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RESEARCH ARTICLE

Cooperative research sheds light on population structure and listing status of threatened and endangered rockfish species

Kelly S. Andrews[1] · Krista M. Nichols[1] · Anna Elz[1] · Nick Tolimieri[1] · Chris J. Harvey[1] · Robert Pacunski[2] · Dayv Lowry[3] · K. Lynne Yamanaka[4] · Daniel M. Tonnes[5]

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Abstract

Population genetics has increasingly become an important tool for determining appropriate taxonomic units for managing species of conservation interest. Yelloweye rockfish (*Sebastes ruberrimus)*, canary rockfish (*S. pinniger*) and bocaccio (*S. paucispinis*) in the inland waterways of Puget Sound (PS), WA, USA were listed under the U.S. Endangered Species Act (ESA) in 2010. These listings relied heavily on evidence from other species that these populations were 'discrete' taxonomic units because little information was available for these species in PS. To fill this data gap, we collaborated with recreational fishing communities in PS to collect tissue samples and used population genetics analyses to determine whether samples from PS were genetically differentiated from samples collected from the outer coasts of the U.S. and Canada. Multiple analyses using restriction-site associated DNA sequencing data showed that yelloweye rockfish in PS and British Columbia, Canada were genetically different from coastal populations, while canary rockfish showed no genetic differentiation. These results support hypotheses that the genetic connectivity of rockfish populations is based on interactions between life-history characteristics and oceanographic conditions. These data also support the ESA designation status and the expansion of protected geographical boundaries for yelloweye rockfish but also suggest canary rockfish in PS are not a 'discrete' population and may not meet the first criterion of the ESA, as initially assumed. Collaboration among agencies and fishing communities, and cost-efficient genetic analyses provided a framework for collecting and analyzing data essential to the conservation and management of threatened and endangered species.

Keywords Endangered Species Act · Population connectivity · Fishing · Local ecological knowledge · Population genetics · RAD-seq

Introduction

One of the greatest challenges in making management decisions related to species or populations of concern is identifying appropriate taxonomic units and their geographical boundaries. Determining whether a specific population is

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✉ Kelly S. Andrews
kelly.andrews@noaa.gov

✉ Krista M. Nichols
krista.nichols@noaa.gov

[1] Conservation Biology Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd East, Seattle, WA 98112, USA

[2] Washington Department of Fish and Wildlife, 16018 Mill Creek Blvd, Mill Creek, WA 98012, USA

[3] Washington Department of Fish and Wildlife, 1111 Washington St SE, Olympia, WA 98501, USA

[4] Fisheries and Oceans Canada, 3190 Hammond Bay Road, Nanaimo, BC V9T 6N7, Canada

[5] Protected Resources Division, NOAA Fisheries West Coast Regional Office, 7600 Sand Point Way, Seattle, WA 98115, USA

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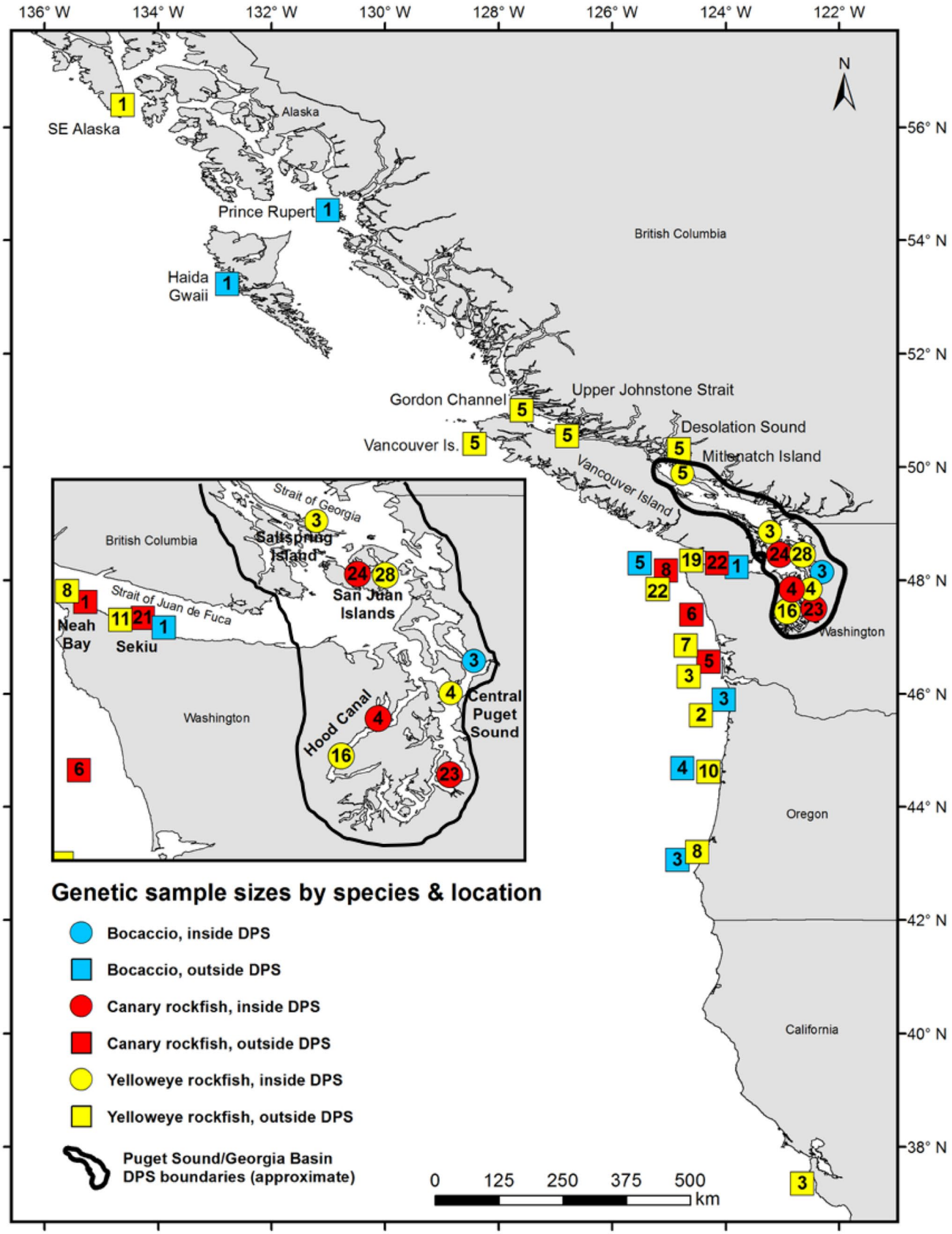
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Mitlenatch Island
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Washington
Oregon
California
Strait of Georgia
Saltspring Island
San Juan Islands
Strait of Juan de Fuca
Neah Bay
Sekiu
Hood Canal
Central Puget Sound
Genetic sample sizes by species & location
Bocaccio, inside DPS
Bocaccio, outside DPS
Canary rockfish, inside DPS
Canary rockfish, outside DPS
Yelloweye rockfish, inside DPS
Yelloweye rockfish, outside DPS
Puget Sound/Georgia Basin DPS boundaries (approximate)
0 125 250 375 500 km

◂**Fig. 1** Map showing the Puget Sound/Georgia Basin distinct population segment (outlined) in waters of Washington State, USA and British Columbia, Canada; general location and number of samples collected and used in final analyses for each species (blue = bocaccio, red = canary rockfish, yellow = yelloweye rockfish) in each region (open circle = inside DPS, open square = outside DPS). Inset shows Puget Sound region in more detail with sample sizes in the Strait of Juan de Fuca

a discrete unit and where its boundaries lie is a common first step in listing a population for protection under various conservation laws, such as the U.S. Endangered Species Act (ESA). It is often the case, however, that identifying discrete management units and ultimately making decisions to list or not to list a species or population under these laws must be done based on the best available evidence, which is often incomplete due to a general lack of data to test hypotheses for rare, threatened or endangered populations (Doremus 1997; Lowell and Kelly 2016).

Several factors can be considered to determine the discreteness of a population, including marked differences in physical, physiological, ecological, behavioral, morphological and/or genetic characteristics between populations of the same species, as well as being delimited by international borders (USFWS-NMFS 1996). Testing hypotheses for differences between populations for most of these factors would require intensive sampling and experimental designs that would be difficult and most likely cost-prohibitive to examine for rare species, and potentially detrimental if sampling effort carries a high risk of being lethal. However, following a number of technological advances, the study of population genetics increasingly has been used as a primary piece of evidence in determining whether a population is a 'discrete' unit (Fallon 2007; Kelly 2010).

The recent ESA listing of three rockfish (*Sebastes* spp.) species in Puget Sound, WA, USA is a case study that illustrates the difficulty of making management decisions for species of great conservation interest in the absence of direct information on population connectivity (Kelly et al. 2017). In general, rockfish in the northeastern Pacific Ocean have been of conservation concern for several decades; they have been subjected to intensive commercial and recreational fishing pressure since the mid-twentieth Century (Love et al. 2002; Levin et al. 2006; Williams et al. 2010), despite exhibiting life history traits that place them at risk to severe local or regional depletion due to overfishing. Such traits include long lifespans, late maturity, episodic recruitment success, and for many species, high site fidelity and small home range sizes (Parker et al. 2000).

The connectivity of most marine populations, including rockfish, is determined through interactions between life history characteristics and oceanographic conditions that facilitate or inhibit adult and larval dispersal (Morgan and Botsford 1998; Love et al. 2002; Palumbi 2003; Cowen et al. 2007). The life history characteristics most relevant to the connectivity of rockfish populations include adult movement (Palumbi 2004; Grüss et al. 2011), timing and depth of larval release (Petersen et al. 2010), larval swimming ability (Leis 2007; Weersing and Toonen 2009), and pelagic larval duration (Sponaugle et al. 2002; Galarza et al. 2009). Studies of population genetics have shown that rockfishes often have population structure over regional scales, due in part to the patchiness of settlement habitat (Johansson et al. 2008) and to oceanographic divisions (Rocha-Olivares and Vetter 1999; Withler et al. 2001; Buonaccorsi et al. 2004; Burford 2009). For example, Cape Mendocino in northern California is associated with a genetic break in blue [*S. mystinus*; Cope (2004)] and yellowtail rockfish [*S. flavidus*; Hess et al. (2011)], while Point Conception in southern California represents a strong break in vermillion rockfish [*S. miniatus*; Hyde and Vetter (2009)].

The transition from open coastal waters to the inland waters of the Puget Sound-Strait of Georgia Basin (PSGB; Fig. 1) represents a potentially strong zoogeographic break. PSGB is a network of inland waters (Fig. 1) that is heavily influenced by the intrusion of deep oceanic waters from the Strait of Juan de Fuca (Alford and MacCready 2014) across several shallow sills that constrict and control circulation dynamics (Masson 2002; Sutherland et al. 2011). In addition, freshwater discharge from numerous rivers in the PSGB watersheds leads to net outflow of surface waters (Masson 2002; Banas et al. 2015). These general circulation patterns vary seasonally and set the stage for complex oceanographic dynamics capable of affecting the dispersal of planktonic organisms, such as fish larvae (Engie and Klinger 2007), and ultimately influencing genetic connectivity and diversity among populations located inside and outside of the PSGB region. This influence is evident in rockfish genetics. For example, copper (*S. caurinus*) and brown rockfish (*S. auriculatus*) from Puget Sound exhibit genetic divergence from populations located along the outer coast (Seeb 1998; Buonaccorsi et al. 2002, 2005). In fact, genetic divergence was found between Puget Sound and coastal populations for every rockfish species that had been studied in this manner to date (e.g. Seeb 1998; Buonaccorsi et al. 2002, 2005).

In 2010, the National Marine Fisheries Service (NMFS) listed yelloweye rockfish (*Sebastes ruberrimus*) and canary rockfish (*S. pinniger*) as 'threatened' and bocaccio (*S. paucispinis*) as 'endangered' in PSGB (Fig. 1) in accordance with the ESA (NMFS 2010). These listings were based on conclusions that the PSGB population of each species was a listable unit under the ESA, and that they faced a moderate-to-high risk of extinction (Drake et al. 2010). The definition of a listable unit under the ESA includes taxonomically identified species and subspecies, as well as distinct population segments (DPS; USFWS-NMFS 1996). The three PSGB rockfish species were designated as DPSs. Two

criteria must be met in order for a vertebrate population to be designated a DPS: it must be 'discrete' from other populations of the same species; and it must be 'significant' to the remainder of the species (USFWS-NMFS 1996). The discreteness conclusion was drawn largely from information from other PSGB rockfish species because at the time of the listing, little to no information had been collected in PSGB specifically to evaluate the discreteness criterion for the three rockfish species of interest (Drake et al. 2010). The most direct evidence was a comparison of allelic variation at nine microsatellite loci that showed subtle differences in genetic structure between yelloweye rockfish populations in waters east and west of Vancouver Island, British Columbia (Yamanaka et al. 2006), which was subsequently verified in a more recent study (Siegle et al. 2013). However, no samples from U.S. waters were included in this analysis to determine whether Puget Sound individuals also showed genetic divergence from coastal individuals.

The primary goal of this study was to fill the gap in genetic information in Puget Sound and determine whether the PSGB region was genetically differentiated from coastal regions for each of the ESA-listed rockfish species, and in the case of genetic differentiation, to better define the possible geographic boundaries separating genetically differentiated populations. We took advantage of the local ecological knowledge of the local fishing and research communities to locate and collect these rare species and then used multiple analytical tools to test whether genetic population differentiation existed in yelloweye rockfish, canary rockfish and bocaccio collections between PSGB and the outer coast. Our study provides a useful template of how management agencies can collaborate with stakeholders to identify and collect information needed to make informed management decisions in an adaptive framework.

Methods

Biological sampling

Because these species occur in very low densities and live in complex, rocky habitats, traditional sampling operations (e.g., bottom trawl surveys) are not capable of collecting enough individuals for adequate analysis and trawling generally results in high mortality rates. Thus, we relied on the collective knowledge of the fishing and research communities to collect tissue samples from these rare species with comparably low direct mortality. We partnered with recreational fishing guides in Puget Sound and the Strait of Juan de Fuca to collect samples. Prior to fishing, we met with local fishing captains, angler clubs, SCUBA divers, scientists and marine managers to collect information to focus our fishing effort. This information included historic locations of catch as reported to port samplers, catches from research and monitoring surveys, sightings from remotely operated vehicles, observations from SCUBA divers, and 20–30-year-old memories from local anglers. This resulted in a map of historical and current "hotspots" of abundance for each species. Identifying sites among the basins of Puget Sound was important because of the potential for regional genetic differentiation. We then spent 76 days between April and October of 2014–2016 fishing at these "hotspots".

We used common bottom hook-and-line fishing methods to capture specimens, including jigging hooks with bait (herring and squid) and artificial lures at depths generally between 30 and 100 m. For each fish collected, we recorded latitude and longitude of the boat, the bottom depth at the time of capture, fork length, weight and sex (if visually distinguishable). A small tissue sample from the caudal fin was collected for each fish and stored in 95% ethanol. For each ESA-listed rockfish, we also attached an external Floy® T-bar anchor tag into the dorsal musculature, in order to prevent duplicate sampling of individuals. After sampling, rockfish were returned to depth using a Seaqualizer® descending device, allowing rockfish to be released at or near their capture depths to prevent mortality of individuals incapable of descending on their own, due to expansion of gases in the swim bladder during capture.

In addition to the samples collected during these cooperative-fishing trips, we obtained fin clips of each species in regions outside of Puget Sound, captured in other monitoring programs conducted by various state and federal agencies in the United States and Canada (Fig. 1; Table 1).

Table 1 Number of samples successfully sequenced from each region and used in subsequent analyses for each species

Region of collection	Yelloweye	Canary
Southeast Alaska	1[d]	0
Inland British Columbia, Can	18[b]	0
Coastal British Columbia, Can	10[b]	0
U.S. West Coast	55[c]	19[c]
Neah Bay, WA	8[a]	1[a]
Sekiu, WA	11[a]	21[a]
San Juan Islands	28[a]	23[a]
Hood Canal	16[a]	4[a]
Central Puget Sound	4[a]	23[a]
South Puget Sound	0	0
Total samples	151	91

[a]Cooperative fishing, this study

[b]Department of Fisheries & Oceans Canada (Yamanaka et al. 2006)

[c]Northwest Fisheries Science Center (Bradburn et al. 2011)

[d]Nichols opportunistic sampling

Preparation, sequencing and analyses of genetic samples

DNA was extracted from fin tissue using the Qiagen DNeasy blood and tissue kit (Qiagen, Valencia, CA) and 500 ng of DNA was used to prepare restriction-site associated DNA sequencing (RAD-seq) libraries, as described by Miller et al. (2007). Briefly, genomic DNA was digested with *SbfI* and a unique barcoded adapter was ligated to the restriction cut site for each individual run within the same sequencing lane. Digested, barcoded DNA from multiple individuals (n = 12–72) was pooled and sheared to an average size of 300–500 bp with a sonicator (QSonica, Newtown, CT). Sheared DNA was used for Illumina sequencing library preparation using the KAPA Hyper Prep library preparation kit (Kapa Biosystems, Inc., Wilmington, MA). Individual libraries were sequenced in a single lane on the Illumina MiSeq or Illumina HiSeq2500, using single end sequencing for 100 bases.

Raw sequence data were visualized for quality using fastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Raw sequencing data were de-multiplexed and processed to produce haplotype genotypes using Stacks v. 1.35 (Catchen et al. 2013), and subsequently filtered as described below. The program process_radtags was used to trim (to 75 bases), quality filter, and de-multiplex each lane of data. For each species, reads from each individual were processed with the program ustacks, with a minimum depth of coverage for individual alleles set at -m 5 for the initial building of the catalog (all other parameters in ustacks were at the default values). Catalogs for identifying SNPs were constructed for each species, using every individual sequenced, with the module cstacks and the number of mismatches allowed between sample tags set to two (-n 2). After building the catalogs for each species, ustacks was used to reconstruct alleles in each individual with minimum depth of coverage of 2 (-m 2), and then using sstacks to align each individual to the species-specific catalog. Finally, the populations module was run with a minimum depth of 7 per site per individual and a minimum global minor allele frequency of 0.05 per site to extract RAD-tag haplotype genotypes for filtering and analysis (--vcf_haplotypes --min_maf -m 7 in populations); this approach produced haplotype genotypes for each RAD-tag using only polymorphic sites within a tag that exceeded MAF 0.05. Using haplotype genotypes, locus and individual genotyping rates were summarized in vcftools [v. 0.1.14; (Danecek et al. 2011)]. For the yelloweye and canary rockfish datasets, any locus missing more than 30% data and individuals missing more than 30% data were removed. Haplotype genotypes were lastly filtered to remove loci not in Hardy Weinberg Equilibrium (HWE); tests for HWE were implemented using Fisher's exact test in the R package pegas [v. 0.9; (Paradis 2010)], and exact test p values were adjusted for multiple testing with a Benjamini Hochberg (Benjamini and Hochberg 1995) false discovery rate (FDR). Any locus with an adjusted p value < 0.05 was removed from further analyses. We were unable to collect enough samples from bocaccio inside the DPS to test our hypotheses; however, we describe the (slightly different) methods used and available data for bocaccio in the Supplemental Material.

The final dataset for each species was evaluated to assess whether related individuals and possible inter-specific hybrids were contained in the dataset. To evaluate whether large proportions of highly related individuals were included in the dataset (and whether related individuals should be pruned for population genetic analyses), genetic relatedness between all pairs of individuals [A_{jk}; (Yang et al. 2010)] was calculated using the R package StAMPP (Pembleton et al. 2013). F_{IS} (also called the inbreeding coefficient) was calculated in vcftools (Danecek et al. 2011); individuals with unusually small F_{IS} are an indicator of highly heterozygous individuals, which could indicate hybridization between species or members of highly differentiated groups, while high F_{IS} is an indicator of inbreeding. Individuals with questionable species identification from the field, or observed as outliers in initial principal components analysis (described below), were independently verified by sequencing the mitochondrial DNA cytochrome b region using primers developed by Rocha-Olivares et al. (1999). The final data were converted from vcf to other formats (e.g. GenePop and STRUCTURE, described below) using the R package stackr (v. 0.2.9.1; https://github.com/thierrygosselin/stackr).

We used three analytical approaches to determine population structure for each species: (1) principal components analysis (PCA), (2) a population genetics-based clustering analysis, and (3) calculation of population differentiation (F_{ST}) among geographic groups. These parallel approaches were used to compare the revealed genetic population structure to the designated DPS boundaries and to other possible boundaries associated with oceanographic or geographic features, and to identify the possible numbers of populations within the samples sequenced (Lamichhaney et al. 2012; Vincent et al. 2013).

First, we used PCA, performed with adegenet (v. 2.0.2; Jombart 2008), to summarize variation across the thousands of RAD-seq loci among all individuals within species. This approach distilled information about the diversity in genetic markers without relying on the assumptions of many population genetics models.

Second, we used a model-based Bayesian genetic clustering analysis, implemented in STRUCTURE v. 2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009), to evaluate the possible number of genetic groups (K = 2–5) within each species. Twenty replicate runs of STRUCTURE were performed for each K value, each run with 100,000 iterations

and a burn-in of 10,000 iterations. STRUCTURE was run without using population as a prior (LOCPRIOR = 0), allowing admixture (NOADMIX = 0), and using the admixture model. The most likely number of populations from all replicate runs across values of K was evaluated from the likelihoods [L(K)] from replicate runs and the method by Evanno et al. (2005), which evaluates the rate of change in the log probability of the data between successive values of K (ΔK). CLUMPAK (Kopelman et al. 2015) and STRUCTURE HARVESTER (Earl and Vonholdt 2012) were used to summarize replicate runs of STRUCTURE across K, and to calculate model statistics.

Finally, we calculated mean pairwise F_{ST} values (Weir and Cockerham 1984), 95% bootstrap confidence intervals, and empirical p values for tests of significant pairwise differentiation using the R package StAMPP (Pembleton et al. 2013). Tests for allele frequency differentiation between regions were also calculated in GenePop (Rousset 2008). For each species showing significant population differentiation, we mapped F_{ST} values among finer-scale collection sites using individuals collected near San Francisco, CA as the reference group.

Results

We successfully sequenced fin clip samples from 151 yelloweye rockfish (inside DPS: n = 48; outside DPS: n = 103), 91 canary rockfish (inside DPS: n = 50; outside DPS: n = 41) and 21 bocaccio (inside DPS: n = 3; outside DPS: n = 18) (Table 1). For each species, some individuals were excluded from the genetic analyses due to insufficient sequence read numbers (Supplementary Material 1). Biological, sequencing, and genetic summary information for each fish are provided in Supplementary Material 1. In all species analyzed, no close genetic relationship was observed in the individuals used for analyses described below (Fig. S1).

Yelloweye rockfish

Yelloweye rockfish samples were collected within the DPS from 22 females, 28 males and 10 individuals of indeterminate sex ranging between 27 and 76 cm fork length indicating that our samples were taken across multiple age classes (Fig. S2a). The length frequency distribution of individuals within the DPS was similar to the length frequency distribution of samples outside the DPS, although individuals < 25 cm total length were missing from the DPS samples. The length-weight relationship for yelloweye rockfish in the DPS was indistinguishable from the length-weight relationship used in the population assessment of yelloweye along the U.S. outer coast [Fig. S3a; Taylor and Wetzel (2011)].

We found strong evidence that yelloweye rockfish collected from the PSGB were genetically different from individuals collected on the outer coast (Figs. 2, 3, 4, 5). The PCA used 7405 loci and showed three distinct clusters of individuals. One cluster consisted entirely of individuals from outside the DPS, caught at coastal sites or in the Strait of Juan de Fuca (Fig. 2a, upper right). A second cluster (Fig. 2a, upper left) consisted of individuals from both inside the DPS (Central Puget Sound, San Juan Islands) and from outside the DPS; most of the outside-DPS individuals in this cluster were from inland marine waters in British Columbia northwest of the DPS boundary (Desolation Sound and Upper Johnstone Strait; see Fig. 1). This cluster also included three individuals from the Strait of Juan de Fuca (Neah Bay and Sekiu), two from the Washington coast, and one from the northwest coast of Vancouver Island. The third cluster consisted almost entirely of individuals from the Hood Canal sub-basin within the DPS (Fig. 2a, lower left). PC1 shows clear genetic differentiation between yelloweye rockfish in inland waters of PSGB and individuals from the outer coast, while PC2 shows clear genetic differentiation between yelloweye rockfish in Hood Canal and the rest of the PSGB DPS. In the yelloweye dataset, there were five individuals with unusually low F_{IS} (and high heterozygosity) in the dataset (Fig. S4a, Supplementary Material 1), and one of these (YI_648 from Hood Canal) was a clear outlier in the PCA plot (Fig. 2a).

The STRUCTURE analysis revealed that two (K = 2) was the most likely number of genetic groups based on mean likelihood of the model and ΔK for yelloweye (Fig. 3a, b); and, the STRUCTURE plot resolves these two groups geographically (Fig. 4). One group consisted of individuals from SE Alaska, Canadian, Washington, Oregon and California coastal sites, along with two sites in the Strait of Juan de Fuca (Neah Bay and Sekiu). The second group consisted of individuals from four inland-waters sites of Canada (Upper Johnstone Strait, Desolation Sound, Mitlenatch Island and Saltspring Island) and sites within Puget Sound (San Juan Islands, Central Puget Sound and Hood Canal). Though the model statistics support K = 2 when all geographic groups are run, when K = 3, Hood Canal individuals show a clearly different pattern of admixture than the other individuals from within the PSGB (Fig. 4). In Fig. 2a, the PC1 axis explained the greatest proportion of variation in the data and differentiated individuals into coastal and inland waters populations. Though STRUCTURE reveals this differentiation when K = 2 with all collections in the analysis, STRUCTURE did not resolve the finer-scale differentiation between Hood Canal and the other inland waters individuals, which were observed along the PC2 axis in the PCA (Fig. 2a). When STRUCTURE was run with only the Hood Canal and other inland waters samples, model statistics support

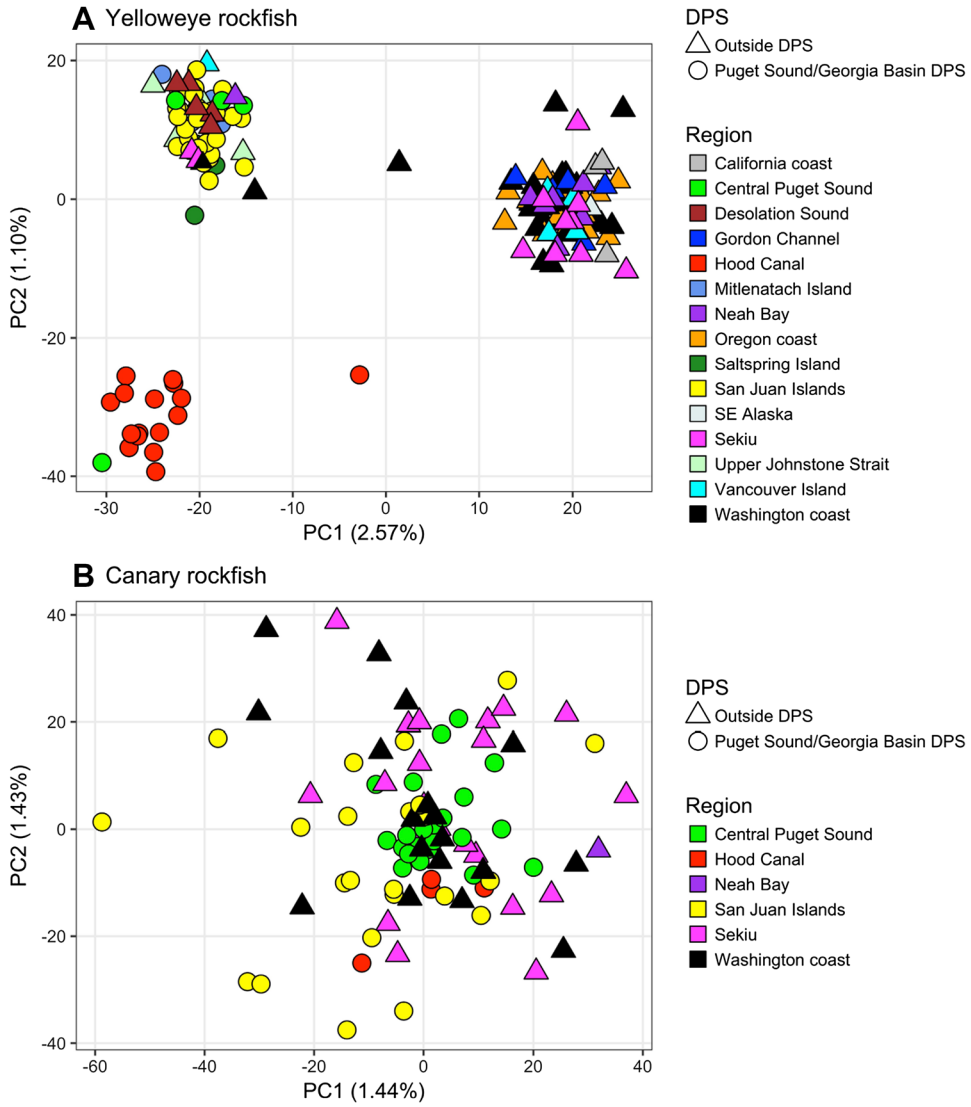

Fig. 2 Principal components analysis reveals **a** three population clusters for yelloweye rockfish and **b** no population structure for canary rockfish across sampled geographic regions. Each symbol represents an individual fish

K = 2 across this subset of sites (Fig. 3c, d) and the STRUCTURE plot differentiates Hood Canal from the other PSGB collections (Fig. S5).

Mean pairwise F_{ST} values showed significant differentiation between individuals collected in inland waters of PSGB compared to individuals collected outside the DPS (Fig. 5; Table 2A). In addition, as observed in the PCA and genetic clustering, F_{ST} values indicated finer-scale structure between yelloweye rockfish in Hood Canal and other inland waters (Table 2A). Pairwise F_{ST} between finer-scale collection sites within regions generally showed an order of magnitude smaller F_{ST} values and non-significant differences between collection sites, further supporting the broader-scale conclusions (Table S1). Tests for allelic frequency differentiation were also highly significant ($p < 0.001$) for each of the three regional comparisons using methods employed by StAMPP and Fisher's method (Table 2A).

Canary rockfish

Canary rockfish samples were collected within DPS waters from 17 females, 11 males and 23 individuals of indeterminate sex ranging between 18 and 47 cm fork length indicating that our samples were taken across multiple age classes (Fig. S2b). The length-frequency distribution within the DPS was similar to the lower end of the distribution of samples outside the DPS, but we were unable to collect larger individuals (> 50 cm) within the DPS. The length-weight relationship for canary rockfish in Puget Sound was indistinguishable from the length-weight relationships used in the most recent population assessment for canary rockfish along the outer coast [Fig. S3b; Thorson and Wetzel (2016)].

We found no evidence that canary rockfish collected within the DPS were genetically distinct from canary rockfish collected outside the DPS. The PCA, using 7397 loci, showed no distinct clustering of individuals (Fig. 2b).

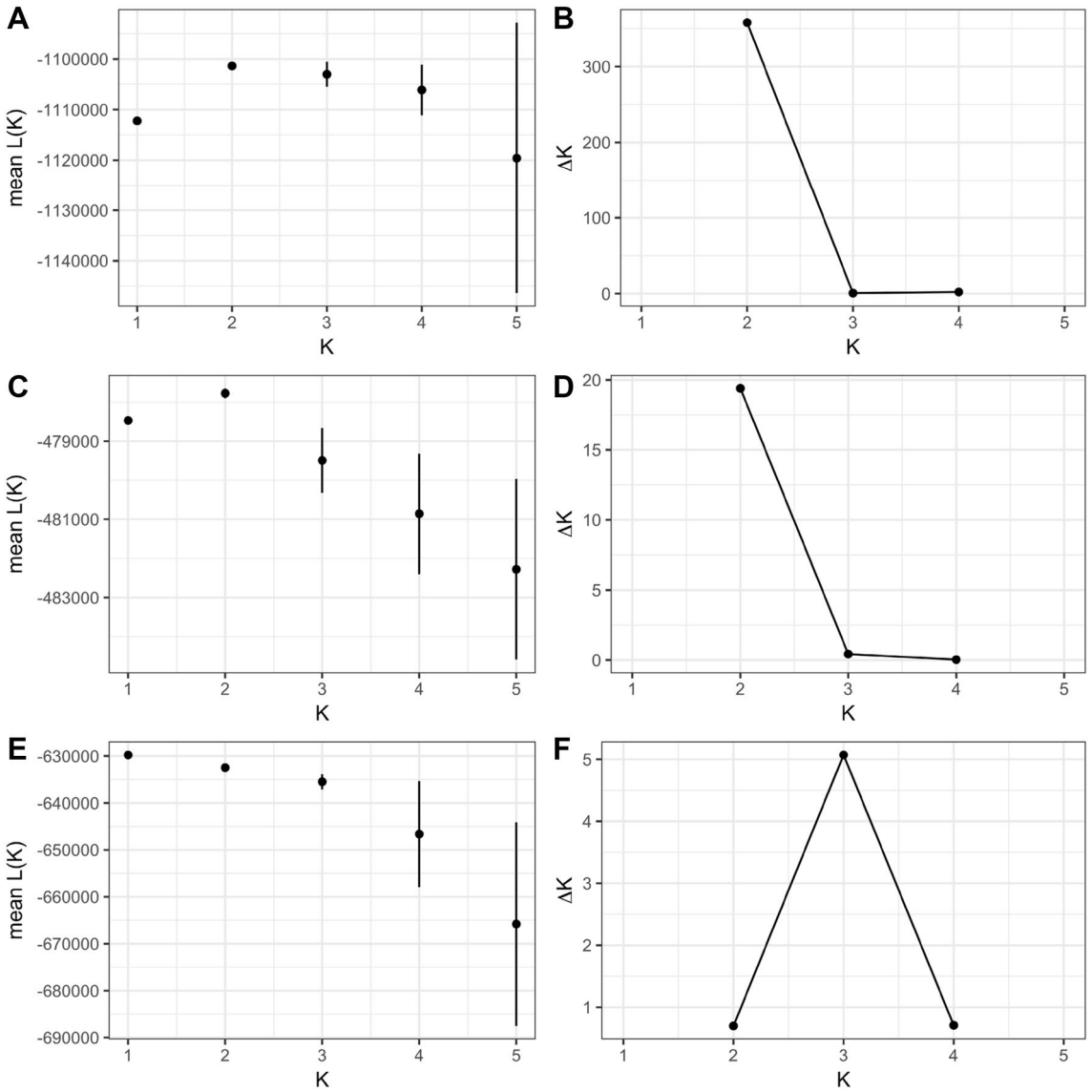

Fig. 3 Mean likelihood (± 1 SD) and ΔK for the replicate runs of STRUCTURE at each K for yelloweye including all collections (**a, b**), yelloweye in the Hood Canal and PSGB only (**c, d**) and for all canary rockfish (**e, f**)

Individuals within each region spanned the range of variation along both PC axes, although San Juan Island individuals showed the greatest variation across both axes. No distinctive outliers were observed in the PCA plot, but two samples (CI_702 and CI_22841_1144014) had very low F_{IS} (Fig. S4b). STRUCTURE showed that genetic variation in canary rockfish was best explained by one population (Fig. 3e, f); the greatest average L(K) was achieved for K = 1, and ΔK was greatest at K = 3, though the Evanno et al. (2005) method is unable to evaluate K = 1. The pairwise F_{ST} values between Hood Canal and Puget Sound collections were both statistically significant from coastal collections using bootstrap estimates (calculated in STAMPP; Table 2B). However, the F_{ST} values were one to two orders of magnitude smaller than values observed in the yelloweye comparisons; and, the confidence intervals overlapped with 0 (Table 2B). Moreover, the test for allelic frequency differentiation among all regional comparisons was not significant (calculated using Fisher's method in GenePop).

Discussion

Through a cooperative sampling effort between Puget Sound's recreational fishing community and state and federal agencies we were able to examine whether two of three ESA-listed rockfish populations in the Puget Sound/Georgia Basin region were genetically differentiated from coastal

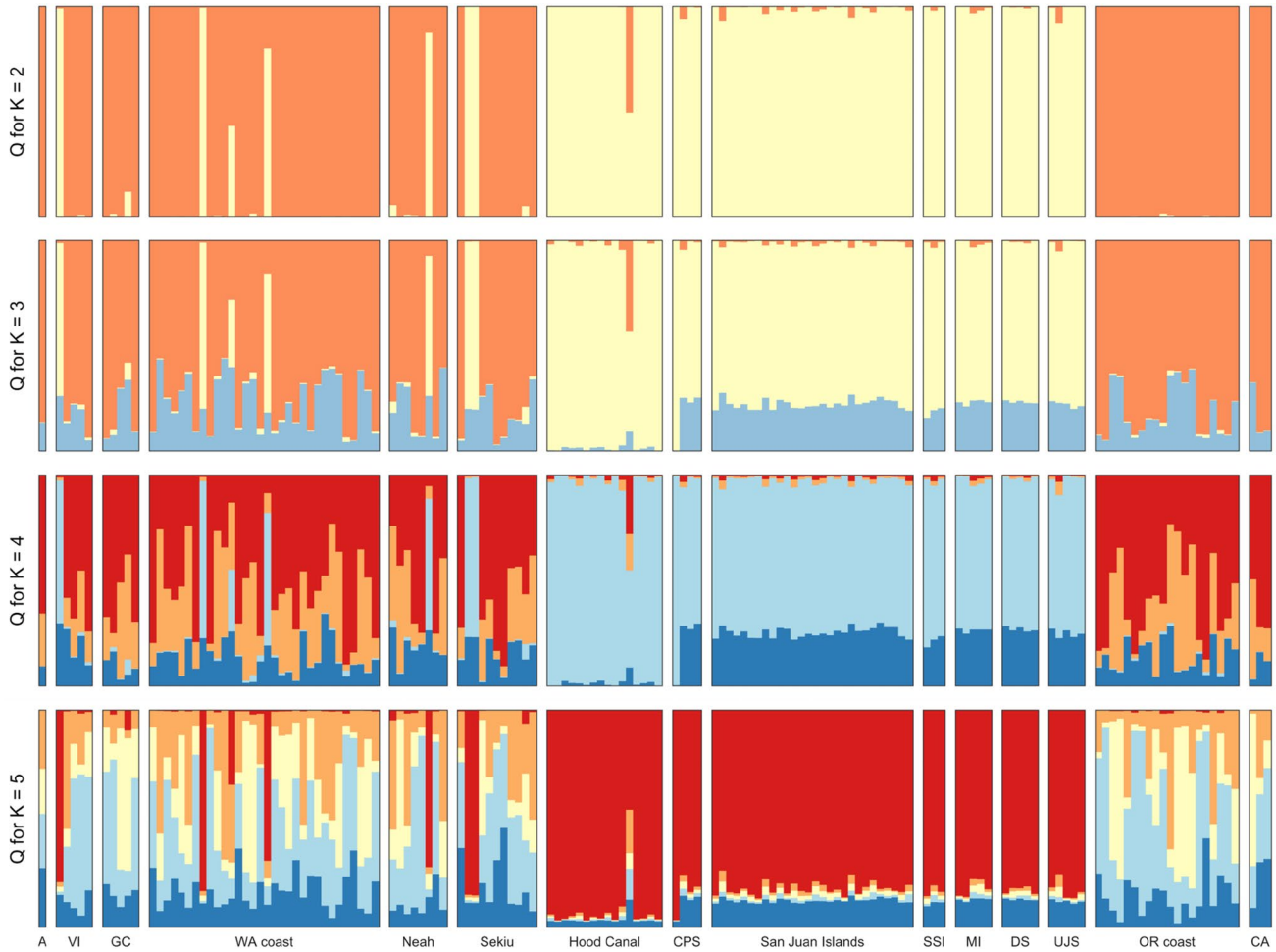

Fig. 4 Assignment of yelloweye rockfish to each region given the number of genetic groups (K = 2–5). Each vertical bar represents an individual, and each color the mean proportion (Q) across replicate STRUCTURE runs for which that individual was assigned to genetic groups. The full dataset was run for 20 replicates at each K value, and 100,000 MCMC iterations per replicate run. Populations are ordered from the north, into Puget Sound and Georgia Basin, and south along the U.S. West Coast: *A* Southeast Alaska, *VI* outer coast of Vancouver Island, *GC* Gordon Channel, *WA* coast outer Washington coast, *Neah* Neah Bay, *Sekiu* Sekiu, *CPS* Central Puget Sound, *SSI* Saltspring Island, *MI* Mitlenatch Island, *DS* Desolation Sound, *UJS* Upper Johnston Strait, *OR* Oregon coast, *CA* California coast

populations. Our results for yelloweye and canary rockfish differed, while we lacked sufficient numbers of samples to answer this question for bocaccio. The preponderance of evidence supported no genetic differentiation between canary rockfish found in Puget Sound as compared to the outer coast, whereas yelloweye rockfish collected in inland marine waters of Puget Sound and British Columbia were genetically differentiated from coastal individuals. The findings for canary rockfish are contrary to the findings for every rockfish species studied in this region to date; all previous studies have shown genetic differentiation between individuals found within the inland waters of the PSGB region and those found on the outer coast (e.g. Seeb 1998; Buonaccorsi et al. 2002, 2005).

The observed differences in genetic differentiation may reflect life history differences between canary and yelloweye rockfish, coupled with the unique geographic and oceanographic characteristics of the PSGB region. Adult canary rockfish have been characterized as transient with wide-ranging spatial movements (Hannah and Rankin 2011) that may cover hundreds of kilometers over the span of multiple years (Lea et al. 1999; Love et al. 2002). In contrast, adult yelloweye rockfish are characterized by low rates of migration (Black et al. 2008) and high site fidelity (Coombs 1978) with little month-to-month variability in horizontal and vertical movements (Hannah and Rankin 2011). Our results are consistent with these characteristics and suggest adult movement is a likely mechanism for population connectivity in canary rockfish and for population differentiation in yelloweye rockfish.

There is no specific information on the timing and depth of larval release, larval swimming ability and the pelagic

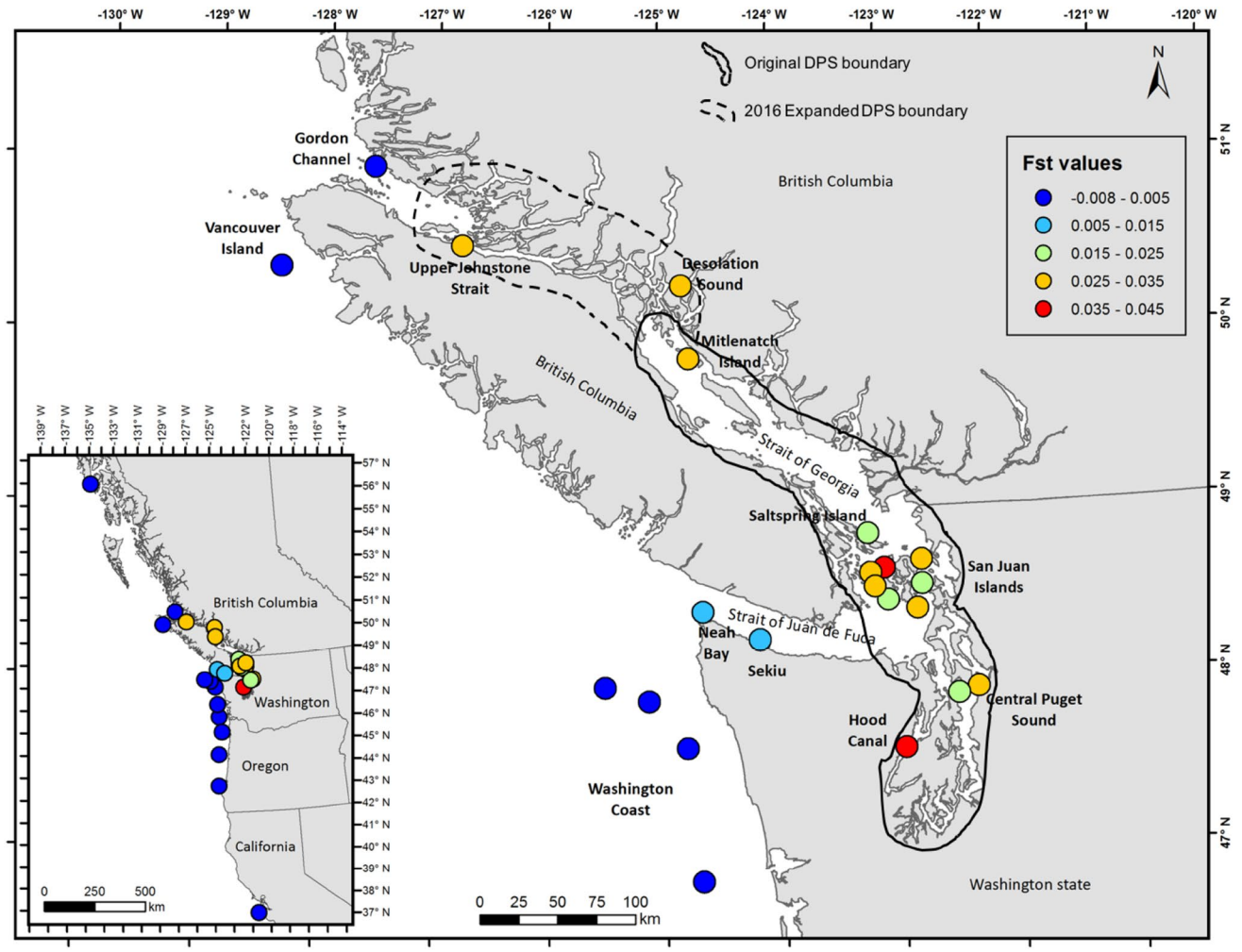

Fig. 5 F_{ST} values for yelloweye rockfish across collection sites (circles) using samples from San Francisco as the reference for comparison. Sampling locations have been lumped into broader collection sites to keep from identifying exact locations of protected species as required by some government agencies

Table 2 Pairwise F_{ST} values (95% bootstrap confidence interval; top line in each cell) and empirical p values (bottom line in each cell) between collection regions for (A) yelloweye and (B) canary rockfish for the test of the hypothesis that pairwise F_{ST} is significantly different from 0 using StAMPP

	Coastal	Hood Canal
(A) Yelloweye		
Hood Canal	0.0276 (0.0264–0.0289) p < 0.001; **	
Other inland waters of PSGB	0.0191 (0.0184–0.0198) p < 0.001; **	0.0128 (0.0120–0.0137) p < 0.001; **
(B) Canary		
Hood Canal	0.00112 (−0.00094–0.00334) p = 0.037; #	
Puget Sound	0.00029 (−0.000003–0.037) p = 0.037; #	0.00069 (−0.00136–0.00284) p = 0.267; #

Results (p values) from additional test for allele frequency differentiation using Fisher's method in GenePop are shown as symbols in bottom line of each cell

#p > 0.05; **p < 0.001 using Fisher's method in GenePop

larval duration for these species in PSGB waters. However, larval dispersal for yelloweye rockfish along the outer coast of British Columbia peaks in the summer months of May and June, while canary rockfish show peak dispersal in the winter months of February and March (Love et al. 2002). If similar temporal patterns occur within PSGB waters, these differences in larval release timing may contribute to differences in population structure between the two species due to seasonal differences in oceanographic processes. Horizontal and vertical volume transport of oceanic and estuarine waters varies seasonally in Puget Sound (Babson et al. 2006). Horizontal advection is greatest in summer and early autumn, while vertical advection is more negative (waters moving from surface to deep) in May/June as compared to relatively no net vertical advection in February/March. More research is needed to understand whether interactions between larval release timing, larval behavior and swimming ability, and oceanographic conditions provide a mechanism for differential larval dispersal that might explain the observed genetic differences for these species in the PSGB region.

Transport mechanisms may also explain the fine-scale genetic differentiation we observed between yelloweye rockfish in Hood Canal and the rest of the PSGB DPS. Hood Canal is a long fjord-like channel within Puget Sound (Fig. 1), with a prominent sill at the northern end that limits the exchange of water. These geological constraints create an environment of low mean transport volumes (Babson et al. 2006) and long water residence times (Sutherland et al. 2011), which likely decreases the potential for larval dispersal into and out of Hood Canal (sensu Engie and Klinger 2007). Physical isolation of the Hood Canal yelloweye population is a likely mechanism for genetic differentiation from other yelloweye rockfish within the DPS. The differentiated loci are primarily associated with the second axis of the PCA (Fig. 2a). Identifying those loci and their functional significance is the subject of further research. In contrast, the canary rockfish that were collected in Hood Canal did not show any differentiation with any other region. Based on these results, we can think of four possible hypotheses for the differences in genetic differentiation observed between the two species: (1) canary rockfish adults move in and out of Hood Canal and we just happened to have captured them while inside Hood Canal, whereas yelloweye rockfish do not move in and out of Hood Canal, (2) canary rockfish adults move or migrate in a way that allows interbreeding to occur across these geological and oceanographic boundaries, whereas yelloweye rockfish adults do not move across boundaries to interbreed, (3) differences in timing of larval dispersal and/or larval behavior between these species creates dispersal pathways into and out of Hood Canal (and the greater PSGB region) for canary rockfish, whereas yelloweye rockfish larval dispersal is constrained, or (4) an interaction between rates of adult movement and the influence of seasonal oceanographic patterns on larval dispersal creates conditions that connect inland and coastal populations of canary rockfish but isolate populations of yelloweye rockfish.

Of all the yelloweye rockfish collected within the PSGB region, none were characterized genetically as coastal fish. However, six individuals collected in coastal waters had PSGB genetic signatures. Three of those individuals were collected from the westernmost portion of the Strait of Juan de Fuca, two from the northern Washington coast and one from the northwest coast of Vancouver Island. From our limited sample size, these results suggest that any connectivity between yelloweye rockfish in PSGB and the outer coast is primarily unidirectional with larvae and/or adults moving from PSGB to the outer coast. If true, this relationship would suggest that a diminished PSGB yelloweye population is unlikely to be replenished by a healthy coastal population. Currently, yelloweye rockfish along the U.S. West Coast are considered overfished and have a 50% probability of being rebuilt by 2067 (Taylor 2011). Similarly, yelloweye rockfish in inland marine waters of British Columbia, Canada have been designated as a species of Special Concern (COSEWIC 2008) as the species was at 12% of unexploited biomass in 2010 (Yamanaka et al. 2012), well below the 40% management target (DFO 2006). Clearly, successful management and conservation of yelloweye rockfish in the inland marine waters of the PSGB should include coordinated international efforts between the United States and Canada.

In addition to questions related to the biology and connectivity of these populations, these data have a direct application to the 2010 ESA listing of yelloweye and canary rockfish. The original listing process carried considerable uncertainty due to a lack of species-specific data (Drake et al. 2010). Specifically, NMFS's Biological Review Team concluded that yelloweye and canary rockfish and bocaccio met the first criterion of the Endangered Species Act of being 'discrete' populations based heavily on the fact that every other rockfish species in the PSGB region that had been examined to date showed genetic differentiation between the PSGB region and outer coastal waters. However, our results suggest that canary rockfish in Puget Sound are not genetically differentiated from canary rockfish on the outer coast and thus, provide one piece of new information that canary rockfish in the PSGB region may not meet the first criterion of the ESA. In contrast, our data support the original designation of a DPS for yelloweye rockfish in Puget Sound and inland marine waters of British Columbia, and further suggest connectivity with and support the extension of the DPS northward into Queen Charlotte Strait (see Fig. 5). The yelloweye rockfish results also suggest that Hood Canal individuals are differentiated from other individuals in the PSGB.

These results provide a better understanding of the geographic boundaries for the yelloweye rockfish DPS, which will increase confidence in the spatial framework for recovery efforts and the design of scientific research (NMFS 2016). In addition, the ESA listings have had significant implications for management of recreational fishing in Puget Sound. To reduce mortality and bycatch of the ESA-listed species, the Washington Department of Fish & Wildlife (WDFW) prohibited fishing for, retaining or possessing any rockfish species and prohibited all "bottomfish" fishing deeper than 36.6 m within DPS waters (WDFW 2015). Increased confidence in the geographical boundaries for each species' DPS should also help ensure that geographically based regulations do not put undo constraints on human activities.

Answering questions related to the status and conservation of threatened and endangered species is generally difficult due to the limited number of samples available from rare species (e.g. Tear et al. 1995; Hernandez et al. 2006). Our sample sizes for yelloweye and canary rockfish within the boundaries of the PSGB DPSs (56 and 51 individuals used in final analyses, respectively) exceeded our expectations, but were still relatively low compared to other population genetics studies (Gharrett et al. 2007; Jasonowicz et al. 2016). However, with the advances in genomic analyses (Miller et al. 2007), we were able to genotype > 7000 loci for each species. This methodology is especially important to the research of species of concern because it allows for detailed examination across the genome of each individual and considerably increases the power of the analyses to detect population structure among geographic regions as compared to traditional analyses which have generally focused on many fewer loci. Using a large number of loci is not a solution on its own to the potential problems associated with small sample sizes (e.g. representative sample, level of uncertainty), which was apparent in our inability to make any conclusions for bocaccio.

This research has highlighted the importance of interactions between life history characteristics, timing of reproduction, and oceanographic processes in determining the connectivity and conservation of populations at multiple spatial scales. In addition, this research has filled an important data gap that was present during the original ESA listing of these species. Working cooperatively with the local recreational fishing community enabled the collection of genetic data from threatened and endangered species and provided a strong foundation for objective, transparent, credible scientific research to be performed within the context of broader societal participation (Johnson and van Densen 2007). This framework has led to subsequent collaboration and research projects with the recreational fishing community to address timely questions relevant to the recovery of rockfish populations in Puget Sound.

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