundity was measured as the total number of pups in utero for pregnant females. Fecundity was described as a linear relationship between total length and total number of pups in utero. Mean number of pups per mature female for each region was calculated from pup count data.

The effects of fishing mortality were approximated by incorporating a harvest matrix **H** into the Leslie matrix for each population. The matrix **H** is a diagonal matrix where the elements of the diagonal h_{ii} are the survivorships associated with a given rate of instantaneous fishing mortality in each year of life. The matrix **H** was constructed by establishing an age of entry for the fishery t_c and a level of fishing mortality for the remaining ages. To simplify the assumptions involved, knife-edge fishing mortality was assumed, and all ages recruited to the fishery were subjected to the same level of harvest mortality ($h_{jj}=h_{ii}$, $j>i\geq t_c$). Years of the life history that are not recruited to the fishery were represented by a diagonal entry with a value of 1. The effects of harvest were estimated by constructing the matrix **H** and combining this matrix with the deterministic Leslie matrix to obtain an augmented life history matrix

$$\mathbf{A'=HA}$$

The dominant eigenvalue of the augmented matrix **A'** was obtained. This eigenvalue gives the estimated rate of growth of the population under the harvest conditions imposed by **H**, and the values taken by the eigenvalue of **A'** over different harvest conditions are therefore considered to be a metric for sensitivity of the population to perturbation.

2.3. Socioeconomics

A methodological approach defined by John Gaddis in "The Landscape of History: how historians map the past", 2002, was a guiding document. The dogfish has a longer history of fisheries for utilization than any other fish since they went through a history of being used by: indigenous people (skin, sandpaper, food), early settlers (lantern oil, food), later settlers (Vitamin A) and then recent times (export market of belly flaps to Europe), for different purposes. Between the later settlers and recent times the salmon and other fisheries arose in Washington State and dogfish were perceived as killers of juvenile salmon and eradication programs were put in place in Washington State and in British Columbia. Of course, this was done with no ability to anticipate the modern export market that currently exists. It is against this background that the state of the stocks, processors, markets, and management policies were examined. Management today is based upon balancing the differing interests of stakeholders, a complex legal structure

defined within the bounds of treaty rights guaranteed indigenous people, the ex-vessel dollar value of other fish relative to the dogfish, the availability of labor for labor intensive value added processing before export, and export markets. The importance of other fish is based upon the relative value of the different fisheries since fishermen will make decisions about the fish to target for any day based upon their anticipated net financial return. Recommendations are made based upon lessons from the crashed east coast fishery and from the principles of precautionary management.

3. PROJECT MANAGEMENT

The Principal Investigator of the project was Dr Lorenz Hauser, who has worked previously on marine pelagic fish, and has extensive experience in genetic stock identification and population genetics. The molecular genetics work was carried out at MMBL (Molecular Marine Biotechnology Laboratory) of the University of Washington.

Prof. Vincent Gallucci was co-PI, and supervised the stock assessment and socioeconomic work. He has wide expertise in fisheries management and stock assessment, has worked extensively on tropical artisanal fisheries especially of sharks, and has supervised other dogfish policy, biology, and stock assessment theses. Although his primary academic appointment is in the School of Aquatic and Fishery Sciences, he is also a faculty member in the School for Marine Affairs where he carries out research on management polices that must mesh with stock assessment findings and projections.

James H Franks was the graduate student working with Dr Hauser on the molecular genetic aspect of the study. James has defended his Master's thesis at the School of Aquatic and Fisheries Sciences in August 2006, and is now preparing several manuscripts for publication.

Nichole Vega was a graduate student working with Dr. Gallucci on the demography aspects of the study. Nichole defended her Master's thesis in the Quantitative Ecology and Resource Management group in June, 2006, and is now preparing manuscripts for publication.

Serra Morrison was a graduate student working with Dr. Gallucci on the socioeconomic aspects of the study. Serra defended her Master's thesis in the School for Marine Affairs in June, 2005, and is now preparing a manuscript for publication.

A range of agencies and individuals worldwide were instrumental in supplying genetic samples of dogfish (Tables I and II). Fin clippings were shipped by courier and mail to the molecular lab for analyses.

4. FINDINGS

4.1. Molecular Genetics

4.1.1. Broad scale population structure and phylogeography

4.1.1.1. Taq polymerase error

Taq errors were found in three of four re-amplified individuals (CA03, ARG05, and NZ213; UK01 had no errors), with five, four, and two base substitutions, respectively, per 1146-bp sequence (0.44%, 0.35%, and 0.17% of bases, respectively). There were seven transitions

and four transversions, and a transition-transversion ratio of 1.75. Two separate clones sequenced from each of the initial cloning reactions showed no substitutions, indicating that errors were due to *Taq* error in the initial PCR prior to cloning, and that the *Taq* error occurred early in the reaction. The errors occurred at random throughout the sequence and never at the same site, and we concluded that removal of all singleton sites would eliminate most of the *Taq* error from our dataset.

Although preliminary, our *Taq* error data provide some interesting and somewhat worrisome insights into the nature and frequency of sequencing errors. Usually, high fidelity polymerases are used for sequencing projects, though with such high-fidelity polymerases errors still occur (e.g. published Promega *Pfu Taq* error rates range from 2.5×10^{-5} to 6.5×10^{-7}, whereas standard Promega *Taq* error rates vary from 2×10^{-4} to 2×10^{-6}, Pavlov *et al* 2004). Nevertheless, most sequencing studies do not estimate *Taq* or other sequencing errors and do not even state the exact polymerase used, even though deposition in sequence databases make the effects of such errors more far-reaching than those in microsatellite genotyping that recently attracted considerable attention (Bonin *et al* 2004, Hoffman & Amos 2005). If *Taq* errors are considered, it is usually by sequencing several clones from the same PCR and cloning reaction – our results indicate that this approach seriously underestimates the error, as no difference was found within cloning reactions, but three of four individuals showed several substitutions between cloning reactions. This pattern suggests that *Taq* error occurs early in the reaction, and that it may therefore also affect results obtained by direct sequencing rather than just those from cloned libraries. It is clear that *Taq* error affects different application to a varying degree: for example, phylogeographic reconstructions presented here were not affected (data not shown), while mismatch distributions would be affected. However, the use of DNA sequences, once submitted to DNA databases, is not predictable and thus caution should be exercised. Given the likely ubiquity of sequencing errors and the potential consequences, more directed empirical and simulation studies are urgently required.

4.1.1.2. Summary statistics

The original dataset (not corrected for *Taq* error and including outgroups) consisted of 44 1146-bp *S. acanthias* sequences; 43 unique haplotypes were identified resulting in a haplotype diversity $h = 0.999$. Out of 130 polymorphic sites, 22 were parsimoniously informative, and there were 88 sites with *S. acanthias*-specific singleton mutations (7.68% of sites). Nucleotide diversity π was 1.03% and the transition-transversion ratio was 3.02.

Dataset 2 (with singletons removed) consisted of 44 1058-bp sequences. Removal of singletons resulted in 33 unique haplotypes and a haplotype diversity of $h = 0.966$. The number of variable sites decreased to 36 (22 of which were parsimoniously-informative) giving an overall nucleotide diversity π of 0.72%, and a transition-transversion ratio of 2.78.

4.1.1.3. Phylogeographic trees

After removing the singleton mutations (presumed *Taq* error, dataset 2), MODELTEST identified the TVM+I+G (variable base frequencies, variable transversions, transitions equal, gamma distributed rate variation among sites, some invariate (static) sites). The tree recovered for dataset 2 was similar to the tree determined by the original dataset (Figure 2). Percent bootstrap support at major nodes was largely unchanged; additionally, trees constructed using the simpler Jukes-Cantor (JC) and HKY85 (HKY) models of nucleotide substitution to determine the robustness of the tree topologies for dataset 2 showed little change in bootstrap support.

All phylogeographic trees showed two distinct clades: one in the north Pacific (CA, PS, AK, JP, RU), and one in the south Pacific and Atlantic (NZ, CH, ARG, UK, NW). There were four fixed differences, and 0.7% sequence divergence, between clades. No geographic structure was apparent within the two clades from these trees. Two individuals sampled in the north Pacific appeared to have south Pacific/Atlantic haplotypes (JP98 and RU02, from Japan and Russia, respectively). Haplotypes of two dogfish sampled in Puget Sound, Washington (PS86 and PS87) appeared to be intermediate to the two clades, as they grouped independently from the S Pacific/Atlantic clade (i.e., between the N Pacific and S Pacific/Atlantic clades) with high bootstrap support.

The two distinct clades correspond to the marked life history differences between N Pacific and S Pacific/Atlantic dogfish (Table V), and thus suggest a genetic split within *S. acanthias*. Our results indicate sequence divergence of 0.7% between clades; it is uncertain at this time whether this level of differentiation justifies distinct species status of the two clades. In comparison, estimates of control region sequence population divergence among sharks range from 0.030- 0.128% (corrected) in makos (Heist *et al.*, 1996), 0.214% in blacktip (*Carcharinus limbatus,* Keeney *et al.,* 2005), to 4% in white sharks (*Carcharadon carcharias*, Pardini *et al.*, 2001). Among species, divergence ranges from 5.6-7.5% in cryptic species and 7.8- 24.3% in known species of hammerhead (*Sphyrna* spp., Quattro *et al.*, 2006); additionally, *Squalus* species in the south Pacific show 4% sequence divergence at COI despite morphological similarity (Ward *et al.*, 2005). Remarkably, some of the highest levels of divergence occur in sympatric species (e.g. cryptic hammerhead and south Pacific *Squalus*), whereas in our geographically distant populations we observed a comparatively low degree of divergence. Clearly, more data on nuclear markers are needed to answer the species question in *S. acanthias*; nevertheless, our results and known life history differences suggest that the two clades at least deserve the status of DPSs (distinct population segments, Waples, 1991) and thus protection as separate entities.

Our data suggest a north Pacific origin of *S. acanthias*. South Pacific/Atlantic haplotypes sampled in the N Pacific (JP98 and RU02, from Japan and Russia respectively) and haplotypes collected in the N Pacific that are intermediate to both clades (PS86 and PS87, from Puget Sound, WA) may represent ancestral haplotypes. In addition, the phylogeographic tree (Figure 2) showed the S Pacific/Atlantic clade nested within the N Pacific clade, suggesting that the N Pacific clade gave rise to the S Pacific/Atlantic clade. While we cannot dismiss migration from the south to the north Pacific as a potential explanation of the S Pacific/Atlantic haplotypes in the

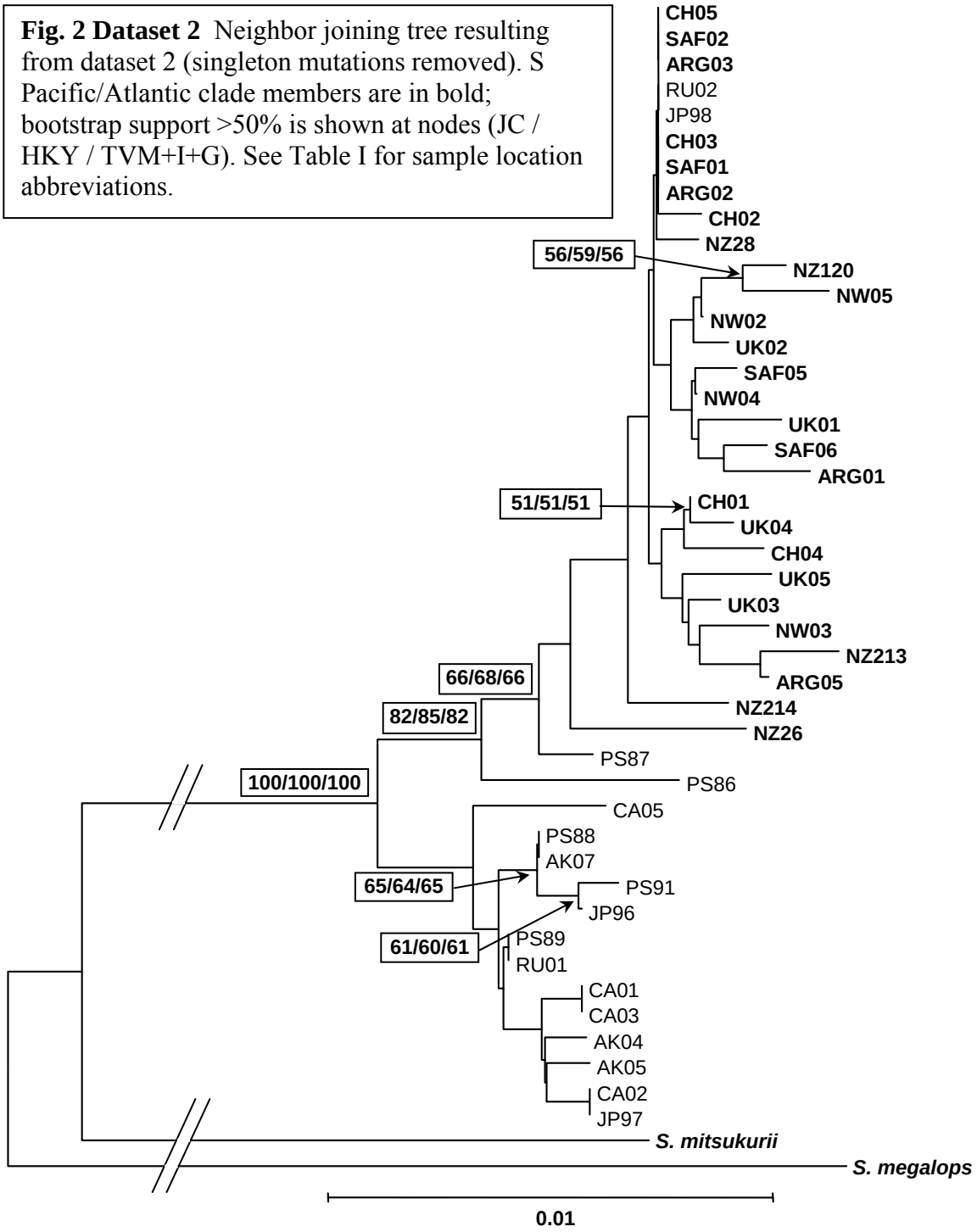
Table V. Life history parameter differences observed in female *Squalus acanthias.*

	Pacific			Atlantic		
	NW Pacific	**NE Pacific**	**New Zealand**	**NW Atlantic**	**NE Atlantic**	**S Africa**
Age at 50% maturity	19 y[a]	30-36 y[a,c,g,h]	10 y[d]	10 y[c]	13 y[a,c]	n/a
Length at 50% maturity	99 cm[b]	87-93 cm[c,g,h]	73 cm[e]	77-80 cm[c,f,h]	78.5 cm[c,f]	57.5 cm
Maximum length	130-140 cm[b]	120-124 cm[b]	111 cm[e]	108 cm[b]	110 cm[b]	n/a

[a] Ketchen 1975, [b] Ketchen 1972, [c] Saunders and McFarlane 1993, [d] www.fishbase.org, [e] Hanchet 1988, [f] Da Silva 1993, [g] Tribuzio 2004, [h] Vega 2006

N Pacific, the basal position of the two Puget Sound haplotypes supports the idea of ancestral haplotypes in the N Pacific. Although we could not perform more detailed phylogeographic analyses (such as investigating mismatch distributions of pairwise differences to infer demographic history, Rogers and Harpending 1992) because of *Taq* error, the genetic data indicated that *S. acanthias* may have originated in the N Pacific and then colonized the Atlantic and S Pacific.

Fig. 2 Dataset 2 Neighbor joining tree resulting from dataset 2 (singleton mutations removed). S Pacific/Atlantic clade members are in bold; bootstrap support >50% is shown at nodes (JC / HKY / TVM+I+G). See Table I for sample location abbreviations.



4.1.2. Small scale population structure

4.1.2.1. Genetic diversity and conformance to population genetic expectations

A total of 433 *S. acanthias* from 8 populations were genotyped at 6 to 8 microsatellite loci. Allelic richness per locus ranged from 3.8 (*DF J451*) to 11.3 (*DF U273*). Both heterozygosity and allelic richness were higher in the south Pacific and Atlantic than the north Pacific.

Out of 64 tests for deviation from Hardy-Weinberg equilibrium, six populations were nominally significant (P<0.05); no individual population tests were significant after Bonferroni correction. The Chilean sample showed nominal deficiencies of heterozygotes at three of the eight loci, possibly indicating a Wahlund effect, although the presence of null alleles in the Atlantic clade cannot be excluded because all these three loci had also positive F_{IS} values in the north Atlantic sample. Over all Pacific samples pooled, only one locus was out of HWE (*DF T289*), though none of the individual samples showed a significant result. The overall F_{IS} was significantly higher in the south Pacific/Atlantic clade than the north Pacific clade (south Pacific/Atlantic F_{IS} = 0.071, north Pacific F_{IS} = 0.005; P<0.05). Micro-checker analysis suggested potential null alleles but found no evidence for large-allele dropout or scoring errors due to stuttering for those samples and loci which had the highest F_{IS} values (Appendix I, north Atlantic *DF H429,* Chile *DF J445*). Null alleles were also suggested for north Atlantic *DF U273*, which had a small but positive F_{IS}.

Thirteen of 224 tests for deviations from linkage equilibrium were nominally significant (P<0.05), and none were significant after Bonferroni correction. Using Fisher's method, probabilities from exact tests were combined over all samples and three locus pairs were nominally significant (*DF V296* and *DF T289*; *DF V296* and *DF U285*; *DF H429* and *DF J445*); none were significant after Bonferroni correction for multiple tests. Out of 94 individuals genotyped twice at *DF T289, DF H429, DF J445*, two errors were found (California/Oregon, locus *DF T289*, and Alaska, locus *DF H429*; both presumably due to large allele dropout), yielding an estimated per reaction genotyping error rate of 0.7%.

4.1.2.2. Genetic differentiation

Within the northeast Pacific, no evidence for population structure was found (overall F_{ST}

Table VI. Pairwise estimates of genetic differentiation among dogfish populations are given, using R_{ST} (above diagonal) and F_{ST} (below diagonal). For F_{ST}, *P < 0.05, **P < 0.01; bold values indicate significance after Bonferroni correction of alpha for multiple tests (adjusted alpha = 0.00179).

		R_{ST}							
	Population	AK	BS	CO	PS	SG	WA	CH	NA
F_{ST}	AK	------	-0.016	-0.002	-0.006	-0.009	-0.009	0.158	0.163
	BS	-0.007	------	0.003	-0.005	-0.012	-0.005	0.164	0.175
	CO	0.002	0.006*	------	-0.003	0.005	0.001	0.127	0.144
	PS	-0.002	0.003	0.002	------	-0.005	-0.005	0.164	0.171
	SG	-0.003	-0.005	0.005	0.001	------	-0.006	0.179	0.184
	WA	-0.001	-0.002	0.002	0.000	-0.002	------	0.167	0.165
	CH	**0.136****	**0.132****	**0.156****	**0.142****	**0.130****	**0.145****	------	0.029
	NA	**0.135****	**0.133****	**0.158****	**0.144****	**0.128****	**0.144****	**0.013****	------

= 0.000). Tests of pairwise F_{ST} among northeast Pacific samples revealed only one nominally significant (P<0.05) comparison (Bering Sea vs. California/Oregon, F_{ST} = 0.006, P=0.011), and no significant differentiation was observed among all other samples. There was no correlation between genetic differentiation and geographic distance among populations (F_{ST} vs. linear geographic distance r^2= 6 x 10^{-6}, P=0.99).

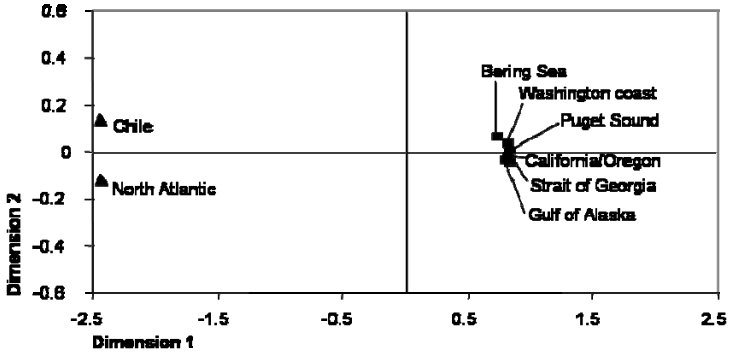
In contrast, all pairwise comparisons between northeast Pacific samples and south Pacific (Chile) or north Atlantic dogfish resulted in values of F_{ST} two orders of magnitude higher than those within the northeast Pacific (Table VI). Pairwise tests were highly significant (P<0.01) and remained significant after Bonferroni correction. North Atlantic and Chilean *S. acanthias* were also significantly differentiated from one another (F_{ST} = 0.013, P= 0.00178). Overall, R_{ST} values were similar to estimates of F_{ST} (Table VI). Tests for differentiation within and between larger regions (northeast Pacific vs. the south Pacific/north Atlantic) using AMOVA found significant heterogeneity among samples (F_{ST} = 0.147, P= 0.000), among samples within regions (F_{SC}= 0.003, P=0.028), and between regions (F_{CT} =0.145, P=0.000).

Multidimensional scaling plots constructed from Cavalli-Sforza distances (microsatellite data, Figure 3) suggest low heterogeneity within the northeast Pacific (indicated by the relatively small distances between northeast Pacific populations), but considerable differentiation between northeast Pacific and northwest Atlantic and Chilean populations, respectively.

4.1.2.3. Genetic assignment

Assignment of individuals to populations by exclusion using GENECLASS v2.0 found that 430 of 433 individuals were assigned to the respective region from which they were sampled (99.3%) (Table VII). Only three dogfish (one each from the northeast Pacific, south Pacific, and northwest Atlantic) were assigned outside of their sampled clade Within the northeast Pacific, individual assignments to samples were not significantly different from random (χ^2=27.23, df=25, P=0.344), while correct assignments between Chile and the northwest Atlantic were significantly more frequent than expected by chance (χ^2=12.10, df=1, P=0.0005).

a) Microsatellite pairwise Cavalli-Sforza chord distance



b) mtDNA control region D_{XY}

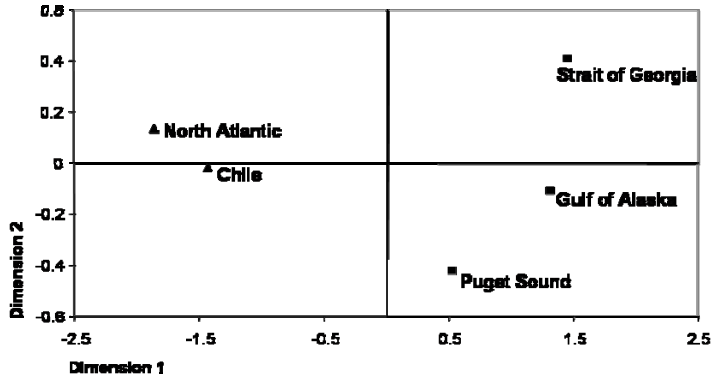


Figure 3. Multidimensional scaling plots of (a) pairwise Cavalli-Sforza chord distance from microsatellite allele frequencies of Pacific and Atlantic *S. acanthias* samples (r^2 =0.999, stress 0.011) and (b) D_{XY} from mtDNA control region sequences (r^2 =0.999, stress 0.008).

Table VII. Assignment of dogfish to clade using GENECLASS v2.0. 430 of 433 dogfish were assigned to populations out of their sampled clade (99.3%).

Sample	Assigned population								
	N Pacific						S Pacific/Atlantic		
	Alaska	Bering Sea	California/ Oregon	Puget Sound	Strait of Georgia	Washington coast	Chile	North Atlantic	*n*
Alaska	5	11	6	10	9	6	0	0	47
Bering Sea	4	5	3	2	5	5	0	1	25
California/Oregon	12	7	20	10	8	14	0	0	71
Puget Sound	15	8	20	24	8	13	0	0	88
Strait of Georgia	6	5	10	12	7	7	0	0	47
Washington coast	3	9	10	11	7	12	0	0	52
Chile	1	0	0	0	0	0	30	13	44
North Atlantic	0	1	0	0	0	0	19	39	59
								Total *N*	433

4.1.3. Interpretation

4.1.3.1. Broad scale structure

Significant differentiation was detected between north Pacific and south Pacific/Atlantic clades at microsatellite loci (Table VI), confirming previous mtDNA data (mtDNA AMOVA: among samples F_{ST} = 0.572 (P=0.000), between clades F_{CT}= 0.551([P=0.000), and among samples within clades F_{SC}= 0.047 (P=0.04), Franks *et al.*, submitted). Multidimensional scaling plots calculated from microsatellite allele frequency data (Figure 3a) and mtDNA control region sequences (Figure 3b) illustrate the large divergence between the two clades shown by both markers. While this result is not surprising given the geographic separation between areas, it is interesting that equatorial waters appear to be an effective barrier to gene flow in the Pacific but not the Atlantic. The small F_{ST} detected between north Atlantic and south Pacific (Chile) populations, although significant, suggests some degree of connectivity across the Atlantic equator, or that these populations may have only recently been separated. The antitropical distribution of dogfish is due to their preference for cooler waters (6°C -15°C, Jensen 1966), and thus warm equatorial waters should represent an effective thermal barrier to migration (Franks *et al.* submitted). The small yet significant genetic differentiation between south Pacific and north Atlantic dogfish may imply that gene flow between these regions across the Atlantic equatorial region has recently occurred, possibly during the last broad-scale glaciation when dogfish would have experienced a decrease in available habitat.

Because of the comparable genetic structure found in the worldwide distribution of dogfish at both mtDNA and microsatellite markers, we find little evidence for the sex-biased dispersal that has been suggested in other wide-ranging shark species (white, Pardini *et al.*, 2001, and mako, Schrey, Heist, 2003). In those studies, female philopatry was implied from genetic structure at mtDNA loci, while 'roaming males' homogenized allele frequencies at microsatellite markers. Little is known about the mating behavior of white and mako sharks, but the genetic dissimilarity between these species and dogfish could be a result of behavioral differences. Wide-ranging, largely solitary species such as white and mako sharks might encounter potential mates infrequently, and reproductive events might occur wherever sharks meet by chance.

Philopatry by females to pupping nurseries is well documented in sharks (Feldheim *et al.*, 2002, Keeney *et al.*, 2005), so matings that occur on the open sea with subsequent homing to natal pupping grounds could explain a pattern of sex-biased dispersal in white and mako sharks. Dogfish, on the other hand, often associate in mixed-sex schools, and as such are in close proximity to potential mates. Mating opportunities appear to be limited to just after the short post-parturition period, which follows a 22-month gestation (Tribuzio, 2004), though there is evidence that a small proportion of females undergoes a resting phase between pregnancies (Hanchet, 1988). Competition among males for available, non-pregnant females is presumably high, and matings close to pupping grounds soon after parturition could result in the genetic structure at both mtDNA and microsatellite loci that we observed.

4.1.3.2. Fine scale structure

An F_{ST} of 0.000 and random genetic assignment within northeast Pacific samples suggest little genetic differentiation. Indeed, this lack of differentiation was not due to low power of the genetic markers: a power analysis (Powsim, (Ryman, Palm, 2006) suggested that we had 92% power to detected differentiation as low as F_{ST} = 0.003. In comparison, genetic differentiation was considerably higher in sharks where differentiation was detected: e.g. lemon shark, F_{ST}=0.016, (Feldheim *et al.*, 2001), blacktip shark, F_{ST}=0.007, (Keeney *et al.*, 2005). Nevertheless, it is important to note that failure to detect genetic structure does not necessarily imply that all northeast Pacific dogfish comprise a single self-recruiting population for management purposes. The migration (and mating) of only a few dozen individuals per generation is adequate to homogenize allele frequencies among populations and achieve the genetic homogeneity we observed (Mills, Allendorf, 1996). Such migration is certainly plausible given the extent of movement between inshore and coastal areas (McFarlane, King, 2003) and long generations times (10-25 years) seen in dogfish; even if most displayed site fidelity, a few migrating individuals could effect the low genetic differentiation we detect within the northeast Pacific. However, such low exchange among essentially self-recruiting populations is not sufficient to compensate for localized fishing pressure via immigration, and for management purposes such populations have to be considered independent units even in the absence of identifiable genetic characteristics (Carvalho, Hauser, 1994). Although there is thus good evidence for no genetic differentiation in the northeast Pacific, there still may be relatively isolated populations that would react independently to demographic perturbation and are thus relevant to management (Bentzen, 1998).

The classification of these independent population units is uncertain given the lack of genetic differentiation; however, dogfish populations in the northeast Pacific could be considered 'harvest stocks,' defined as "locally accessible fish resources in which fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource" (Gauldie, 1988). Support for such harvest stocks in the northeast Pacific stems from differences in growth and reproductive characters along a latitudinal gradient from California to Alaska (Vega, 2006), which could indicate the presence of self-recruiting dogfish populations along the western coast of the U.S. Moreover, the large-scale tagging effort of 71,000 individuals by McFarlane and King (2003), perhaps the most comprehensive study of dogfish migration in the north Pacific, provided evidence that discrete dogfish stocks occur in coastal waters of the U.S. and Canada, and that inshore waters harbor populations which receive little replenishment from offshore stocks. Further research, using a several independent approaches (Cadrin, 2005) is required to address this question.

4.2. Demographic parameters

4.2.1. Comparison of growth rates

Regressions of the Ketchen (1975) equation for wear point correction were created based on data for unworn spines. There were insufficient unworn spines from the Monterey and Conception regions to make an assessment; the curve obtained from the Oregon data was used for these regions. For the Washington data, the resulting curve was:

$$Age = 1.1761 * X^{1.9454}$$

For the Oregon data, the resulting curve was

$$Age = 0.8402 * X^{2.186}$$

Fits of the versatile growth equation (VGM) of Schnute (1981) and the traditional VBGF were calculated for each region (Figure 4). Bootstrap distributions for parameter estimates were created using 3000 resamples. For all regressions, residuals appeared homoscedastic and approximately normally distributed.

A set of hypothesis tests based on the LRT (Cerrato 1990) (# tests=14) were made between pairs of coastal regions (Washington, Oregon, Monterey, and Conception) and between coastal regions and inshore regions (Table VIII). The null hypothesis of no difference could not

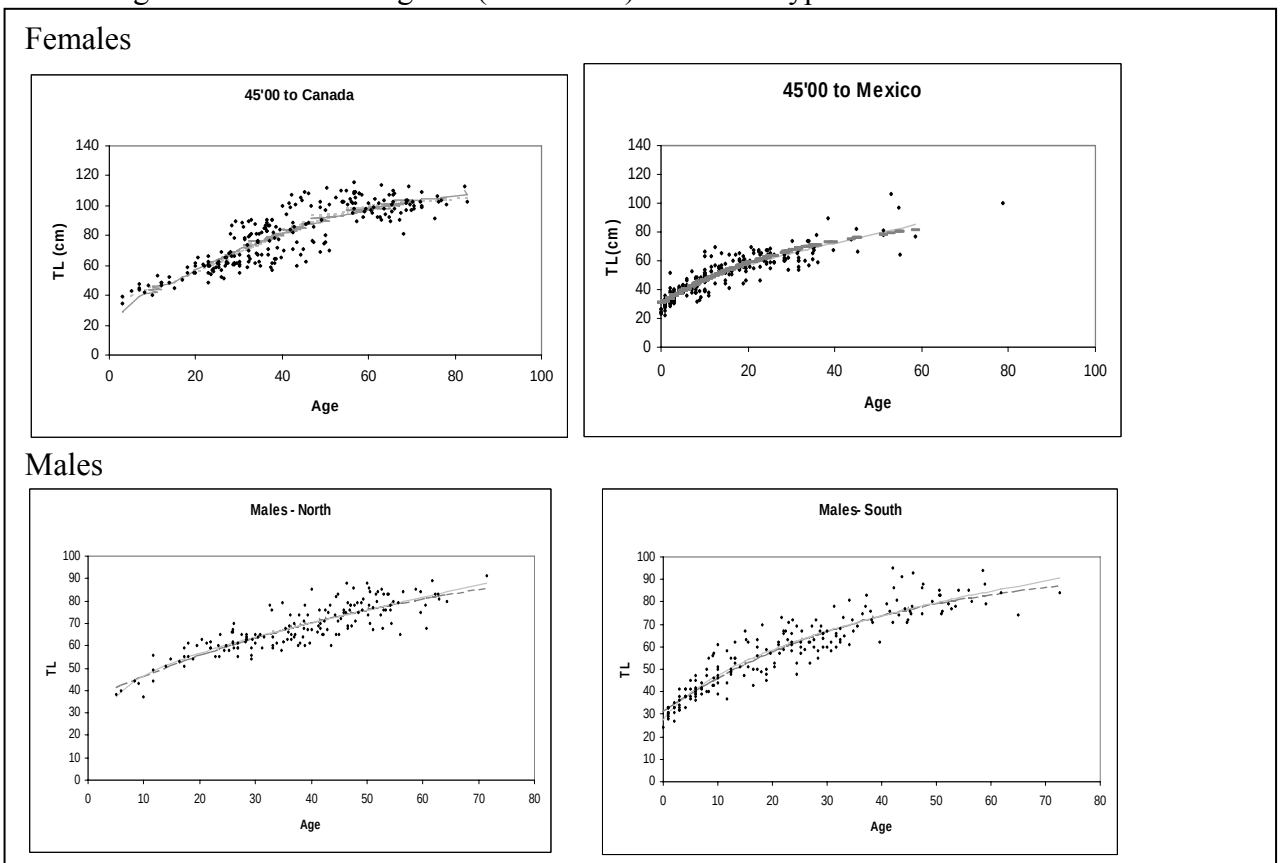


Figure 4: Growth curves of females and males in the northern (left) and southern (right) region of the study area.

Table VIII. Results of tests based on null hypothesis of no difference in growth between regions. Cells give p-values associated with test statistic. Bolded cells indicate paired tests where H_0 was not rejected.

# tests = 14						
	WA	Oregon	Monterey	Conception	NPS	SPS
WA	NA	<0.0001	<0.0001	<0.0001	**0.109**	**0.106**
Oregon		NA	**0.012**	**0.177**	<0.0001	**0.066086**
Monterey			NA	**0.006**	<0.0001	<0.0001
Conception				NA	<0.0001	<0.0001
NPS					NA	**0.093**
SPS						NA

be rejected in any of the pairwise tests between the Oregon, Monterey, and Conception regions; data from these regions were therefore combined for further analysis.

The offshore region between Canada and Mexico was therefore divided into two subregions. The first ("north", 236 females) extended from the Canadian border to 45'00 N and comprises the whole of the "Washington" data set from the initial analyses. The second subregion ("south", 238 females) extended from 45'00 N to the California-Mexico border. A set of hypothesis tests based on the LRT (Cerrato 1990) was made between all pairs of regions (Table IX). The northern and southern regions in this data do appear to show different patterns of growth, as do the southern region and North Puget Sound ($p<0.001$, both tests). Results indicate that the null hypothesis of no difference between regions cannot be rejected between North and South Puget Sound and between offshore Washington and Puget Sound. However, results from other comparisons with the South Puget Sound data set and analyses of data quality for this region indicate that comparisons based on female growth data from South Puget Sound may be of low power due to high variability within the data and low number of data points.

Table IX. Results of tests based on null hypothesis of no difference in growth between regions. Cells give p-values associated with test statistic. Bolded cells indicate paired tests where H_0 was not rejected.

# tests = 6				
	North	South	NPS	SPS
North	NA	<0.0001	**0.109**	**0.106**
South		NA	<0.0001	<0.0001
NPS			NA	**0.093**
SPS				NA

4.2.2. Life History analysis and matrix population models for north-east Pacific dogfish

Results of the augmented matrices incorporating harvest into the population model indicated that the degree of fishing mortality that can be sustained varies by region (Table X, Figure 5). The North Puget Sound dogfish appears to be unable to sustain much if any harvest; North Puget Sound currently sustains a commercial dogfish harvest and may be overfished (WDFW 2003), and the life history parameters we have obtained for this region's dogfish may reflect the condition of the stock due to overfishing. The "northern" dogfish appears to be able to sustain a fishing mortality of up to 0.02 over all ages in the population (Table X); the effect of fishing mortality in this model is greatly lessened after approximately age 40, as most of the reproductive potential of the average individual female in this model has been realized after the first few years of reproductive activity. The NW Atlantic dogfish are predicted to be able to sustain a high level of harvest as compared with other regions; the rapid growth to breeding size

Table X. Results of harvest matrix analyses, all regions.

	Leslie matrix				Harvest	
	tmax	R0	Generation	lambda	Marketable age	Max F
North	103	2.444	41.085	1.022	21	0.020
South	82	1.466	13.634	1.028	30	0.025
NPS	92	2.330	40.570	1.021	17	0.015
NWA	50	1.498	8.003	1.052	7	0.050

and the low generation time associated with observed life history in this region may indicate an ability of this dogfish stock to respond quickly to perturbation.

4.2.3. Interpretation

Based on comparisons of growth curves, dogfish in waters from British Columbia/Washington State to southern California/Mexico were broken into four demographic units. Dogfish within Puget Sound were divided into North Puget Sound and South Puget Sound due to differences in growth patterns; growth in South Puget Sound is poorly characterized and will require further research. Dogfish in North Puget Sound have a growth pattern closely resembling that observed in the Strait of Georgia and may not be demographically distinguishable from dogfish in this region. The offshore region between Canada and Mexico was divided into two regions, "north" and "south".

It must be noted that these latitudinal divisions are intended to allow analyses of differences in growth rather than to suggest concrete physical boundaries. It is frequently difficult to determine physical boundaries for marine organisms due to the lack of clearly-defined impassable regions and the time-dependent fluxes of the marine environment, and there are enormous difficulties in observation of individual movement in marine organisms. Moreover, it appears unlikely that there is any completely impassable boundary to movement between regions in this case, as small amounts of exchange have been observed in tagging studies (McFarlane and King 2003).

We therefore tentatively propose the existence of two demographic types in ocean waters from Washington State/BC to Southern California/Mexico, northern and southern, which may be distinct from the dogfish in North and South Puget Sound. The boundary between the tentative northern and southern demographic regions is expected to be a leaky boundary off the coast of Oregon or southern Washington State, as data from tagging studies (McFarlane and King 2003) indicates that movement along the coast does occur. It is not presently known whether the northern clade extends to British Columbia and Alaska; further data and analyses are required to extend the range of these conclusions. We propose that these rare long-distance north-south movements are random events rather than indicators of a more universal north-south migratory tendency in the spiny dogfish of the Eastern Pacific.

Data for southern regions (Oregon, Monterey, Conception) were obtained entirely from bottom trawl surveys conducted by NMFS. It is possible that there is a bias in the sizes of dogfish captured in the trawl surveys, as very large animals are rare in this data, even in northern regions where dogfish in excess of one meter are known to occur. It is possible, therefore, that the parameter estimates for growth in the southern dogfish are biased. In the absence of further

information on the size structure of dogfish in this region, it is not possible to say with certainty whether bias is present in the data, or whether this bias may be significant for our results. However, the results of the comparison of growth in males provide support for the conclusions of analyses based on females-only data.

Examination of vital rates and model results indicates a shift in life history strategies along the latitudinal gradient. The model results for the North Puget Sound data and the North data were very similar, though median value model results indicate that the spiny dogfish in ocean waters around Washington State is slightly larger, longer lived, and slower growing than its nearby conspecific inside Puget Sound. These regions are in proximity and experience the same climactic regime, and dogfish in both regions are subject to directed fishing effort. It is unsurprising that dogfish in these regions should exhibit fairly similar phenotypes. Life history theory indicates that organisms may maximize fitness in a variable environment by increasing age at maturity and life span; it is possible that the differences in life history characteristics reflect the differences between the ocean environment and the less variable conditions experienced within the enclosed Sound.

The model for the South data does not resemble the models for the northern dogfish. Our results indicate that the Southern dogfish is comparably small and fast-growing with a probable low age and size at maturity. This life history pattern is more characteristic of the East Coast stock of spiny dogfish than the life history patterns characterized for the dogfish of Washington State and North Puget Sound.

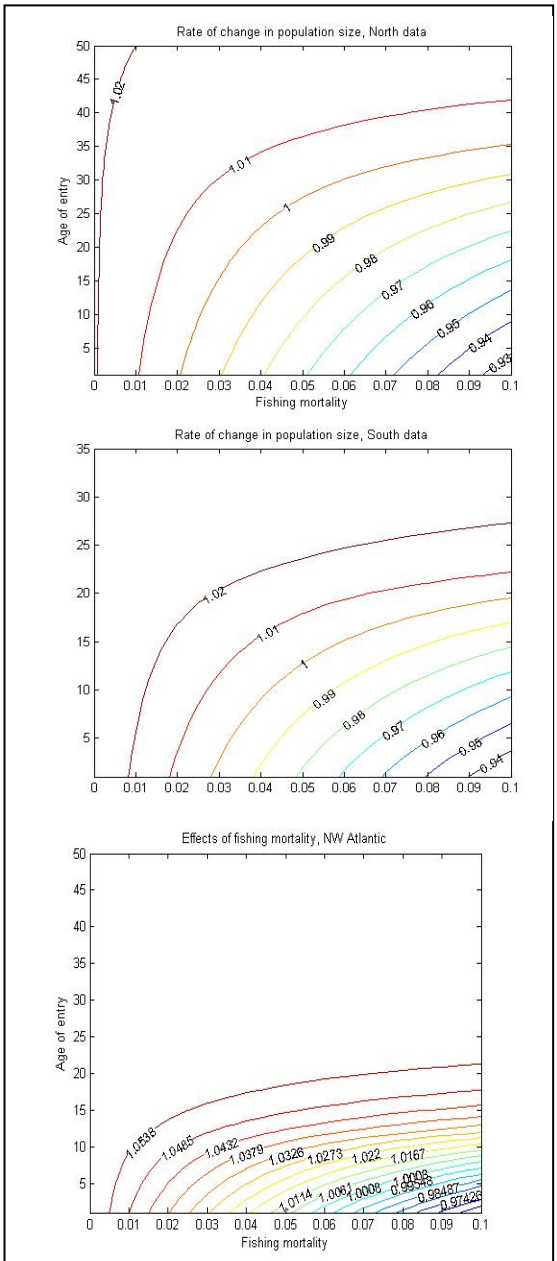


Figure 5: Rate of change in population size in northern (top) southern (middle) and North Atlantic dogfish, depending on age of entry and fishing mortality. These results suggest that Pacific dogfish are more vulnerable to fishing, especially the northern stock.

The predictions of life history theory indicate that units of a species in the north of a naturally occurring northern-hemisphere geographic range should be prone to exhibiting a "risk-averse" life history, where age and size at maturity will be high, allowing an organism to devote multiple years of resources toward somatic development before undertaking the risky and expensive business of reproduction (Castillo 1995). The "south" dogfish, existing in waters nearer to the equator and experiencing less seasonal variation, and the highly migratory "NW

Atlantic" dogfish, would be expected under this theory to grow and mature more quickly than the "north" dogfish, exhibiting a less pronounced *K*-selected life history than their northern conspecifics, which does appear to be the case for these data. Caution is advised in interpreting these results, as data for several regions is incomplete, most field data was collected over only a few years and cannot reflect naturally occurring temporal variability, and none of these models have been validated against abundance data.

Comparison of demographic results with genetic data indicates that the demographic variation observed in this species does not correspond to observable genetic variation at the loci examined. Over the Northeastern pacific latitudinal gradient observed in this study, no differentiation is observable at neutral genetic markers. There are several possible explanations for this result. It is possible that differentiation has occurred at loci that are expressed phenotypically; this would occur as an adaptive response to differing selective pressures and would not be visible in tests using neutral markers. It is also possible that exchange of individuals between regions occurs at a high enough rate to prevent differentiation and that the observed demographic variations are due to phenotypic plasticity - given that the generation time of the North clade, for example, was calculated at approximately 41 years, it would theoretically require a very small number of migrants annually to maintain genetic homogeneity.

The populations defined in this study are statistical and demographic, and these results must be interpreted accordingly. The existence of regions where growth can be observed to occur at different rates can imply that organisms within regions tend to remain within those regions for large portions of their life, growing and developing in response to local conditions. Based on this study, we cannot make any definitive statement about possible levels of gene flow between regions beyond the statement that gene flow appears to be sufficient to prevent differentiation at neutral markers.

4.3. Socioeconomics

The market for dogfish in the recent past, including today, is limited to export markets since the chemical contamination is in excess of that allowable. Mercury concentration in the Washington and in some Atlantic coast state's waters has been above allowable levels (Director, 2001). Other nations accept fish and shellfish with mercury levels as high as one part per million, allowing U.S. export to their markets.

There is a pending CITES action that may influence future commercial trade of the species but under current circumstances there is an active export market. Differing opinions on the status will continue to draw discussion among managers, conservationists, industry, and interestingly, fishers of other commercial or recreational species. In Washington State, it is recommended that five changes/ modifications occur in management. One, is greater attention be given to recreational catch and to by-catch in other fisheries which are usually wasted and are not usually recorded. Two, the fishery should be viewed in an ecosystem context, since it is carried out in environments where rock fish co-occur, thus balanced judgments are needed to balance value, protected status, and costs of recovery. Three, precautionary management is essential given the species' age structure and longevity (Director, 2001). Over fishing for very short time periods can take extra-ordinarily long time periods for recovery. Four, transboundary coordination with British Columbia fishery managers should be increased since the populations undoubtedly move from one side of the border to the other and thus, unequal fishing effort on

one side will affect the population on the other side of the border, and thus influence national market exports. Five, the Strait of Georgia, the Puget Sound, and the ocean fisheries should be managed as separate units. And, finally, six, management should restrict open seasons to the times when female pupping is at minimal levels rather than to times defined by other criteria (Tribuzio, 2004).

The above description is different from the status of the dogfish in British Columbia and in Alaska where the populations are generally more abundant but where the exploitation history is less clear and less available (Bonfil, 1999).

5. SIGNIFICANT PROBLEMS OR NEGATIVE RESULTS

Our study of the genetic population structure of dogfish in the northeast Pacific essentially produced a negative result, that is, no significant differentiation was detected between Alaska and California. This result is important given the high power of our molecular marker set. Nevertheless, in large marine populations, lack of genetic differentiation cannot be equated to a lack of population structure: as outlined above, even the exchange of a few dozen individuals between demographically independent populations (migration rate less than 10%, Hastings, 1993) may result in genetic homogeneity. It is therefore difficult and dangerous to draw any management conclusions from the lack of differentiation reported here.

Our study of the demographic parameters for life history of dogfish in the northeast Pacific was not one hundred percent successful. While potentially discrete stocks were found along the latitudinal gradient from Canada to Mexico. Results indicate differences in growth between dogfish in the northern and southern regions of the latitudinal range and between Puget Sound and outside waters of the open coast. On the other hand, insufficient samples were available from the Bering Sea and thus we were not able to examine the entire latitudinal gradient for growth differences, especially at the northern limit of occurrence. Further, insufficient samples were available to examine the entire world wide range over which genetic analyses were carried out.

6. POTENTIAL ADDITIONAL WORK.

Research on dogfish molecular ecology is still in its infancy and the data available to date have to be viewed as preliminary. There is thus much scope for further molecular genetic research, targeting taxonomic revisions, intraspecific phylogenies, population genetics and mating behavior. However, the real scope of dogfish molecular ecology lies in its use as a model system for gene expression analyses and genome evolution, and thus the availability of extensive EST databases. These databases are not only a tool to overcome limitations to molecular studies caused by low variability in sharks of many molecular markers, but also offer the opportunity to investigate adaptive genetic differentiation in dogfish populations.

For all applications of molecular markers, it is important to integrate molecular data with information on biology, ecology and demography of dogfish as well as pertinent features of the environment. For example, the interpretation of patterns of molecular differentiation in many shark species relies on detailed knowledge on the reproductive behavior, in particular mating and nursery areas and seasonal migrations. Similarly, an investigation of dogfish population structure is much more powerful if genetic and phenotypic data are combined (see Vega *et al.*, this

volume). While there is a plethora of demographic and life-history data on dogfish, much of their biology is still relatively unknown and needs further research.

The integration of information from different biological fields and thus an interdisciplinary approach will likely become even more important once genomic resources are applied to dogfish research. Although dogfish transcriptional assays have been primarily aimed at phylogenetic comparisons with higher vertebrates, environmentally oriented studies could exploit the same resources to investigate responses to osmotic or thermal stress, adaptation to estuarine environments or to stressors at the edge of their distribution (e.g. Bering Sea and equator). Such data would provide valuable insights not only into the limits of the physiological tolerance and thus distribution of dogfish, but also into likely reaction to anthropogenically or naturally changing environments. However, recent research has shown that depending on the nature of environmental stressors different gene or gene groups may be involved in stress response (Podrabsky, Somero, 2004), illustrating the need for detailed environmental and biological information (or experimental manipulation which is difficult in dogfish). Interdisciplinary research on a species with world-wide distribution, relatively high abundance, economic importance, taxonomic interest and ecological adaptability may establish the lowly dogfish as one of the prime marine model species of ecological and evolutionary research.

Demographic analyses can be extended to reach definitive results about prospective harvesting of stocks over the geographic range. At present it is clear that further sampling in the Bering Sea and the Gulf of Alaska would complete the picture of how growth and biomass will increase over the latitudinal gradient. This information would allow the prediction of recovery and sustainable yield values from Alaska to Northern California.

7. EVALUATION

7.1. Attainment of project objectives

Like many other science projects, our effort was more successful than expected in some regards, while falling short of expectations in others. For example, because microsatellite markers were isolated by another research group (who kindly provided these markers) we were able to perform a large scale phylogeographic study investigating genetic differentiation between Atlantic and Pacific dogfish, and demonstrating correspondence between demographic parameters (growth, longevity, fecundity) and genetic differentiation on a broad scale. Similarly, we were able not only to compare growth rates among regions, but also use this information to make prediction on the vulnerability of different stocks to fishing pressure. On the other hand, both demographic and genetic analyses suffered from the difficulty of obtaining samples at the extremes of the dogfish distribution in the north east Pacific (Bering Sea and Mexico). Dogfish from these areas are most likely to show phenotypic and genetic differentiation and are thus most interesting scientifically. However, these areas are also unlikely to contribute significantly to an expanded dogfish fishery and the lack of data from these regions is unlikely to affect our general conclusions.

Specifically, we attained the first objective of our study, the identification of self-recruiting populations of dogfish in the northeast Pacific. While genetic markers failed to reveal any differentiation, demographic data strongly suggested the existence of a gradient in growth along the Pacific coast. Such a gradient shows that dogfish along the coast are not randomly

mixing and therefore should be managed independently, though gene flow may be sufficiently high to prevent genetic differentiation among stocks.

We also achieved our second objective to compare population parameters along a latitudinal gradient. These comparisons showed highly significant growth rate differences among regions. The socioeconomic objectives were largely accomplished to the extent that they could be with the data sources that currently exist. The fact is that data exist where there are fisheries. Thus, while detailed discussions about Puget Sound management can take place based on data, little can be said about management where a fishery has never existed.

In addition, we also addressed several questions which were not part of the original proposal. For examples, we carried out a broad scale phylogeographic study that demonstrated genetic differentiation between Atlantic / South Pacific and north Pacific dogfish. Furthermore, we evaluated the effect of differences in life history characters on sensitivity to fishing pressure. Both these results are of great importance to the management of north east Pacific, because they demonstrate (i) that Atlantic dogfish are sufficiently differentiated from their Pacific counterparts as not be to usable as predictor for reaction to fishing pressure and (ii) that Pacific dogfish are indeed more vulnerable to fishing than Atlantic dogfish, a cautionary result given plans to expand this fishery.

7.2. Dissemination of Project Results

7.2.1. Papers and books

- A conference proceedings from the *First International Symposium on the Management and biology of Dogfish Sharks* organized by Vincent Gallucci is currently being edited.
- A paper on geographic variation in growth parameters of dogfish (Vega *et al.*) has been submitted to the above volume and is accepted pending revisions.
- A review on dogfish genetics (Hauser) in the above volume reviews the current status of research in dogfish genetics and provides an outlook for future research. This paper is also accepted pending revisions.
- We have submitted a manuscript on the mitochondrial DNA and broad scale phylogeography to the *Transactions of the American Fisheries Society* (Franks *et al.*).
- A manuscript presenting the microsatellite data is currently in preparation (Franks *et al.*).
- A manuscript presenting the demographic analysis with estimation of life history rates is currently in preparation but awaiting more data from the Bering Sea (Vega *et al*).

Theses

- Nicole Vega defended her thesis entitled 'Variation in demographic characteristics of the spiny dogfish, *Squalus acanthias,* along a latitudinal gradient in the eastern Pacific," on June 1st, 2006.
- James Franks defended his MS thesis entitled 'Phylogeography and population genetics of spiny dogfish (*Squalus acanthias*)' on Aug 17th, 2006.
- Serra Morrison defended her MS thesis entitled 'A historical analysis of the Pacific Northwest Spiny Dogfish (*Squalus acanthias*) fishery. Its co-management regimes and lessons for future management' in 2004.

Oral presentations

- Hauser *et al.* (2005) *Genetic differentiation and phylogeography of spiny dogfish (Squalus acanthias).* First International Symposium on the Biology and Management of Dogfish Sharks, Seattle, Washington.
- Franks *et al.* (2005): *Phylogeography and genetics of spiny dogfish (Squalus acanthias).* American Fisheries Society Annual Meeting, Anchorage
- Vega *et al.* (2005): *Biogeographic Analysis of the Demography of Spiny Dogfish Over Extreme Latitudinal Variation.* American Fisheries Society Annual Meeting, Anchorage
- Franks *et al.* (2005) Sharks in the Salish Sea: phylogeography of the spiny dogfish (*Squalus acanthias*). Puget Sound Georgia Basin Research Conference, Seattle.
- Franks *et al.* (2004): *A global phylogeography of spiny dogfish shark* (Squalus acanthias). EVO-WIBO meeting, Port Townsend.
- Hauser *et al.* (2004) *Managing a globally distributed species: population genetics and demographic variation in spiny dogfish.* Symposium on spiny dogfish, AAAS meeting Seattle.

Acknowledgements

We are deeply grateful to our colleagues worldwide who went through considerable efforts to obtain samples and data for us. Some of these collaborators are listed in Tables I and II, but others, too numerous to name individually, also helped our sampling efforts. Funding for this study was provided by NOAA, National Marine Fisheries Service, Saltonstall Kennedy Program. Additional graduate student support was provided by SAFS endowment scholarships.

Appendix 1. Summary of microsatellite loci data for all populations

Sample	Param.	*DF U273*	*DF V296*	*DF J451*	*DF T289*	*DF H429*	*DF J445*	*DF U285*	*DF H434*	All loci
AK	*N*	47	46	46	47	47	47	45	45	45-47
	AR	9.940	5.636	4.128	2.426	3.320	6.454	4.855	7.402	11.267
	H_O	0.894	0.500	0.413	0.532	0.298	0.702	0.681	0.822	0.605
	H_E	0.876	0.506	0.462	0.515	0.299	0.694	0.612	0.810	0.597
	F_{IS}	-0.021	0.012	0.108	-0.032	0.004	-0.011	-0.114	-0.016	-0.014
BS	*N*	25	22	20	25	25	24	25	25	20-25
	AR	9.557	6.720	4.000	2.000	3.599	7.499	4.793	6.599	7.238
	H_O	0.920	0.318	0.650	0.480	0.200	0.708	0.680	0.880	0.605
	H_E	0.879	0.434	0.568	0.509	0.223	0.746	0.650	0.789	0.600
	F_{IS}	-0.047	0.272*	-0.148	0.059	0.105	0.052	-0.048	-0.119	-0.008
CO	*N*	62	70	64	60	71	71	70	67	60-71
	AR	9.116	6.434	2.529	2.667	2.955	4.999	5.313	6.563	3.802
	H_O	0.871	0.471	0.359	0.467	0.197	0.732	0.657	0.746	0.563
	H_E	0.855	0.526	0.377	0.501	0.207	0.669	0.660	0.766	0.570
	F_{IS}	-0.019	0.105	0.047	0.068	0.046	-0.095	0.004	0.027	0.013
PS	*N*	87	88	80	86	88	88	74	85	74-88
	AR	8.612	6.204	2.500	2.877	3.522	5.571	4.648	7.762	4.132
	H_O	0.828	0.545	0.488	0.465	0.273	0.693	0.676	0.776	0.593
	H_E	0.859	0.572	0.414	0.518	0.275	0.698	0.653	0.821	0.601
	F_{IS}	0.037	0.046	-0.180	0.102	0.008	0.007	-0.035	0.054	0.014
SG	*N*	47	47	46	46	47	47	47	47	46-47
	AR	9.94	5.636	4.128	2.426	3.32	6.454	4.855	7.402	11.267
	H_O	0.894	0.500	0.413	0.532	0.298	0.702	0.681	0.822	0.605
	H_E	0.876	0.506	0.462	0.515	0.299	0.694	0.612	0.810	0.597
	F_{IS}	-0.021	0.012	0.108	-0.032	0.004	-0.011	-0.114	-0.016	-0.014
WA	*N*	50	52	46	52	52	51	49	51	46-52
	AR	9.502	5.115	2.000	2.385	3.772	6.667	4.290	6.510	6.856
	H_O	0.900	0.404	0.457	0.423	0.288	0.804	0.673	0.745	0.587
	H_E	0.877	0.395	0.400	0.504	0.260	0.739	0.644	0.807	0.578
	F_{IS}	-0.027	-0.022	-0.143	0.162	-0.110	-0.088	-0.046	0.077	-0.015
CH	*N*	43	44	44	43	44	43	44	44	43-44
	AR	13.000	8.955	4.000	8.000	5.977	6.000	5.977	9.999	5.653
	H_O	0.721	0.818	0.705	0.372	0.750	0.488	0.523	0.886	0.658

	H_E	0.831	0.797	0.699	0.464	0.757	0.644	0.644	0.867	0.713
	F_{IS}	0.133*	-0.027	-0.008	0.200	0.009	0.244*	0.190*	-0.022	0.078**
NA	*N*	44	44	51	59	59	57	57	55	44-59
	AR	11.977	8.955	4.000	7.575	6.981	6.436	9.364	13.024	8.322
	H_O	0.818	0.727	0.686	0.339	0.661	0.509	0.702	0.855	0.635
	H_E	0.869	0.756	0.645	0.374	0.781	0.620	0.756	0.862	0.709
	F_{IS}	0.059	0.039	-0.065	0.094	0.154*	0.181*	0.072	0.008	0.065**
Total N Pacific	*N*	318	325	302	316	330	328	310	320	302-330
	AR	9.640	5.790	3.060	2.543	3.402	5.945	4.752	7.068	5.288
	H_O	0.884	0.456	0.463	0.483	0.259	0.724	0.675	0.799	0.593
	H_E	0.870	0.490	0.447	0.510	0.260	0.707	0.639	0.800	0.590
	F_{IS}	0.004	0.041	-0.044	0.097*	0.007	-0.019	-0.047	0.019	0.005
Total S Pacific/ Atlantic	*N*	87	88	95	102	103	100	101	99	87-103
	AR	12.134	8.716	4.000	7.757	6.780	6.585	8.422	11.750	6.869
	H_O	0.770	0.773	0.695	0.356	0.706	0.499	0.612	0.870	0.660
	H_E	0.850	0.777	0.672	0.419	0.769	0.632	0.700	0.864	0.710
	F_{IS}	0.095**	0.005	-0.038	0.144**	0.094*	**0.209****	0.119*	-0.005	**0.071****
Total	*N*	405	413	397	418	433	428	413	419	389-433
	AR	11.267	7.238	3.802	4.132	5.209	6.856	5.653	8.322	n/a
	H_O	0.856	0.536	0.521	0.451	0.371	0.667	0.659	0.817	0.610
	H_E	0.866	0.562	0.503	0.488	0.388	0.688	0.654	0.816	0.620
	F_{IS}	0.022	0.030	-0.041	0.106	0.055*	0.028	-0.005	0.012	0.022*

H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = inbreeding coefficient (heterozygote excess = negative values, heterozygote deficiency = positive values). Asterisks indicate nominal to high significance (*= P<0.05, **=P<0.01); values in bold are significant after Bonferroni correction. Allelic richness (AR) is based on a minimum sample size of 20 individuals. Samples within larger regions (e.g., N Pacific) were pooled to estimate parameters for entire region.

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