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## Using Otolith Chemistry to Investigate Population Structure of Quillback Rockfish in Puget Sound

### Abstract

We assess the potential of using otolith chemistry to differentiate quillback rockfish (*Sebastes maliger*) within Puget Sound, Washington, where two distinct population segments (DPS) have been identified. Using opportunistic collections (1993-2003) of quillback rockfish (n=77; age range of 2-65 yrs.) we first sought to determine whether fish from different sites and regions could be differentiated based on the trace elemental concentrations at the edge of their otoliths (i.e., the chemical record of the fish's recent history). Results of our quadratic discriminant function analysis (QDFA) indicated significant spatial variability for fish collected at relatively large (regions) and small (sites) spatial scales. Specifically, fish collected from regions in 2002 (San Juan Islands and southern Puget Sound) and 2003 (eastern and western Strait of Juan de Fuca) were correctly classified with 100% and 65% accuracy (based on jack-knife classification), respectively, while fish collected from sites in 1998 (Mukilteo and Foulweather) were classified with 100% accuracy. We also investigated whether we could differentiate fish that were collected from different DPS and regions by using elemental concentrations from their whole otolith (which represents environmental information over the lifetime of a fish). Results from the QDFAs indicated relatively high classification success (80%) when comparing fish collected from either different DPS (i.e., Northern Puget Sound and Puget Sound Proper DPS) or regions (i.e., western and eastern Strait of Juan de Fuca). Findings from this study highlight the value of otolith chemistry in the study of population structure of quillback rockfish in Puget Sound.

### Introduction

Quillback rockfish (*Sebastes maliger*) is a commercially and recreationally exploited species that is distributed from central California to the Gulf of Alaska (Love et al. 2002). Over the last 20 years, quillback rockfish in Puget Sound (defined as the geographical region in the inland waters of Washington State, U.S. and British Columbia, Canada) has experienced significant declines in abundance and mean size, which are generally attributed to harvest (Palsson and Pacunski 1995, Stout et al. 2001, Palsson et al. 2009). Amid concerns about its sustainability the National Marine Fisheries Service convened, in 2001, a Biological Review Team (BRT) to conduct a status review on quillback rockfish. From this status review the BRT highlighted three distinct population segments

(DPS; a subgroup of a vertebrate species that is treated as a species for purposes of listing under the Endangered Species Act); a coastal DPS, a Puget Sound proper DPS, and a northern Puget Sound DPS (Figure 1).

In their review the BRT also concluded that the Puget Sound proper DPS was vulnerable (under the International Union for Conservation of Nature) and quillback rockfish has since been listed as a species of concern by both state and federal fisheries management agencies. Further, the BRT highlighted areas of uncertainty. In particular, they suggested that more information was needed to clarify the spatial extent of the northern Puget Sound DPS, and the degree to which the Puget Sound proper DPS is different from those of other regions (Stout et al. 2001).

The majority of evidence used in the status review took advantage of natural variability in gene frequencies (i.e., allele and microsatellite

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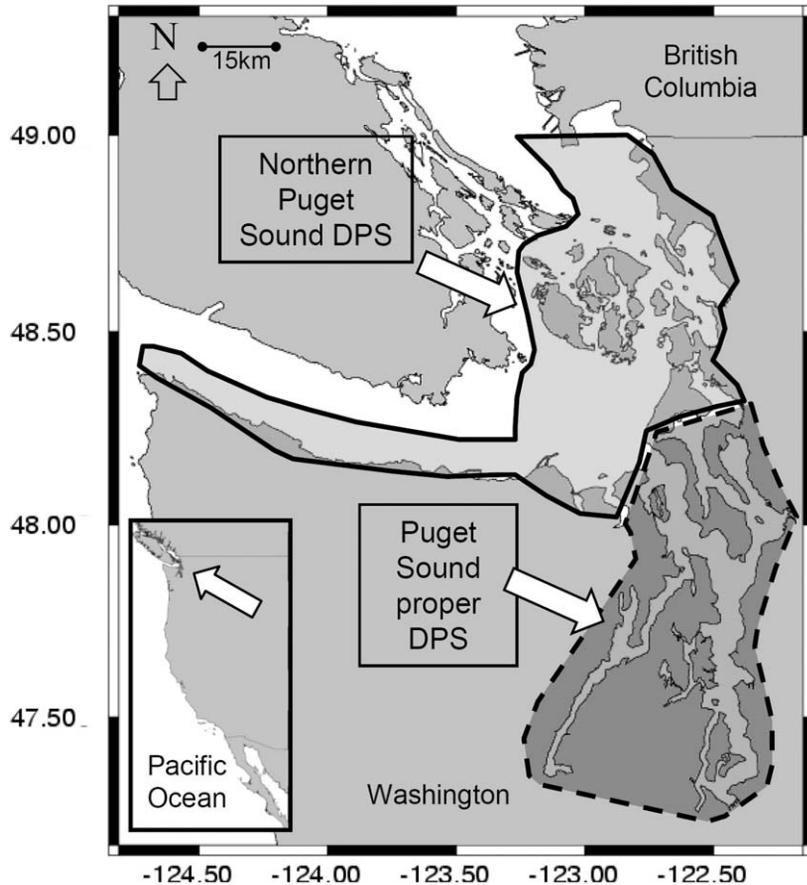


Figure 1. Generalized DPS boundaries for quillback rockfish in Puget Sound. Modified from Stout et al. (2001).

work; Seeb 1998) while here we aim to assess the potential of using otolith chemistry to differentiate collections of quillback rockfish in Puget Sound. Otoliths (or earstones), are found in most teleosts, and are paired, calcified structures that grow throughout the life of the fish (Campana 1999). As otoliths grow they not only form visible increments that can be used to estimate age and somatic growth, but they are continually depositing environmentally derived isotopes (see Farrell and Campana 1996, Geffen et al. 1998, Kennedy et al. 2002, Elsdon and Gillanders 2003). In addition, otoliths are metabolically inert, and therefore the combination of visible increments and an environmentally sequestered chemistry results in otoliths being a chronological record of the environment a fish occupied (Campana 1999).

Because of the natural variability found in both the genes and otolith chemistry, they are often used

to estimate population structure and movement of individuals (for otolith chemistry see Thorrold et al. 1998, 2001; Rooper et al. 2008; Chittaro et al. 2009; for genetics see Taylor and Hellberg 2003, Buonaccorsi et al. 2005, Curley and Gillings 2009). However, these two methods reflect processes occurring at different time scales; the genetic approach provides information on an evolutionary time scale, while otolith chemistry provides information on the lifetime of an individual. In light of the relatively recent decline of quillback rockfish in Puget Sound and its resultant conservation status, we believe that the contemporary nature of otolith chemistry information can potentially provide new insights into the population structure and movement of this species.

Despite the growing use of otolith chemistry in ecological research we know of relatively few studies on long-lived fish in Puget Sound (see Volk

et al. 2000, Gao et al. 2001, Chittaro et al. 2009). Our aim was therefore to first assess the applicability of otolith chemistry to the ecological study of quillback rockfish by quantifying the extent to which there exists geographical variability in trace element concentrations. Specifically, using opportunistic collections of quillback rockfish throughout Puget Sound (and over a number of years; 1993-2003), we quantified the trace element concentrations at the otolith edge (corresponding to the chemical record of the fish's recent history) and for the whole otolith (which represents environmental information over the lifetime of a fish) in order to assess the extent to which fish could be spatially differentiated. The ultimate goal of our study was to provide support

for a technique that could further resolve population structure and thus address the uncertainties raised by the BRT.

## Methods

### Specimen Collections

Seventy-seven adult quillback rockfish (age range: 2-65 years) were collected between 1993 and 2003 from 22 sites within six regions of two distinct population segments (DPS) (Figure 2 and Table 1). Distinct population segments were delineated based on genetic work by Seeb (1998) and Burr (1999). Because collections of quillback rockfish were made opportunistically, sample sizes were relatively small, and sites were not sampled in

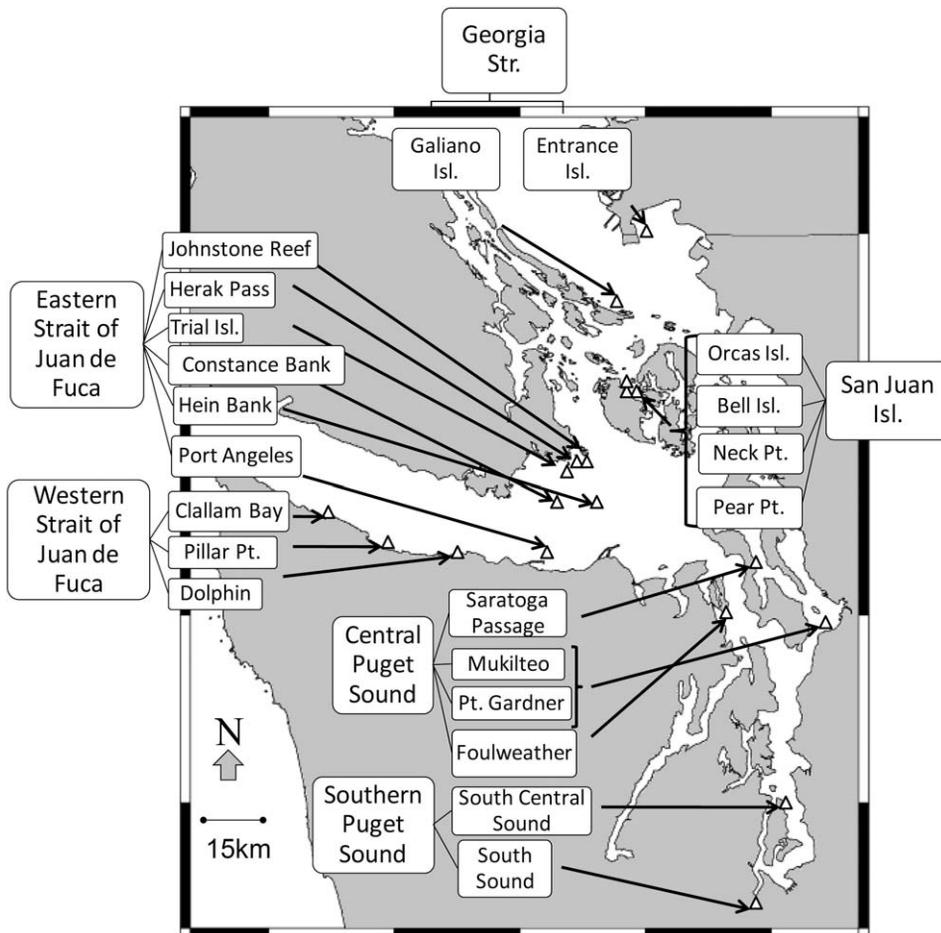


Figure 2. Puget Sound sites and regions from which quillback rockfish were collected (1993-2003). Within the Puget Sound Proper DPS two regions (central and southern Puget Sound) are indicated. Within the Northern Puget Sound DPS four regions (eastern and western Strait of Juan de Fuca, Georgia Strait, and San Juan Islands) are indicated.

TABLE 1. Collections of quillback rockfish from Puget Sound distinct population segments, regions, and sites.

Distinct population segment	Region	Site	Year	N	Age range	Size range (TL mm)
Puget Sound Proper	Central Puget Sound	Point Gardner	1996	4	6-8	202-269
		Mukilteo	1998	5	8-16	266-350
		Foulweather	1998	7	5-11	256-330
		Saratoga Passage	1998	2	6-8	235-255
	Southern Puget Sound	South Central Sound	2002	5	6-21	255-370
		South Sound	2002	3	7-18	260-309
Northern Puget Sound	San Juan Islands	Orcas Island	1994	4	5-32	274-437
		Bell Island	2002	3	NA	244-262
		Neck Point	2002	1	NA	217
		Pear Point	2002	1	NA	252
	Eastern Strait of Juan de Fuca	Trial Islands	2003	10	8-42	253-470
		Constance Bank	2003	3	12-34	373-449
		Port Angeles	2003	1	4	265
		Hein Bank	2003	1	2	145
		Herak Pass	2003	3	10-39	350-451
		Johnstone Reef	2003	3	11-65	309-475
	Western Strait of Juan de Fuca	Pillar Point	2003	2	5-10	300-350
		Clallam Bay	2003	5	5-42	233-384
		Dolphin	2003	1	12	349
	Georgia Strait	Galiano Island	1993	1	8	237
		Entrance Island	2000	12	11-37	330-409

NA indicates 'not aged.'

different years. Collections were made by the Washington Department of Fish and Wildlife and by the Department of Fisheries and Oceans Canada during fishery-independent trawl surveys. Specimens were obtained between May and August using an otter trawl towed at approximately 3-5 km/hr. Total length of each fish was measured, and sagittal otoliths were removed for ageing and chemical analysis.

#### Otolith Structural and Chemical Analysis

The right sagittal otolith from each specimen was sonicated for 5 min in ultra-pure water and embedded in epoxy resin. Otoliths were fixed to microscope slides using Crystal Bond and then ground in the transverse plane with 1200  $\mu\text{m}$  silicon carbide paper until the core was exposed. Otoliths were then sequentially polished with 6, 1 and 0.05  $\mu\text{m}$  diamond suspensions. Age was estimated for each fish by counting the number of bands presumed to be annual increments (Kerr et al. 2005).

The left sagittal otolith from each fish was chemically analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) following the protocol outlined in Sanborn and Telmer (2003). The LA-ICP-MS system used was a VG Elemental PQ II S+ high sensitivity ICP-MS (Thermo Electron Corporation, Waltham, MA, USA) coupled to a Merchantek UV laser ablation system (New Wave Research, Fremont, CA, USA). The laser system operates at a wavelength of 266 nm and a maximum energy output of 4 mJ. All otolith analyses were conducted at a frequency and power of 20 Hz and 75%, respectively. Average energy while operating at these conditions was 0.70 mJ. Laser line scans were made at  $\sim 5.3 \mu\text{m}/\text{s}$  across the surface of each otolith, running from core to edge along the longest axis. The core-to-edge line scan corresponds to elemental tracking through the life of a fish, with the core portion corresponding to the natal period.

Isotopes measured in the otolith included calcium ( $^{43}\text{Ca}$ ), manganese ( $^{55}\text{Mn}$ ), zinc ( $^{66}\text{Zn}$ ), strontium ( $^{86}\text{Sr}$ ) and barium ( $^{137}\text{Ba}$ ). Calcium was used as an internal standard, with background intensities collected for 30 s prior to operating the laser. Data collection and reduction were completed using VG Thermo Electron PlasmaLab Software 2003, Version 1.06.007. The Fully Quantitative Analysis option was used, and an SRM613 NIST glass was selected as the known standard. Two SRM 613 NIST glasses were analyzed, both at the beginning and end of each run. These certified standards were used to complete an external drift correction to compensate for any changes in machine sensitivity. Ten otoliths were analyzed between each set of standards. An SRM 611 NIST glass was also analyzed as an unknown sample during each run of 10 structures as a quality assurance test to help ensure measurement accuracy and precision.

#### Statistical Analyses

*Otolith Edge Chemistry*—One of our objectives was to quantify the level of spatial variability in otolith chemistry. To do this we first selected the otolith edge from each otoliths' core-to-edge line scan (i.e., ~150  $\mu\text{m}$  of the total core-to-edge line scan), and determined average concentrations of Mn, Zn, Sr, and Ba. The otolith edge represents a time in the life of the fish prior to its collection (weeks to months depending on the size/age of the otolith/fish). Because of the relatively small home ranges reported for quillback rockfish in Puget Sound (~700-1250 $\text{m}^2$  for adult fish with a standard length of 260-320mm; see Tolimieri et al. [2009] and references therein) we expect that the otolith edge chemistry corresponds to the site (or at least proximal) from which the fish was collected.

We next statistically assessed whether there was sufficient spatial variability in these trace elemental concentrations to allow individuals (collected in the same year) to be grouped according to their region or site of collection. Regions were delineated using information on bathymetric and circulation patterns (Thomson 1994) (e.g., a sill in the Strait of Juan de Fuca separates eastern and western regions), while sites were designated by the latitude and longitude of sampling. Our statistical analysis was limited to years in which we had an adequate sample size and at least two groups (i.e., region or site) (Table 1). Specifically,

we compared elemental concentrations between fish collected from a) two sites (Mukilteo and Foulweather) within the central Puget Sound region of the Puget Sound proper DPS (1998), b) two regions (eastern and western Strait of Juan de Fuca) within the northern Puget Sound DPS (2003), and c) a region (San Juan Islands) of the northern Puget Sound DPS and a region (southern Puget Sound) of the Puget Sound proper DPS (2002).

These analyses were performed using a quadratic discriminant function analysis (QDFA), a multivariate approach in which the goal is to find axes that best discriminate between groups (i.e., regions or sites) (McCune and Grace 2002). In this analysis, elemental concentrations are the dependent variables and otoliths (individuals) are the replicates. A jackknifed classification matrix was constructed for each QDFA to indicate the percentage of fish correctly identified to the region or site from which they were collected (Systat 2002). To help visualize similarities/differences between groups we plotted factor scores (i.e., combined measure created for each fish on each factor extracted from the dependent variables) that were derived from each QDFA. Overlap in factor scores highlights similarities in otolith concentrations and the extent to which fish are misclassified. All variables were  $\log_{10}$  transformed to improve normality.

*Whole Otolith Chemistry*—Another objective of this study was to assess the degree to which fish collected from different DPS (or regions) had overlapping elemental concentrations that corresponded to their entire life (i.e., concentrations derived from chemical analysis of the entire otolith). Before determining whole otolith elemental concentrations from our core-to-edge line scans, we first inspected the core of each otolith for elevated concentrations of manganese (see Brophy et al. 2004, Ruttenberg et al. 2005, Chittaro et al. 2006, Ben-Tzvi et al. 2007). Line scans that did not contain elevated concentrations of manganese were removed from our dataset because they were suspected of failing to pass through the core of the otolith, and thus were likely unsuccessful at capturing the chemistry corresponding to the juvenile and natal life history.

Due to the nature of our opportunistic sampling, it was not possible to analyze a group of fish of the same age collected in the same year. Consequently, in this analysis we used only those otoliths for

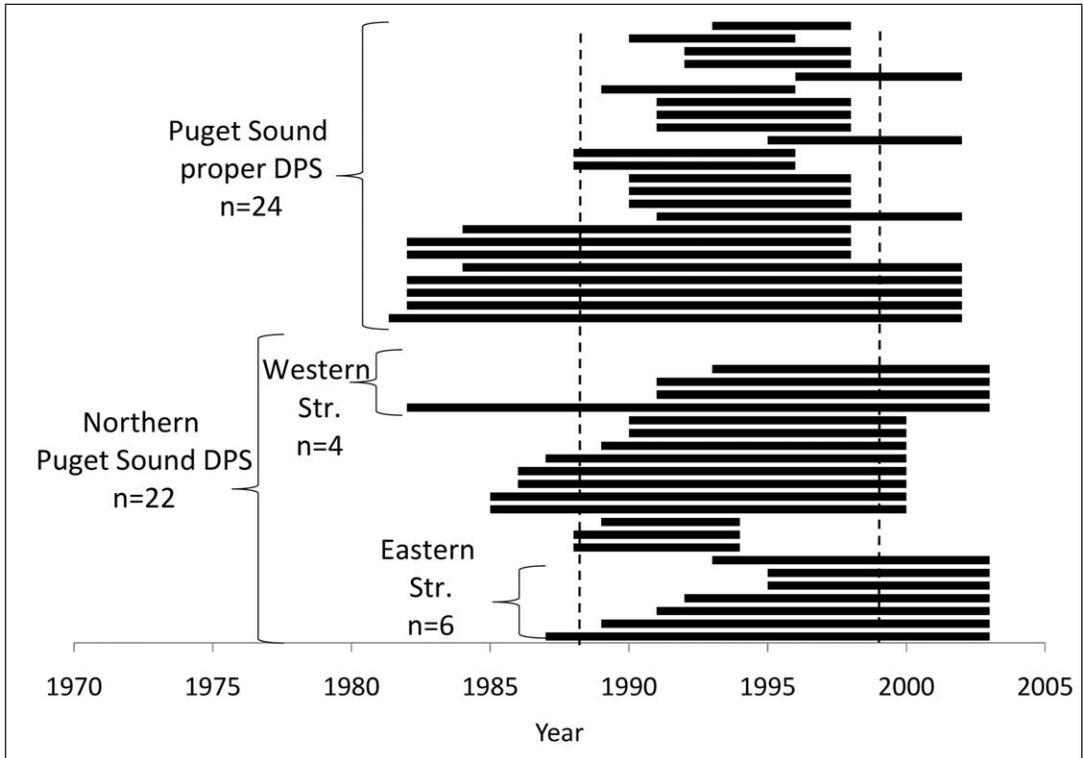


Figure 3. Fish age and thus number of years of otolith chemistry data for each quillback rockfish (each bar) and the DPS from which they were collected. Dashed lines indicated average year of collection and average back-calculated hatch dates. Only those regions used in the statistical analysis are labeled.

which the whole-otolith chemistry corresponded to an interval of time that was represented by fishes from the northern Puget Sound DPS and the Puget Sound proper DPS. Specifically, otoliths from 46 individuals collected from 1994 to 2003, and ranging in age from 5 to 21 years, provided elemental signatures corresponding to an average age of  $11.5 \text{ yrs} \pm 4.7$  (northern Puget Sound DPS =  $11.5 \text{ yrs} \pm 3.7$ ; Puget Sound proper DPS =  $10.8 \text{ yrs} \pm 5.5$ ), with an average hatch year of  $1988 \pm 4.0$  years (northern Puget Sound DPS =  $1989 \pm 3.3$  yrs; Puget Sound proper DPS =  $1988 \pm 4.5$  yrs), and collection year of  $1999 \pm 2.8$  yrs (northern Puget Sound DPS =  $2001 \pm 3.0$  yrs; Puget Sound proper DPS =  $1999 \pm 2.2$  yrs) (Figure 3). We used QDFA to determine whether whole otolith concentrations varied sufficiently to permit the differentiation of individuals between a) the northern Puget Sound DPS and the Puget Sound proper DPS, and b) between regions (eastern and western Strait of Juan de Fuca) within the northern Puget Sound DPS.

## Results

### Otolith Edge Chemistry

Elemental concentration of the otolith edge varied among sites and regions (Figure 4), which allowed for individuals to be classified with relatively high accuracy to the site or region from which they were collected. Specifically, the QDFA of fish collected (in 1998) from regions within the Puget Sound proper DPS, but between two sites (Mukilteo and Foulweather) revealed 100% classification accuracy (Wilks' Lambda = 0.436;  $df = 4, 47$ ;  $F = 2.260$ ;  $P < 0.16$ ; Figure 5a; Table 2). In other words, fish collected from Mukilteo and Foulweather had unique elemental concentrations at the otolith edge that allowed fish from these two sites to be differentiated. Our QDFA of fish collected (in 2003) from two regions (western and eastern Strait of Juan de Fuca) within the northern Puget Sound DPS, indicated an average classification accuracy of 65% (Wilks' Lambda =

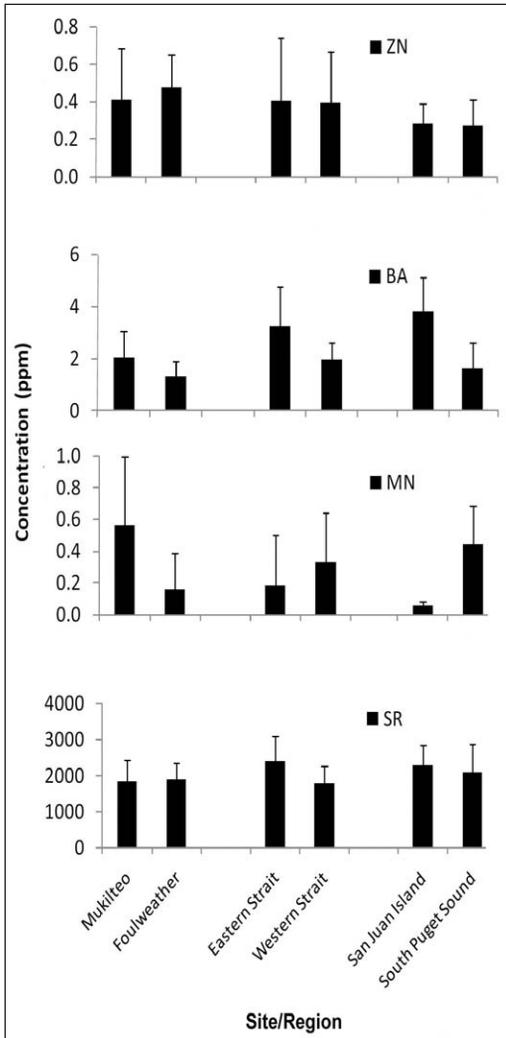


Figure 4. Average elemental concentrations (whiskers are standard deviations) of manganese, zinc, strontium, and barium from the otolith edge. Fish were collected from Mukilteo and Foulweather (1998), eastern and western regions of the Strait of Juan de Fuca (2003), and San Juan Island and South Puget Sound regions (2002) of the Northern Puget Sound DPS and the Puget Sound Proper DPS, respectively.

0.610;  $df = 4,21$ ;  $F = 3.34$ ;  $P < 0.03$ ; Figure 5b; Table 2). Ten individuals were misclassified (out of 29), of which seven and three were collected from the eastern and western Strait of Juan de Fuca, respectively (Figure 5b). Finally, the QDFA that compared otolith chemistry of fish collected (in 2002) from regions (San Juan Islands and southern Puget Sound) within the Puget Sound proper DPS

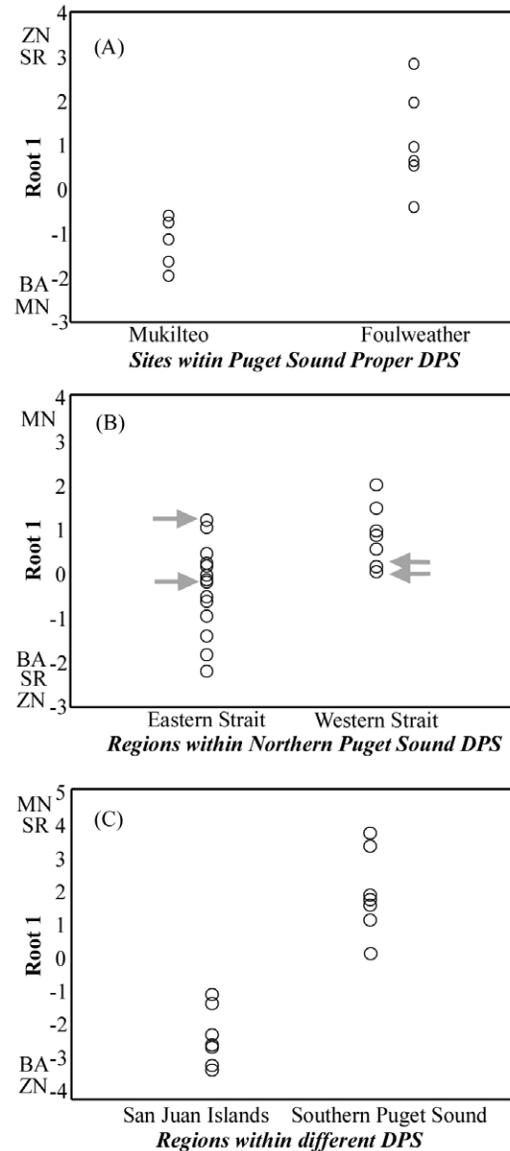
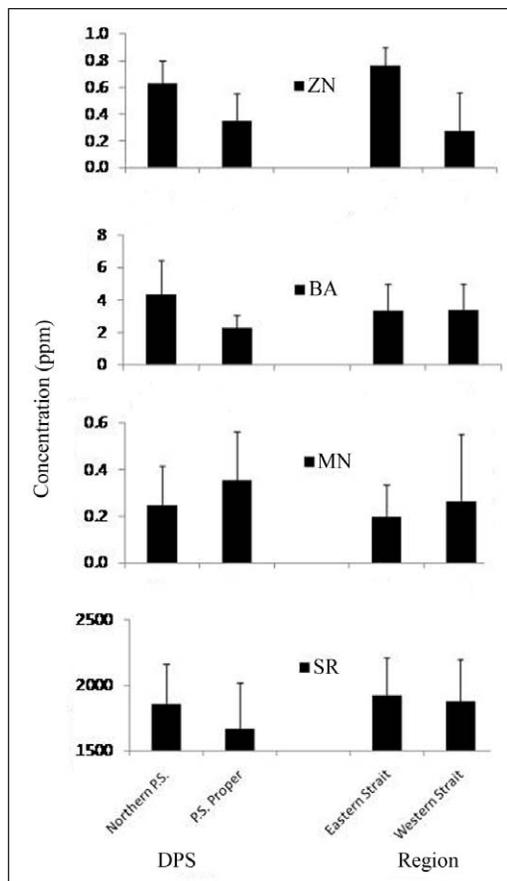


Figure 5. Plots of factor scores that were produced from the QDFAs using chemical concentrations derived from the otolith edge to discriminate quillback rockfish that were collected from a) two sites (Mukilteo and Foulweather; 1998), b) two regions (eastern and western Strait of Juan de Fuca; 2003), and c) two regions (San Juan Islands and Southern Puget Sound; 2002). In each plot, trace elements are listed on the y-axis to identify whether they were positively or negatively associated with root 1. Arrows indicate the range of root 1 values within which fish were misclassified.

TABLE 2. Results of the quadratic discriminant function analyses that used chemistry from the otolith edge or whole otolith to classify fish to the distinct population segment, region, or site from which they were collected. Jack-knife percent classifications are provided, and elements that were useful in the classification of fish are listed in decreasing order of discriminatory ability.

Otolith section	Type of analysis	No. of groups	No. of fish	% correct classification	Important elements
Otolith edge	Within Puget Sound proper DPS, within Central Puget Sound region, and between sites sampled in 1998; Mukilteo and Foulweather.	2	15	100	Ba, Mn, Sr, Zn
	Within northern Puget Sound DPS and between regions sampled in 2003; eastern and western Strait of Juan de Fuca.	2	12	100	Zn, Ba, Mn, Sr
	Between regions sampled in 2002; San Juan Island and Southern Puget Sound.	2	9	65	Ba, Mn, Sr, Zn
Whole otolith	Between DPS; Puget Sound proper and Northern Puget Sound.	2	46	80	Mn, Ba, Zn, Sr
	Within Northern Puget Sound DPS and between regions; eastern and western Strait of Juan de Fuca.	2	10	80	Zn, Ba, Sr

and the northern Puget Sound DPS, respectively, indicated 100% classification accuracy (Wilks' Lambda = 0.166;  $df = 4, 10$ ;  $F = 12.556$ ;  $P < 0.01$ ; Figure 5c; Table 2).



### Whole Otolith Chemistry

Elemental concentration of the whole otolith varied among regions and DPS (Figure 6), and results from our QDFA also revealed relatively high classification success. Specifically, when we compared the elemental concentrations for fish collected from both the Northern Puget Sound DPS and the Puget Sound Proper DPS, we found significant differences that enabled 80% of individuals to be correctly classified to the DPS from which they were collected (Wilks' Lambda = 0.558;  $df = 4, 41$ ;  $F = 8.135$ ;  $P < 0.001$ ; Figure 7a, Table 2). Nine individuals were misclassified (out of 46), of which five and four were collected from the eastern and western Strait of Juan de Fuca, respectively (Figure 7a). Analysis of fish from the western and eastern Strait of Juan de Fuca (within the northern Puget Sound DPS) also indicated a jackknife classification success of 80% (Wilks' Lambda = 0.582;  $df = 3, 6$ ;  $F = 1.435$ ;  $P < 0.03$ ; Figure 7b, Table 2). Two individuals were misclassified (out of 10), both of which were collected from the eastern Strait of Juan de Fuca (Figure 7b).

Figure 6. Average elemental concentrations (whiskers are standard deviations) of manganese, zinc, strontium, and barium obtained from the analysis of whole otoliths of fish collected a) throughout the Northern Puget Sound DPS and Puget Sound Proper DPS, and b) eastern and western Strait of Juan de Fuca represent a subset of otoliths within the Northern Puget Sound DPS.

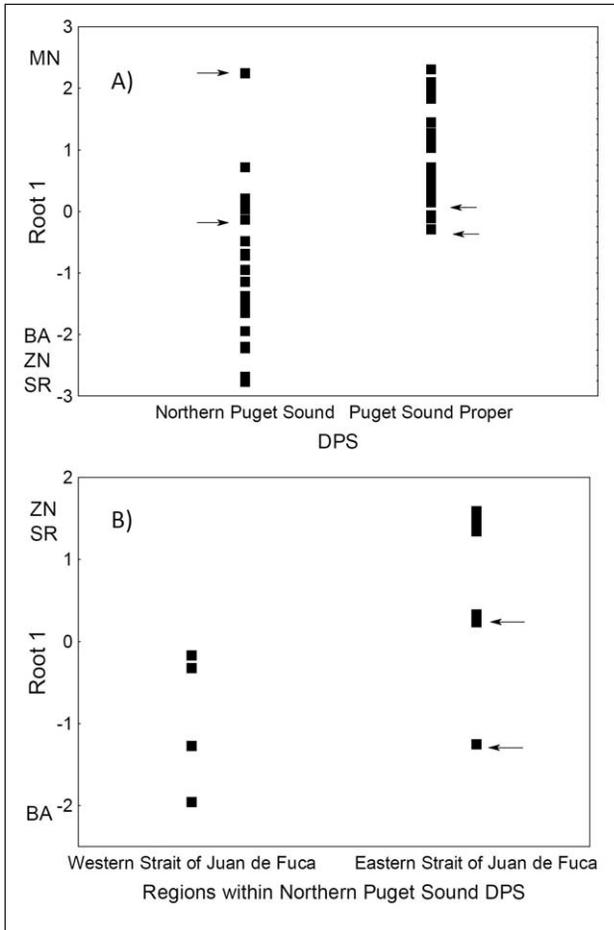


Figure 7. Plots of factor scores that were produced from the QDFAs using chemical concentrations derived from whole otoliths to discriminate quillback rockfish that were collected from a) the Puget Sound Proper DPS and the Northern Puget Sound DPS, and b) between regions (western or eastern Strait of Juan de Fuca). In each plot, trace elements are listed on the y-axis to identify whether they were positively or negatively associated with root 1. Arrows indicate the range of root 1 values within which fish were misclassified.

## Discussion

An important first step towards sustainable long-term fisheries is understanding the structure (i.e., spatial discreteness) of fish populations (Iles and Sinclair 1982, Bergenius et al. 2005), in part because it provides a spatial context by which managers can focus their policies. In this study we sought to assess the suitability of using otolith chemistry to differentiate quillback rockfish from within Puget Sound in order to aid in the understanding of their population structure, with

the ultimate hope of addressing uncertainties raised from the status review of this species (see Stout et al. 2001).

Recent work by Chittaro et al. (2009) using the otolith edge chemistry of English sole (*Parophrys vetulus*) collected from Puget Sound Proper indicated significant spatial variability in trace elemental concentrations that enabled the accurate classification of individuals among regions. Using elemental analysis of otolith edges, we were also able to distinguish quillback rockfish captured from different sites and regions (Table 2; Figures 4 and 5). Some of the variability in otolith chemistry that Chittaro et al. (2009) reported was suggested to be related to the amount of human development, as well as the bathymetry and topography of Puget Sound and the surrounding area. For example, elevated concentrations of barium have been associated with deeper water and upwelling events (see Bruland 1983) as well as to petroleum products (see Dove and Kingsford 1998). But regardless of the mechanisms that influence an environments' elemental concentration, the important finding for our purposes was that spatial variability was present and that differences were detected at spatial scales smaller (i.e., sites and regions) than those used in the management of this species. These findings support the continued use of otolith chemistry to study quillback rockfish movement and population structure. However, because of when and where quillback rockfish were collected there are important limitations to this study that need to be acknowledged.

Because of the opportunistic sampling of quillback rockfish only a small number of collection years had an adequate number of fish for statistical analysis. Therefore, our picture of the spatial variability in otolith edge concentrations was limited to a few years. Further, even in the years for which we had adequate sample sizes, because relatively few sites were sampled, and none were re-sampled in different years, we were limited to assessing spatial variability in otolith chemistry to a few sites/regions, and we were unable to assess temporal variability (i.e., differences in elemental concentrations among years when using fish collected from the same site).

Understanding population structure is important for fisheries management, especially for quillback rockfish given its conservation status in Puget Sound (Gao et al. 2001, Gillanders 2002, Ashford et al. 2008). Using the DPS outlined by the BRT we observed differences in elemental signatures (obtained from the analysis of whole otoliths) that allowed most fish to be accurately classified to the DPS from which they were collected (Table 2; Figures 6 and 7). We also detected differences in whole otolith chemistry between regions (western and eastern regions of the Strait of Juan de Fuca) within the Northern Puget Sound DPS. Overall, these differences in whole otolith signatures suggest that fish from DPS (and regions) occupied environments across their lifetimes that were chemically unique, and thus implies a level of separation or population (sub)structure. However, it is important to note that several individuals were misclassified, suggesting that their lifetime chemical record was more similar to the other sampled DPS (or the other sampled region). Further, our findings of elemental differences between regions are not supported by any genetic evidence (although Seeb 1998 and Burr 1999 did report population substructure from genetic analyses between Puget Sound and San Juan Island sites), and therefore we recommend that further research is needed to illuminate the nature of this substructure.

Although differences in elemental concentrations from whole otoliths were detected, it is important to note that because of the unbalanced design and small sample sizes, this aspect of our study was limited in that the chemical signature of each DPS (or region) consisted of a different number of fish, with different ages, that were collected across several overlapping years. Furthermore, it is possible that the whole otolith chemistry disproportionately emphasizes later life cycle stages (e.g., sub-adult to adult stages) and thus what appears to be population structure may actually be late stage habitat segregation.

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Unfortunately, we were unable to quantify the extent to which these sample-related factors influenced our results. Therefore, our findings from the analysis of whole otolith, particularly that highlighting population substructure within the northern Puget Sound DPS, should be regarded cautiously until future work with more appropriate sampling is conducted.

With the majority of evidence used in the status review of quillback rockfish based on genetics, chemical information from otoliths can provide new and contemporary insights into their ecology. Although our results are based on opportunistic collections (i.e., irregular sampling, both in space and time), we feel that our findings contribute to the growing number of studies that demonstrate the utility of otolith chemistry in investigations of fish life-history and patterns of movement, which are especially important in the context of the conservation status of quillback rockfish in Puget Sound. Specifically, not only did we detect spatial variability in otolith chemistry, but this variability was significantly different at spatial scales relevant to existing management units, as well as at smaller scales (i.e., regions and sites). These results justify the continued use of otolith chemistry to obtain ecological information about quillback rockfish.

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