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Replicate divergence between and within sounds in a marine fish: the copper rockfish (*Sebastes caurinus*)

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Abstract

Understanding the factors that influence larval dispersal and connectivity among marine populations is critical to the conservation and sustainable management of marine resources. We assessed genetic subdivision among ten populations of copper rockfish (*Sebastes caurinus*) representing paired samples of outer coast and the heads of inlets in five replicate sounds on the west coast of Vancouver Island, British Columbia, using 17 microsatellite DNA loci. Overall, subdivision (F_{ST}) was low ($F_{ST} = 0.031$, $P < 0.001$), but consistently higher between paired coast and head of inlet sites (mean $F_{ST} = 0.047$, $P < 0.001$) compared to among the five coast sites (mean $F_{ST} = -0.001$, $P > 0.5$) or among the five head of inlet sites (mean $F_{ST} = 0.026$, $P < 0.001$). Heterozygosity, allelic richness and estimates of effective population size were also lower in head of inlet sites than in coast sites. Bayesian analysis identified two genetic groups across all samples, a single genetic group among only coast samples, two genetic groups among head of inlet samples and two genetic groups within each sound analysed separately. Head of inlet copper rockfish tended to be shorter with lower condition factors and grew more slowly than coast sites fish. Reduced physical connectivity and selection against immigrants in contrasting outer coast–head of inlet environments likely contribute to the evolution of population structure of copper rockfish. Based on genetic connectivity, coast sites appear to be better served by existing marine protected areas than are head of inlet sites.

Keywords: connectivity, dispersal, marine protected areas, population structure, Scorpaenidae, seascape genetics

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Introduction

The study of landscape genetics focuses on the role of habitat features (topography, distance, environmental gradients) in structuring patterns of genetic variation within and among localities (e.g. Manel *et al.* 2003; Holderegger & Wagner 2010). While the same processes (drift, differential selection and barriers to migration) influence genetic variation in all habitats,

the study of seascape genetics has lagged behind genetic studies in the terrestrial realm (Selkoe *et al.* 2008; Manel & Holderegger 2013). From the limited studies in seascape genetics, it is evident that in addition to history (e.g. McCusker & Bentzen 2010) and natural selection (e.g. Nielsen *et al.* 2009), contemporary features of the seascape such as coastline shape, bathymetry and circulation can heavily influence the structure of natural populations (e.g. Banks *et al.* 2007). Such factors, however, need not act independently. For instance, physical features of coastlines or island chains can create physical retention zones that

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influence connectivity and also, through their influence on water temperature and salinity, help to create environmental gradients that generate distinct selective regimes that may influence population structure (e.g. Johanessonn & André 2006; Schmidt *et al.* 2008).

The scorpionfishes or rockfishes (Pisces: Scorpaenidae) are a group of teleost fishes with about 418 species, most of which are found in the Indian Ocean and North Pacific Ocean (Nelson 2006). The genus *Sebastes* consists of about 107 species, all but six of which are part of a species flock endemic to the North Pacific Ocean (Johns & Avise 1998; Hyde & Vetter 2007). *Sebastes* range from the intertidal zone to several thousands of metres in depth, are generally long-lived and slow to mature (some may exceed 200 years of age) and are viviparous (i.e. live-bearing). Juveniles settle in rocky areas after a variable period of larval drift (generally one to a few months, Love *et al.* 2002). The copper rockfish (*S. caurinus*) is found in the eastern North Pacific Ocean and ranges from the Gulf of Alaska south to the Baja Peninsula where it is a common member of the inshore rockfish assemblage from shallow subtidal areas to depths of about 180 m. The inshore distribution of the copper rockfish, its documented site fidelity, apparent preferences for areas of complex rocky substrate coupled with its viviparous mode of reproduction and relatively short larval duration (two-three months) may predispose it to development of substantial population subdivision due to limited gene flow. In fact, Buonaccorsi *et al.* (2002) examined microsatellite DNA variation at six loci across six localities and documented significant population subdivision, particularly between the semi-enclosed waters of Puget Sound, Washington, and coastal samples from British Columbia (BC), Oregon and California, where F_{ST} was about ten times higher than among all coastal samples. Similarly, a major genetic distinction at microsatellite loci occurred between samples taken from the semi-enclosed waters of the Strait of Georgia and outside waters (west coast of Vancouver Island) in the yelloweye rockfish (*S. ruberrimus*, Siegle *et al.* 2013). Taken together, these and related studies suggest that coastline complexity and the potential for local retention zones to form owing to the interaction between topography, circulation and life history may be a major factor driving population subdivision in nearshore marine fishes and in rockfishes in particular (e.g. see also Burford *et al.* 2011; Hess *et al.* 2011). If this is true, then marine fish population structure may be predictable based on geographical features that influence dispersal. In addition, understanding how the seascape influences connectivity, dispersal, and population structure and demography has implications for the design of marine protected

areas (and, in particular, Rockfish Conservation Areas in Canada, DFO 2007; Segelbacher *et al.* 2010).

One of the prominent features of the coast of BC, unlike most other areas of the range of copper rockfish, is the abundance of deeply incised inlets or fjords, of which about 79 have been documented (Thomson 1981). These inlets, especially on the west coast of Vancouver Island in southwestern BC, have a characteristic, deep U-shaped basin, with a glacially derived mud bottom. There is usually a river at the head of the inlet and an underwater ridge, or sill, at the mouth (Thomson 1981). The mouths of such inlets empty onto the continental shelf where there is typically a seasonally variable longshore current (Thomson 1981). Together, the shelf area and inlet constitute a series of 'sounds' along the west coast of Vancouver Island. The topography of the inlets creates unique circulation and environmental gradients from the head to the mouth. For instance, freshwater flows from rivers help to create a surface outflow and a low to high surface salinity gradient from head to mouth (Thomson 1981). A deeper inflow of high-salinity water typically occurs across the sill up towards the head of the inlet. These circulation patterns, coupled with the partial depth constriction created by the sills, and the longitudinal environmental gradients within the inlets, may have a large influence on the extent of genetic exchange between populations of marine organisms whose distributions encompass both the inlets themselves and the adjacent continental shelf. In addition, differential selection pressures at the head and the mouth of inlets may present the possibility for local adaptation to also promote genetic isolation.

In this study, we conducted a test of the idea that the inlet—outer coast seascape transition influences rockfish population structure by sampling copper rockfish from paired head of inlet and outer coast sites in five replicate sounds on the west coast of Vancouver Island, BC (Fig. 1). Owing to the major physical and environmental differences between head of inlet sites and their adjacent outer coast (continental shelf) areas, we predicted that genetic differentiation would be most pronounced between these areas, followed by differences among samples from the heads of different inlets. Further, given the pronounced longshore currents that occur along the continental shelf, we expected that fish sampled from adjacent outer coast areas would be much less genetically differentiated from one another. We also measured length, weight and age of fish to assess growth rates in copper rockfish in different habitats. Differences in growth may reflect differences in the selective environments of the outer coast and the heads of inlets, or among sounds, which could also promote population differentiation.

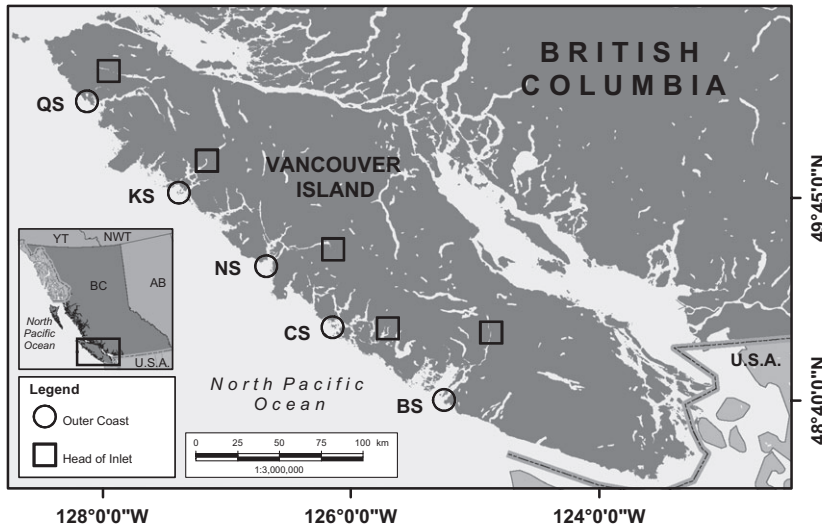


Fig. 1 Copper rockfish (*Sebastes caurinus*) collection locations along the west coast of Vancouver Island, British Columbia (BC), Canada. BS, Barkley Sound; CS, Clayoquot Sound; NS, Nootka Sound; KS, Kyuquot Sound; QS, Quatsino Sound. Inset shows position of Vancouver Island (boxed area) within British Columbia.

Materials and methods

Samples

Copper rockfish were collected from five regions on the west coast of Vancouver Island: Barkley Sound, Clayoquot Sound, Nootka Sound, Kyuquot Sound and Quatsino Sound (Fig. 1). Within each region, two sites were selected for sampling: a 'head of inlet' site at the terminal end of the longest inland inlet and a 'coast' site on the exposed coastline, immediately adjacent to the opening of the sound. Sampling locations were paired in this fashion to allow for comparisons between proximate head of inlet and coast collections, which were separated from each other by an average of 48.0 km (range = 29–70 km). The average distance between adjacent coast sites was 70.5 km (range = 63–84 km), and between adjacent head of inlet sites was 129.5 km (range = 119–140 km). All sites were named using the first letters of the sound (e.g. B, C, N, K, Q) and location (e.g. HI, C). For example, the Barkley Sound head of inlet site was named BSHI.

Six hundred and five copper rockfish were collected between June 1 and November 18, 2009 (Table 1). Two localities [Clayoquot Sound head of inlet (CSHI), Kyuquot Sound coast (KSC)] were also sampled in 2010. All fish were caught by hook and line from a small rigid-hull inflatable boat. Fishing effort occurred over relatively small geographical areas, typically on the order of 0.5–1 km². Due to low catch per unit effort at the Barkley Sound head of inlet site, the sampling area was broadened to approximately 2 km². All fishing took place at depths between 8 and 20 m over rocky-bottom habitats.

Copper rockfish were landed, euthanized immediately and placed in a cooler with ice for processing. All

other species were released immediately. At the end of each day of fishing, all copper rockfish were measured (total length) to the nearest millimetre and weighed to the nearest centigram. Fin clips were taken from the anal fins, washed in deionized water and placed in 1.5-mL vials with 95% ethanol.

Otolith analysis

Otoliths, small calcareous structures within the inner ear of fishes and used in maintaining equilibrium and sound reception, were removed and stored dry. Subsequently, the largest (sagittal) otoliths were cleaned with deionized water and prepared for ageing by counting annular growth rings. As the otolith grows in size, it accretes layers of calcium carbonate, the rate of which varies with the growth rate of the fish. Otoliths, therefore, contain a record of an individual fish's growth (Weatherly *et al.* 1987). In fishes such as copper rockfish that live in seasonally variable thermal environments (colder in winter, warmer in summer), 'tree-ring'-like growth increments are formed in the otoliths that reflect this seasonality. To count these rings and estimate the age of copper rockfish, we used the otolith burnt section technique (MacLellan 1997; Andrews *et al.* 2007). Briefly, the otoliths were snapped approximately in half along the short axis, and the exposed surface gently burned to a uniform light brown colour. At this point, the annuli become dark brown and are easily distinguished from the surrounding matrix, which retains a lighter colour. The number of annual rings was counted using digital images obtained using a JVC 3-CCD camera mounted on an inverted transmission light microscope and the digital imagery software Auto-Montage Pro (version 5.03, Syncroscopy, Beacon House,

Table 1 Locations and sample sizes for collections of copper rockfish (*Sebastes caurinus*) collections from the west coast of Vancouver Island, British Columbia

Region	Location	Abbreviation	Latitude	Longitude	N
Barkley Sound	Head of inlet	BSHI	49°11'26" N	124°49'24" W	30
	Coast	BSC	48°47'35" N	125°13'00" W	51
Clayoquot Sound	Head of inlet	CSHI	49°13'32" N	125°35'55" W	105
	Coast	CSC	49°07'29" N	125°58'05" W	60
Nootka Sound	Head of inlet	NSHI	49°38'03" N	126°04'30" W	49
	Coast	NSC	49°34'45" N	126°40'15" W	52
Kyuquot Sound	Head of inlet	KSHI	50°11'00" N	127°18'05" W	53
	Coast	KSC	49°59'20" N	127°25'11" W	100
Quatsino Sound	Head of inlet	QSHI	50°38'02" N	127°56'54" W	52
	Coast	QSC	50°27'04" N	128°03'45" W	53

Cambridge, UK) after applying a drop of mineral oil to the burnt surface to increase clarity. In age estimation of fishes, it is important to validate that annual rings actually represent approximately 1 year of growth (Beamish & MacFarlane 1983). Although age validation has not to our knowledge been completed for copper rockfish, the burnt section technique has been validated for several closely related *Sebastes* in the North Pacific (e.g. see Kerr *et al.* 2005; Andrews *et al.* 2007). Furthermore, the age-length relationships of our samples (see below) were consistent with that reported for copper rockfish collected in Puget Sound, suggesting that our ageing techniques are valid (see Washington *et al.* 1978).

Microsatellite assays

Total genomic DNA was extracted from fin clip tissue using a standard phenol–chloroform extraction procedure. The polymerase chain reaction (PCR) was used to amplify seventeen microsatellite loci developed for *Sebastes maliger*, *S. pinniger* and *S. rastelliger*: *Sma2*, *Sma3*, *Sma4*, *Sma5*, *Sma10* and *Sma11* (Wimberger *et al.* 1999); *Spi4*, *Spi6*, *Spi10*, *Spi12* and *Spi18* (Gomez-Uchida *et al.* 2003); and *Sra5-9*, *Sra7-7*, *Sra7-25*, *Sra11-103*, *Sra15-8* and *Sra16-5* (Westerman *et al.* 2005). All loci have been previously tested on *S. caurinus* and have been shown to be polymorphic (Wimberger *et al.* 1999; Gomez-Uchida *et al.* 2003; Westerman *et al.* 2005).

Forward PCR primers were fluorescently labelled and amplified in multiplex reactions using the Qiagen™ Multiplex PCR Kit following the manufacturer's protocols, with approximately 150 ng template DNA in 15 µL total volume. Typical cycling conditions included an initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 56 °C for 30 s and 72 °C for 1 min. Final extension was carried out at 72 °C for 10 min.

The PCR products were analysed using a CEQ™ 8000 Genetic Analysis System (Beckman Coulter™). Internal

DNA size standards were included in each well, and fragments were sized using CEQ™ 8000 software (Beckman Coulter™).

Statistical analyses

Condition factor (CF) was determined for each fish as $100[\text{weight}/\text{length}^3]$ (Ricker 1975). We tested for differences in mean length and CF between sounds and between coast and head of inlet sites within sounds using a two-way analysis of variance with 'Sound' ($N = 5$) and 'Habitat' (coast, head of inlet, $N = 10$) as factors in the analyses. In a few cases, distributions departed from normality and homogeneity of variances was detected using Shapiro–Wilks and F-ratio tests, respectively. Log transformations of the data eliminated these situations, but results (i.e. probability levels) of the ANOVAS were identical to those when using non-transformed data so we report statistical results using the nontransformed data. Size at age was modelled using the von Bertalanffy growth equation (Ricker 1975):

$$L(t) = L_{\infty} * [1 - \exp(-K * (t - t_0))]$$

in which $L(t)$ is total length (cm) at age t (years), L_{∞} is the theoretical asymptotic length or the length at which growth approaches 0 in older fish, K is the growth curvature parameter (i.e. the rate at which a fish reaches L_{∞}) and t_0 is the theoretical estimated time at which a fish has 0 length, which can be used to estimate the length at birth. To test for differences in growth rate between head of inlet and coast collections, the von Bertalanffy growth model was fitted separately to length–age data for inlet fish and coast fish using the iterative method described by Schnute (1981). Resulting growth functions were compared using likelihood ratio tests, as described by Kimura (1980). Tests were implemented in R (R Core Team, 2013).

Genetic diversity within sampling locations was characterized using expected heterozygosity (H_E ; Nei 1987) and allelic richness. Allelic richness was calculated for each locus at the smallest collection size ($2N = 60$), using the rarefaction index approach computed in the program `FSTAT` version 2.9.3 (Goudet 2002) to account for the tendency for larger samples to exhibit more alleles. Differences in expected heterozygosity, allelic richness and F_{ST} between coast and head of inlet sites were tested for statistical significance by permutation analyses in `FSTAT`.

Genetic divergence within and among sampling locations was characterized using Weir and Cockerham's (1984) unbiased F -statistics as implemented in the program `GENEPOP` version 4.2 (Raymond & Rousset 1995; Rousset 2008). Estimates of F_{IS} were calculated for each locus–population combination to measure deviation from conformance to Hardy–Weinberg equilibrium (HWE). Estimates of F_{ST} were calculated for: (i) all sites combined (global F_{ST}); (ii) the five coast sites; (iii) the five head of inlet sites; and (iv) the five head of inlet–coast site combinations (pairwise F_{ST}).

Exact probability tests were used to evaluate conformance to HWE and linkage equilibrium and to assess the homogeneity of spatial distributions of genetic variance. Estimates of exact significance probabilities were obtained using the Markov chain algorithm as implemented in `GENEPOP` (1000 batches and 5000 iterations per batch). Significance probabilities over multiple loci were combined using Fisher's method (Sokal & Rohlf 2012). In cases where loci did not conform to Hardy–Weinberg equilibrium, the program `MICRO-CHECKER` was used to assess the presence of nonamplifying (null) alleles or dropout of large alleles as reasons for departures from equilibrium (Van Oosterhout *et al.* 2004). `MICRO-CHECKER` was also used to assess any potential scoring errors or failure to detect large molecular weight alleles. To assess the assumption of selective neutrality of our microsatellite loci, the data were analysed using the program `LOSITAN` (Antao *et al.* 2008), which implements the F_{ST} outlier approach of `FDIST` (Beaumont & Nichols 1996). We employed 50 000 simulations under the infinite alleles model, 95% confidence intervals, a neutral mean F_{ST} and a false discovery rate of 0.1; tests were conducted for all populations together as well as by sound. We also employed the hierarchical population structure model-based tests for selection using `ARLEQUIN` (version 3.5.1.2, Excoffier *et al.* 2005) with the population structure defined as two groups: coast sites and head of inlet sites.

The genetic clustering program `STRUCTURE` version 2.3 (Pritchard *et al.* 2000) was used to determine the number of distinct genetic clusters (K) among the sampling locations. `STRUCTURE` uses a Bayesian clustering

method to assign individuals to genetic clusters based on their genotypes. An individual may be assigned to more than one cluster if its genotype indicates admixture of two or more genetic groups. A Markov chain Monte Carlo method (MCMC) is used to estimate posterior probability distributions for each possible number of clusters. Simulations in `STRUCTURE` were performed using values of K between 1 and 15 for analyses of all 10 localities, between 1 and 10 for analyses examining all coast sites or all head of inlet sites separately and between 1 and 5 for analysis of each individual sound. Each analysis used parameters that allowed admixture, correlated allele frequencies and did not include a location prior. Each run consisted of a 500 000-step burn-in followed by an additional 1 000 000 steps. Ten iterations were run for each value of K . In cases where the highest likelihood value was for a value of $K > 1$, we used the method of Evanno *et al.* (2005), which calculates the second-order rate of change in the log probability of successive K values (ΔK), to evaluate different solutions for K . We also used factorial correspondence analysis (FCA) as a multivariate measure of population differentiation to visualize samples in 'allelic space' with `GENETIX` (Belkhir *et al.* 2004).

We employed `GENECLASS` (Piry *et al.* 2004) to test for potential migration between populations by calculating the likelihoods of individuals being first-generation migrants in a modification of the basic assignment test procedure (see Hansen *et al.*, 2001). Using variation across loci, the likelihood score was calculated as L_{ij} ; the likelihood of drawing a particular individual's genotype from the population residing in the locality from which it was sampled given the observed allele frequencies. This approach is the most appropriate when not all possible source populations have been sampled (Paetkau *et al.* 2004). To identify putative immigrants to a locality, we used the genotype resampling procedure; an individual fish was rejected as a member of the population from which it was sampled if the observed likelihood of population membership was represented by <1% of 10 000 genotypes simulated randomly from allele frequencies within the sample population (Paetkau *et al.* 2004).

We quantified variation in allele frequencies attributable to each sound (five levels) and location within sound (coast or head of inlet) using the hierarchical approach in the analysis of molecular variance (AMOVA) of `ARLEQUIN`. Given the relatively small spatial scale of study and relatively low levels of genetic divergence, no isolation by distance (IBD), identified as a significant correlation between F_{ST} and geographical distance, was apparent among coast localities (data not shown). Furthermore, given the structural complexity of the head of

inlet localities relative to coast localities, we did not attempt to derive IBD relationships across all locations, as distance would likely be confounded by the geomorphological complexity of the coastline.

We derived estimates of effective population size (N_e) using the linkage disequilibrium (LD) method using LDNe (Waples & Do 2008) because our samples consisted largely of single time points. The LD method operates on the principle that departures from random association between alleles across loci (linkage disequilibrium) will be inversely proportional to N_e . Because our samples consisted of multiple cohorts (generations), the estimates derived are more properly referred to as the effective number of breeders (N_b) rather than N_e *per se*. (see Waples 2005). In addition, to minimize the effect of admixture between coast and head of inlet samples in each sound, we removed individuals that had admixture coefficients >0.20 (i.e. those individuals in one genetic group that STRUCTURE analysis suggested had at least 20% of their genome from the other genetic group). Finally, significance levels for any multiple simultaneous tests were adjusted following Narum (2006).

Results

Microsatellite diversity

Across the 605 individuals assayed, each of the 17 loci was polymorphic. The average number of alleles per locus was 13.1, with a range of 3–36 (Table S1, Supporting information). A total of 223 alleles were detected across all loci and localities. Expected heterozygosity ranged from 0.08–0.94, and the average over all loci was 0.72 (Table S1, Supporting information). MICRO-CHECKER found no evidence of scoring error due to stutter or large allele dropout.

Average expected heterozygosity over all collections was 0.61, with a range of 0.57–0.94 (Table S1, Supporting information). Expected heterozygosity was lower for head of inlet collections (mean (\pm SD) = 0.60 ± 0.01) than coast collections (mean = 0.64 ± 0.01 , $P = 0.025$). Allelic richness calculated at a minimum sample size of $2N = 60$ was also lower for head of inlet collections (mean = 6.8 ± 0.4) than coast collections (mean = 8.0 ± 0.1 , $P = 0.02$). Average allelic richness over all collections was 7.4 (Table S1, Supporting information).

Three single-locus departures from HWE were significant after corrections for multiple tests: the *Spi4* locus in the BSC collection ($F_{IS} = 0.121$, $P = 0.0018$); the *Sma10* locus in the Kyuquot Sound head of inlet (KSHI) collection ($F_{IS} = 0.362$, $P = 0.0004$); and the *Sra16-5* locus in the Quatsino Sound head of inlet (QSHI) collection ($F_{IS} = 0.088$, $P = 0.0029$). When probabilities were

combined over all collections for each locus (Fisher's method), the *Sma10* locus displayed a significant departure from HWE ($F_{IS} = 0.047$, $P = 0.001$). When probabilities were combined over all loci for each collection, there were no significant departures from HWE. The global P -value for all loci in all collections was low, but significant ($F_{IS} = 0.015$, $P = 0.014$).

MICRO-CHECKER results suggested that the three single-locus departures from HWE may be the result of null alleles. Allele frequencies at these loci were adjusted using the Brookfield (1996) null allele estimator 1 model because at least one allele was observed in all samples (i.e. we observed no null-null homozygotes). The results of allele frequency-based analyses (i.e. F_{ST} and significance of F_{ST}) were very similar between the corrected and uncorrected data sets, so all analyses were performed using the uncorrected data set.

Allele frequencies at all loci conformed to linkage equilibrium after corrections for multiple tests. Results from LOSITAN indicated that in only one comparison was selection suggested to act on any of the loci, divergent selection at *Spi18* between the KSC and KSHI samples. Using the hierarchical population model, a single locus, *Sma11*, was identified as a locus potentially under stabilizing selection ($P < 0.006$). As no locus was consistently identified as potentially the target of selection between all pairs of populations or population groups, we retained all loci in subsequent analyses.

Effective population size

The linkage disequilibrium method produced estimates of N_b from about 100 in the BSHI and Nootka Sound head of inlet samples to over 2800 in the Clayoquot Sound coast sample (Table 2). Several localities produced negative estimates of N_b owing to insufficient linkage disequilibrium with the sample. Point estimates of N_b averaged 1,181 (SD = 1,418) for the coast samples and 401 (SD = 526) for in the head of inlet samples. The only coast vs. head of inlet comparison without overlapping confidence intervals, however, was that involving Kyuquot Sound (Table 2).

Population structure

Genetic variance was strongly partitioned among sampling locations. Comparisons between sample years (2009 and 2010) were not significant either for the CSHI sample ($F_{ST} = 0.0023$, $P > 0.10$) or for the KSC locality ($F_{ST} = 0.0018$, $P > 0.15$). Consequently, both years' samples were pooled for each locality. When all ten collections were contrasted, sixteen of the seventeen loci exhibited significant heterogeneity in allele frequencies

(Table S2, Supporting information). The global F_{ST} for all loci in all populations was 0.031 and highly significant ($P < 0.001$; Table S2, Supporting information). When only the coast collections were contrasted, allele frequencies were homogeneous at all loci; overall F_{ST} was small and not significant ($F_{ST} = -0.001$, $P = 0.737$; Table S2, Supporting information). By contrast, among the head of inlet collections, significant heterogeneity in allele frequency was detected at thirteen of the seventeen loci; overall F_{ST} was significant ($F_{ST} = 0.026$, $P < 0.001$; Table S2, Supporting information) and higher than that among the coast collections ($P = 0.026$). The largest values of F_{ST} were found between paired coast and head of inlet collections (Table 3); pairwise F_{ST} values for the five coast–head of inlet comparisons ranged

from 0.042 to 0.054 (mean = 0.047), and all were highly significant (Table 4).

The pronounced differentiation between coast and head of inlet samples was evident in the FCA plot of allelic variation (Fig. S1, Supporting information) as well as in the STRUCTURE analysis. Across all samples, the most likely number of populations was $K = 2$ (Fig. 2; Table S3, Supporting information). The coast and head of inlet samples were dominated by strikingly different proportions of the two genetic groups across individuals with little apparent structure either within the coast group or the head of inlet group (Fig. 2). When the coast group of localities were examined by themselves, the most likely number of populations was $K = 1$. By contrast, when the head of inlet localities were analysed by themselves, $K = 2$ was the most likely populations structure; the CSHI sample was distinct from all others (Table S3, Supporting information). In each of the analyses conducted separately by sound, the most likely number of populations was also $K = 2$; in each sound, head of inlet and coast localities had strikingly distinct frequencies of fish dominated by one genetic group or the other (Fig. 3; Table S3, Supporting information).

Analysis of molecular variance also suggested that the most significant axis of genetic divergence was between populations grouped into head of inlet and coast localities. Here, variation between the head of inlet and coast groups was significant (3.5%, $P = 0.006$) and higher than variation between samples within these two groupings (1.0%, $P < 0.001$), with the majority of variation residing within individual localities (95.5%, $P < 0.001$, Table S4, Supporting information). An alternative arrangement where coast and head of inlet samples were grouped into the five distinct sounds resulted in nonsignificant variation among groups (–1.8%,

Table 2 Estimates of the effective number of breeders (N_b) in coast and head of inlet localities across five sounds in copper rockfish (*Sebastes caurinus*) assayed at 17 microsatellite DNA loci. Estimates were derived using the linkage disequilibrium method with the lowest allele frequency class of 0.01 used in the calculations. An estimate of ∞ denotes that insufficient linkage disequilibrium was present to estimate N_b

Locality	N_b	95% CI
Barkley Sound head of inlet	∞	210.6– ∞
Barkley Sound coast	305.1	161.6–1809.0
Clayoquot Sound head of inlet	1601.4	442.6– ∞
Clayoquot Sound coast	∞	∞ – ∞
Nootka Sound head of inlet	221.1	67.9– ∞
Nootka Sound coast	391.7	177.2– ∞
Kyuquot Sound head of inlet	194.5	119.8–459.4
Kyuquot sound coast	∞	971.2– ∞
Quatsino Sound head of inlet	742.0	199.8– ∞
Quatsino Sound coast	1912.5	355.7– ∞

Table 3 Genetic differentiation (F_{ST} , θ) estimates for all pairwise comparisons across ten samples of copper rockfish (*Sebastes caurinus*) assayed at 17 microsatellite DNA loci

Comparison	BSHI	BSC	CSC	CSHI	KSC	KSHI	NSC	NSCI	QSC
BSC	0.044								
CSC	0.045	–0.002							
CSHI	0.044	0.036	0.043						
KSC	0.046	–0.001	–0.001	0.041					
KSHI	0.012	0.041	0.046	0.033	0.042				
NSC	0.050	0.001	0.000	0.045	–0.001	0.045			
NSHI	0.019	0.049	0.051	0.032	0.045	0.003	0.051		
QSC	0.051	–0.002	–0.002	0.048	–0.003	–0.003	–0.001	0.054	
QSHI	0.020	0.044	0.048	0.030	0.048	0.048	0.050	0.016	0.054

BSHI, Barkley Sound head of inlet; BSC, Barkley Sound coast; CSC, Clayoquot Sound coast; CSHI, Clayoquot Sound head of inlet; NSC, Nootka Sound coast; NSHI, Nootka Sound head of inlet; KSC, Kyuquot Sound coast; KSHI, Kyuquot Sound head of inlet; QSC, Quatsino Sound coast; QSHI, Quatsino Sound head of inlet. Italicized values are not significantly different from 0 (at $P < 0.0114$).

$P = 0.9$), but significant variation between coast and head of inlet samples within sounds (4.7%, $P < 0.001$), with the majority of variation residing within individual localities (97.1%, $P < 0.001$, Table S4, Supporting information).

Eighteen of 605 fish (2.9%) had less than a 1% chance of being from the genetic population with which they were sampled and, consequently, were inferred to be migrants to the sample locality. Ten of these 18 putative migrants were inferred in the coast populations [with the exception of Quatsino Sound coast (QSC)], and eight were inferred to occur in the head of inlet populations. Only five putative immigrants, however, could be inferred to have originated in only one of the other populations with a probability of at least 0.9; three of these involved fish sampled from coast sites that were inferred to have originated in other coast sites and two were inferred to have moved between coast and head of inlet sites.

Table 4 Pairwise genetic differentiation estimates (F_{ST}) for coast-head of inlet comparisons of copper rockfish (*Sebastes caurinus*) assayed at 17 microsatellite DNA loci. Also shown are the distances in kilometres between paired coast-head of inlet sites (distance in km)

Pairwise comparison	F_{ST}	P	Distance (km)
BSHI vs. BSC	0.044	<0.001	57
CSCI vs. CSC	0.043	<0.001	36
NSHI vs. NSC	0.051	<0.001	48
KSHI vs. KSC	0.042	<0.001	29
QSHI vs. QSC	0.054	<0.001	70
Average	0.047	<0.001	48

BSHI, Barkley Sound head of inlet; BSC, Barkley Sound coast; CSC, Clayoquot Sound coast; CSHI, Clayoquot Sound head of inlet; NSC, Nootka Sound coast; NSHI, Nootka Sound head of inlet; KSC, Kyuquot Sound coast; KSHI, Kyuquot Sound head of inlet; QSC, Quatsino Sound coast; QSHI, Quatsino Sound head of inlet.

Length, condition factor, age and growth

Our samples of copper rockfish averaged 30.5 cm in length (range = 13.2–49.8 cm), 583 g in weight (range = 20–2100 g) and 1.7 in condition factor (range = 1.05–2.28). Inlet fish were on average 9.7 cm shorter and showed 7.3% lower body condition factor than those from the outer coast. There was a significant effect of sound ($P = 0.0004$) and habitat (outer coast vs. head of inlet; $P < 0.0001$) on mean length, as well as a significant sound*habitat interaction ($P < 0.0001$). Fish sampled from the Barkley Sound coast site tended to be the longest fish, and fish from coastal sites tended to be longer than head of inlet sites, especially for Clayoquot, Kyuquot and Quatsino sounds (Fig. 4; Table S5, Supporting information). As with mean length, fish from coast sites tended to be heavier than head of inlet fish, especially for Clayoquot and Quatsino sounds (Table S5, Supporting information). By contrast, average condition factor did not differ by sound ($P = 0.718$), but there was a significant effect of habitat ($P < 0.0001$) as well as an interaction between sound and habitat ($P < 0.0001$; Fig. 4, Table S5, Supporting information).

Ages of 274 copper rockfish were determined from the otoliths and fish ages averaged 10.4 years (range 2–43 years, Table S6, Supporting information). The youngest fish collected were from the Nootka Sound coast site (mean = 8.4 years, no head of inlet fish were aged), and the oldest were from the QSC and QSHI sites (mean = 11.3 years). We calculated an overall von Bertalanffy growth equation that produced an asymptotic total length of 38.2 cm (95% CI = 36.0–40.8) and estimated the growth coefficient (K) at 0.17 (95% CI = 0.15–0.21). The size-at-age curve consisted of two groupings of points; fish collected from coast sites were consistently larger at a given age than were fish from head of inlet sites (Fig. 4). The same pattern of faster growth in coast site fish was consistent across the four sounds for which we had comparable data (Fig. S2, Table S6, Supporting information). For inlet fish, L_{∞} was estimated to be 30.7 cm (95% CI = 28.4–33.7 cm)

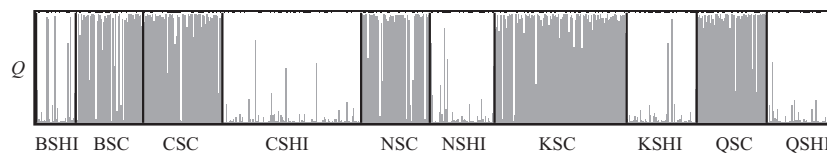


Fig. 2 Analysis of population structure in copper rockfish (*Sebastes caurinus*) sampled from five sounds along the west coast of Vancouver Island, British Columbia, and assayed at 17 microsatellite DNA loci. Each fish's genotype is represented by a thin vertical line, the grey and white portions of which represent the proportional contribution of each of two genetic groups to the admixture coefficient, Q (varying from 0 to 1.0 along the vertical axis), from STRUCTURE analysis (Pritchard *et al.* 2000). BSHI, Barkley Sound head of inlet; BSC, Barkley Sound coast; CSC, Clayoquot Sound coast; CSHI, Clayoquot Sound head of inlet; NSC, Nootka Sound coast; NSHI, Nootka Sound head of inlet; KSC, Kyuquot Sound coast; KSHI, Kyuquot Sound head of inlet; QSC, Quatsino Sound coast; QSHI, Quatsino Sound head of inlet. The thick vertical black lines separate each locality from one another.

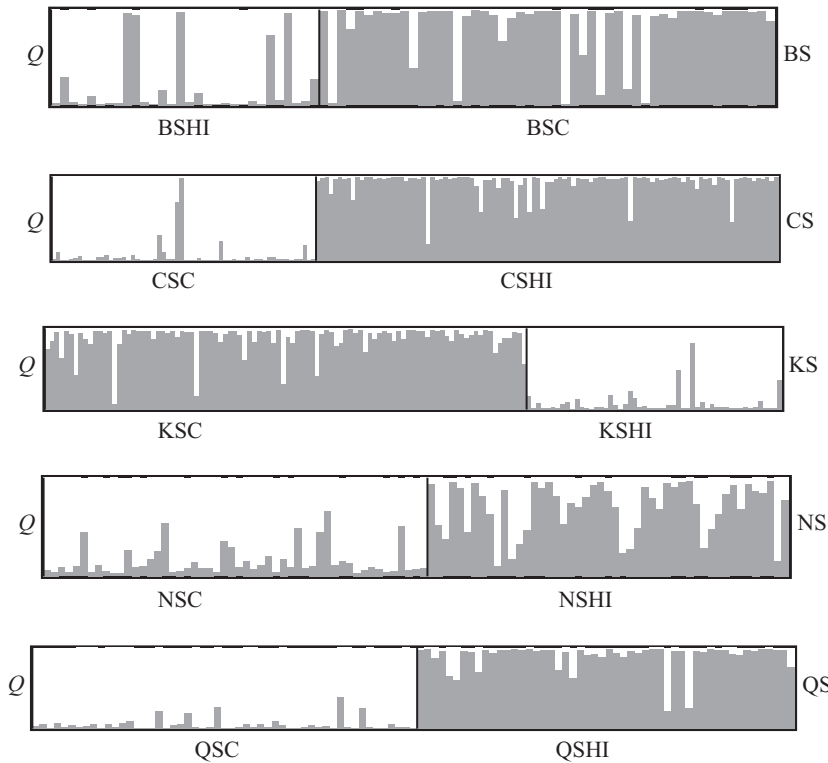


Fig. 3 Analysis of population structure in copper rockfish (*Sebastes caurinus*) sampled from five sounds along the west coast of Vancouver Island, British Columbia, and assayed at 17 microsatellite DNA loci. Each fish's genotype is represented by a thin vertical line, the grey and white portions of which represent the proportional contribution of each of two genetic groups to the admixture coefficient, Q (varying from 0 to 1.0 along the vertical axis), from STRUCTURE analysis (Pritchard *et al.* 2000) of each individual sound. BS, Barkley Sound; CS, Clayoquot Sound; NS, Nootka Sound; KS, Kyuquot Sound; QS, Quatsino Sound; BSHI, Barkley Sound head of inlet; BSC, Barkley Sound coast; CSC, Clayoquot Sound coast; CSHI, Clayoquot Sound head of inlet; NSC, Nootka Sound coast; NSHI, Nootka Sound head of inlet; KSC, Kyuquot Sound coast; KSHI, Kyuquot Sound head of inlet; QSC, Quatsino Sound coast; QSHI, Quatsino Sound head of inlet. The thick vertical black lines separate coast and head of inlet sites from one another within each sound.

compared to 42.2 cm (95% CI = 41.2–43.3 cm) for coast fish. Values of K were estimated to be 0.18 (95% CI = 0.15–0.23) for inlet fish and 0.20 (95% CI = 0.19–0.22) for coast fish. Likelihood ratio tests comparing the two growth curves confirmed that coast fish grow more quickly ($P = 0.021$) and reach a larger maximum size ($P = 0.003$) than inlet fish. Differences in t_0 , the theoretical age when a fish has 0 length, were not significant ($P = 0.06$). Overall, the difference between the two growth curves was highly significant ($P < 0.0001$).

Discussion

Factors influencing population structure in marine environments

The tremendous growth in the study of population genetics of marine organisms in the last 30 years (see review by Hedgecock *et al.* 2007) coupled with recent behavioural and life-history studies have illustrated that marine fishes from diverse regions may have substantial and previously unrecognized diversity (e.g. Bowen *et al.* 2001) including extensive population subdivision (Cowen *et al.* 2000; Gerlach *et al.* 2007). Our study demonstrated repeated divergence between copper rockfish collected from coast and head of inlet sites across relatively small spatial scales in five sounds along the west coast of Vancouver Island. Further, different head of

inlet sites were distinct from one another, while no detectable genetic differentiation was found among coast sites at the mouths of the five sounds. In particular, we have demonstrated a strong association between genetic differentiation and habitat structure (open coast sites vs. more enclosed head of inlet sites) as one component of seascape genetic structure. Further, our data suggest that outer coast fish experience a more favourable growth environment; they were larger, had higher condition factors and grew faster than head of inlet fish. Our analyses across the coast–head of inlet habitat transitions suggest that genetic structure in some marine fish may result from a combination of restricted gene flow driven by limitations on physical movement (through the influence of coastal topography and hydrography) and selection against immigrant phenotypes in contrasting environments.

Historical and contemporary oceanographic and seascape factors are increasingly recognized for their role in structuring current patterns of population subdivision in marine species exposed to natural selection, migration, drift and their interactions across both space and time (e.g. Planes *et al.* 2001; Hellberg *et al.* 2002; Galindo *et al.* 2006; Hedgecock *et al.* 2007; Hess *et al.* 2011). In particular, oceanographic factors influencing the ability and direction of movement during dispersive phases of the life cycle of marine species (e.g. upwelling, currents and gyres) are potentially important

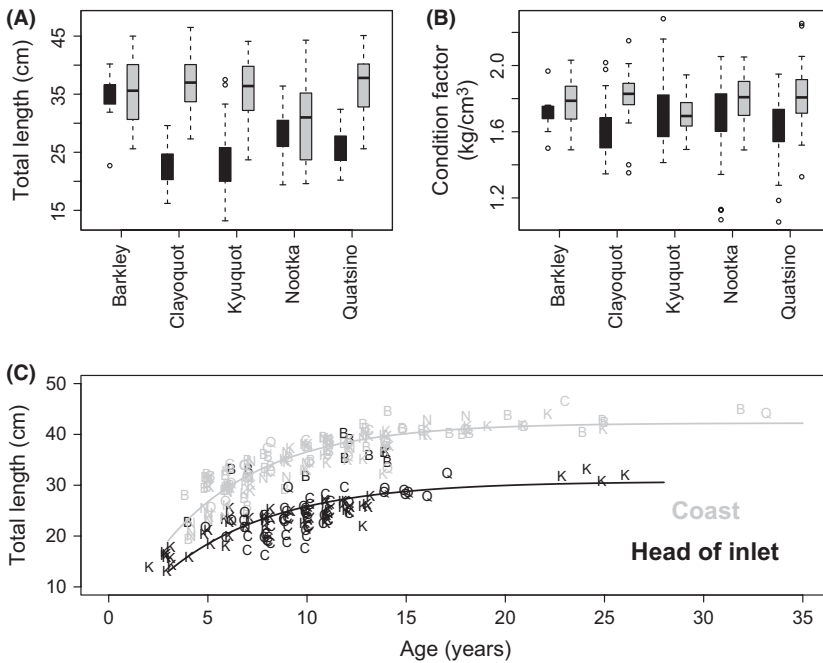


Fig. 4 Variation in (A) total length (cm), (B) condition factor (CF, weight/length³) and (C) Von von Bertalanffy growth in copper rockfish (*Sebastes caurinus*) collected from five sounds along the west coast of Vancouver Island, British Columbia. Grey bars are coast sites, and black bars are head of inlet sites within each of the five sounds (Barkley, Clayoquot, Kyuquot, Nootka, and Quatsino). Small open circles are outliers, heavy horizontal line indicates the median, dashed vertical lines represent the range, and the box represents the second (upper box border) and third (lower box border) quartiles. In C, grey line and letters represent fish from coast sites, and black line and letters are fish from head of inlet sites; and B, Barkley Sound; C, Clayoquot Sound; K, Kyuquot Sound; Q, Quatsino Sound.

influences on marine connectivity and the evolution of population structure (Sinclair & Iles 1989; Stepien 1999; White *et al.* 2010; but see Benzie & Williams 1997). Ocean circulation patterns and connectivity, however, may be heavily influenced by physical landform either in the form of depth profiles, islands, the continental shelf or continental coastline complexity (e.g. Farmer & Freeland 1983; Maier-Reimer *et al.* 1990; Ridgway & Dunn 2003). Thus, physical features of the seascape may be important drivers in the evolution of population genetic structure (e.g. Banks *et al.* 2007; Fontaine *et al.* 2007; Schultz *et al.* 2008) and other aspects of diversification in the marine environment (e.g. Avise 1992; Bernardi *et al.* 2003; Jacobs *et al.* 2004; Banks *et al.* 2007). Our study of copper rockfish illustrates these principles by showing that longshore ocean currents are probably the dominant process linking copper rockfish populations at outer coast sites of sounds. By contrast, physical constrictions on exchange, such as the presence of sills and narrow channels, are associated with much stronger divergence between head of inlet sites and adjacent coast sites within sounds as well as among head of inlet sites across sounds.

Several species of marine fishes have been shown to exhibit substantial differentiation in phenotype and in genetic traits between populations located in coastal areas and those within and among inlets. In Norwegian Atlantic cod (*Gadus morhua*), Atlantic herring (*Clupea harengus*) and pearlside (*Maurolicus muelleri*), morphological and biochemical and molecular data have demonstrated significant differentiation among

fjords and between fjords and adjacent coastal areas (e.g. Jørstad & Nævdal 1989; Girever & Stien 1998; Suneetha & Nævdal 2001). In the North Pacific Ocean, Cunningham *et al.* (2009) reported greater genetic differentiation when samples of Pacific cod (*Gadus macrocephalus*) from fjord-like areas in the inner Strait of Georgia and Puget Sound were compared to open coast samples than when open coast samples, separated by comparable or even much greater distances, were compared to each other. Siegle *et al.* (2013) also noted marked differentiation between Strait of Georgia and outer coast samples of yelloweye rockfish (*Sebastes ruberrimus*). In fact, a previous study on copper rockfish in this same area (Buonaccorsi *et al.* 2002) also identified heightened divergence between the Strait of Georgia and Puget Sound and open coastal areas of the northeastern Pacific Ocean (F_{ST} of ~ 0.025 – 0.098 for Puget Sound/Strait of Georgia vs. coastal compared to -0.001 – 0.022 for coastal comparisons). By contrast, Cunningham *et al.* (2009) reported that differentiation between Puget Sound samples of Pacific cod and outer coast samples was less than that between Strait of Georgia and outer coast samples (although the Puget Sound sample was small, $N = 18$). Our analyses by replicating comparisons between five pairs of adjacent open coast and head of inlet samples of copper rockfish provide compelling evidence of divergence across this environmental gradient occurring in parallel and strongly implicate the role of coastal topography and hydrography on the evolution of population structure.

Genetic divergence within and among sounds

The degree of divergence between coast and head of inlet populations in our study is comparable to that reported by Buonaccorsi *et al.* ($F_{ST} = 0.063$, 2002) although their analysis included some localities that were separated by far greater distances (>2000 km) than in our study. Our paired comparisons between coast and head of inlet sites within individual sounds were also remarkably consistent with one another, varying by a maximum of only about 11% from the maximum pairwise comparison. This argues that the processes influencing genetic divergence between head of inlet and coastal sites are relatively consistent among the five sounds since the inlets were colonized postglacially.

The absence of divergence among the coastal collection sites in the five sounds is consistent with studies of copper rockfish over even larger distances in coastal environments. For instance, Johansson *et al.* (2008) reported slight, but significant IBD and an F_{ST} of only 0.0042 among 12 localities spanning more than 2000 km of coastline in Washington, Oregon and California. Over a small range (450 km, seven localities) in Oregon, F_{ST} , although statistically significant, was only 0.001 and IBD was not detected (Johansson *et al.* 2008). The inshore, shallow-water nature of copper rockfish habitat use, the sedentary nature of adults, behavioural attributes of larvae and habitat breaks along the Oregon coast (rocky vs. sandy areas) may reduce offshore advection of larvae and help generate some population structure despite a larval duration period of two to three months (Buonaccorsi *et al.* 2002; Johansson *et al.* 2008). These processes appeared to act together to generate a mesoscale dispersal pattern in northwest US coastal copper rockfish, in which populations are self-recruiting on a regional scale, but habitat disjunctions may produce genetic breaks or distinctions (Gunderson & Vetter 2006; Johansson *et al.* 2008). Our study sampled across <300 km of coastline and across the five sounds, copper rockfish also appear to exhibit a meso-scale pattern of dispersal because we detected no IBD among the coastal localities, but each coast-head of inlet comparison revealed substantial genetic differentiation.

The widespread dispersal of copper rockfish among the coastal localities in our study is likely facilitated by the pattern of alongshore currents on the west coast of Vancouver Island. The predominant offshore current is the west-east Subarctic Current which bifurcates into the northward Alaska Current and the southbound California Current between 45–50°N latitude and 130–150°W longitude (Thomson 1981). The strength and offshore distance of these predominantly alongshore currents varies seasonally (Thomson 1981), and the more inshore, shallow-water distribution of copper

rockfish compared to congeners such as the black rockfish (*Sebastes melanops*) may make copper rockfish less susceptible to alongshore transport via these vectors. Copper rockfish larvae that were released by females at coastal spawning reefs, however, are probably more exposed to alongshore transport than individuals spawned at the heads of inlets.

Inlets typically have an estuarine-type circulation pattern where low-salinity surface flow generated by freshwater inputs from rivers at the head of each inlet is replaced by deeper, higher salinity water inflow from coastal localities (Thomson 1981; Farmer & Freeland 1983). Such a circulation pattern might facilitate at least some exchange of individual pelagic copper rockfish larvae between coast and head of inlet sites. The level of such exchange would, however, be influenced by the residence time of water within the inlet. For instance, Buonaccorsi *et al.* (2002) argued that because the residence time of water within Puget Sound, WA (50% retention over seven months), exceeded that of the typical copper rockfish larval duration period (two-three months), there would be limited exchange of larvae between Puget Sound and outside waters. Estimates for water residence times for the five study inlets are not available, but they may be considerably lower than those for Puget Sound given their less constrained, direct openings to the continental shelf. For instance, Pedersen (1984) reported that residence times averaged about 13 days for Rupert Inlet, one of the two main arms of Quatsino Sound, which is about one quarter the length of the distance between the head of inlet and the coast collection sites. Consequently, physical water-mass retention within the inlets themselves may not be the most important factor driving divergence between head of inlet and coastal samples of copper rockfish. It is possible that if we had examined multiple localities within each inlet, a more gradual pattern of genetic divergence would have been resolved. Two observations, however, argue against such a scenario. First, we resolved no genetic structure among coastal sites situated at similar or greater geographical distances from each other. Furthermore, the average F_{ST} among coast-head of inlet comparisons was almost twice that observed among just the head of inlet sites despite the greater pairwise geographical distances involved in the latter comparisons.

Head of inlet sites are influenced by seasonally variable freshwater inflows from rivers that affect water temperature, salinity, suspended sediment levels and water clarity, with resultant effects on biotic elements. It is possible therefore that physical dispersal between coast and head of inlet sites is extensive, but effective dispersal is much reduced by contrasting selective

environments and selection against immigrant genotypes. Divergent natural selection acting in contrasting marine environments is common (e.g. Nielsen *et al.* 2006, 2009; Galindo *et al.* 2010; Teacher *et al.* 2013), and consistent phenotypic differences between copper rockfish from head of inlet vs. coast sites suggest the possibility for variation in selection between habitats. Our data showed that head of inlet fish are smaller and have lower condition factors, a general measure of body robustness (Ricker 1975), than fish sampled from coast sites. Further, our size at age analysis indicated that fish at coast sites grow much faster than those at the heads of inlets. These results strongly suggest that conditions for growth are better at coast sites than at head of inlet sites and therefore that the two habitats are not ecologically equivalent and may represent distinctive selective environments (cf. Cadrin *et al.* 2010). While none of the loci that we examined appear to be influenced by natural selection, the contrasting environments of coast and head of inlet sites and phenotypic differences between fish in these environments suggest that selection may favour behavioural traits that reduce passive dispersal between coast and head of inlet sites. Such selection could take the form of spawning site choice by adults that reduces the exposure of larvae to coastward dispersal vectors (e.g. currents) as suggested by Buonaccorsi *et al.* (2002, 2004) for several *Sebastes* including the copper rockfish. At least some rockfishes appear capable of olfaction-based specific habitat recognition (Mitamura *et al.* 2005). Further, selection for larval behaviours such as rheotaxis, substrate orientation or schooling could contribute to reduced dispersal between coast and head of inlet sites (discussed in Miller & Shanks 2004; Cowen & Sponaugle 2009).

Implications for rockfish conservation

Our results have two implications for the conservation of copper rockfish and marine fishes more generally. First, our data document previously unknown spatial genetic diversity in a marine fish: a repeated pattern of divergence across coast-head of inlet habitat transitions. Our results are a further example of cryptic variation in rockfishes and other marine fishes (cf. Zemplak *et al.* 2009; Burford *et al.* 2011). Such biodiversity needs to be considered in conservation plans for marine fishes and their habitats (i.e. the potential existence and recognition of ecotypes and cryptic species) to ensure adequate representation of distinct populations.

Second, considerable effort has been put into the implementation of rockfish conservation areas (RCAs) in British Columbia, which are areas of inshore habitat closed to commercial and recreational fishing for rockfishes (DFO 2007). The development of RCAs was part

of a broader strategy to reverse the decades-long decline in abundance of several rockfish species (Yamanaka & Logan 2010). Given that RCAs are part of an explicit conservation strategy to help rebuild populations coastwide, some understanding of the level of connectivity among RCAs and intervening habitat is essential to their evaluation (Lotterhos *et al.* 2014). In our study area, RCAs are found in close proximity (i.e. a few km) to all of the coast sites, but except for Quatsino Sound, all of the head of inlet sites are located much farther from RCAs (at least a few 10 s of km, see DFO 2007 and <http://www.pac.dfo-mpo.gc.ca/fm-gp/maps-cartes/rca-acsc/areas-secteurs/wc-co-eng.html>). Given that our data suggest widespread effective dispersal among coast sites, inferred from their genetic similarity, all the coastally distributed RCAs are likely well connected to each other and to intervening areas at least when considered over multiple generations. This suggests that coastal RCAs may function to not only reduce rockfish mortality within the RCA, but also provide some export of within-RCA production to non-RCA localities and thus serve as an effective 'network' of reserves (Gaines *et al.* 2010). By contrast, the head of inlet sites appear to be much less well served by RCAs. For instance, there are fewer RCAs located at the head of inlets (DFO 2007); only one is located within one of the three arms that constitute head of inlet areas in Quatsino Sound and one is located within one head of inlet site in Clayoquot Sound. In addition, our data suggest that connectivity between coast and head of inlet sites is restricted and that the coastally biased distribution of RCAs may not function well in terms of exporting larvae to head of inlet sites. Thus, inlet sites may not be part of an effective reserve network (i.e. they have few RCAs themselves and are more isolated from the coastal RCA network). This suggests that the size of individual head of inlet RCAs (to ensure self-sustainability, Botsford *et al.* 2003) and conservation regulations not related to RCA establishment (e.g. fishing regulations in adjacent areas) are especially important for the head of inlet sites, particularly given their low levels of genetic diversity relative to coast sites.

Several recent studies have measured population structure of Pacific coast marine fishes (e.g. Rocha-Olivares & Vetter 1999; Buonaccorsi *et al.* 2002, 2005; Bernardi *et al.* 2003; Cunningham *et al.* 2009; Sivasundar & Palumbi 2010). These studies commonly reveal patterns of isolation by distance across large geographical scales (e.g. Buonaccorsi *et al.* 2002, 2005), relatively sharp 'breaks' in the distribution of genetic variants associated with major physiographic features (e.g. Rocha-Olivares & Vetter 1999; Bernardi *et al.* 2003; Sivasundar & Palumbi 2010) or some combination of such effects (e.g. Buonaccorsi *et al.* 2002). Our work

illustrates how seascape physiography, in particular the incision of coastlines by inlets and the associated variability in terms of connectivity and divergent selective environments, may be a major driver of population subdivision in marine fishes (cf. Teacher *et al.* 2013).

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This study formed the basis of the Masters of Science degree of S. Dick. J. Shurin and E.B. Taylor were co-supervisors on the project. S. Dick collected all the data, performed some of the analyses and contributed to the writing. J. Shurin contributed to the analysis of the size and growth data as well as the writing. E.B. Taylor conducted most of the analyses and wrote the majority of the manuscript.

Data accessibility

All raw genotypic data have been deposited in the Dryad archiving database (DOI:10.5061/dryad.s489b) or are contained in the online supplementary material associated with this article. Individual lengths, weights, condition factors and ages: Tables S5 and S6 (Supporting information).

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Plot of factorial correspondence scores along three axes of genetic variation in copper rockfish (*Sebastes caurinus*) assayed at 17 microsatellite DNA loci.

Fig. S2 Von Bertalanffy growth curves for copper rockfish (*Sebastes caurinus*) collected from coast (stars) and head of inlet (open squares) sites in each of four sounds along the west coast of Vancouver Island, British Columbia: a) Barkley Sound, b) Clayquot Sound, c) Kyuquot Sound, d) Quatsino Sound.

Table S1 Copper Rockfish (*Sebastes caurinus*) microsatellite DNA loci allele frequencies and summary statistics.

Table S2 Genetic differentiation (F_{ST}) estimates for all collections combined, coast collections only and head of inlet collections only for copper rockfish (*Sebastes caurinus*) assayed

at 17 microsatellite DNA loci. Adjusted significance level (α) = 0.0145.

Table S3 Summary of Structure genetic analyses (Pritchard et al. 2000) of population structure in copper rockfish (*Sebastes caurinus*) assayed at 17 microsatellite DNA loci.

Table S4 Results of analysis of molecular variance from ARLEQUIN (Excoffier *et al.*, 2005) summarizing variation in allele frequencies at 17 microsatellite DNA loci assayed in ten samples of copper rockfish (*Sebastes caurinus*) under two grouping hypotheses: (a) groups represent 'coast' sites and 'head of inlet' sites, populations represent the five coast sites or five head of inlet sites within the 'coast' and 'head of inlet' groups and (b) were groups represent the five different 'sounds' and populations represent coast and head of inlet sites within each sound.

Table S5 Individual total length, weights and condition factors.

Table S6 Size at age for Copper Rockfish (*Sebastes caurinus*) collected from nine sites on the west.