

Cowcod Status Report

June 13, 2011

Cowcod (*Sebastes levis*) is managed as a single stock in U.S. waters extending from the U.S.-Mexico border to just north of Cape Mendocino (40° 10' N. latitude). It was declared overfished in 2000 following the first assessment of the substock in the Southern California Bight (SCB), defined as U.S. waters south of 34° 27' N. latitude (Butler et al. 1999). The most recent benchmark or "full" assessment (Dick et al. 2007) has been updated once (Dick et al., 2009), and indicated that spawning biomass of cowcod in the SCB was between 3.8% and 21.0% of its unfished level in 2009.

From 2002-2009, total mortality of cowcod has remained below the target level (Table 1). The majority of discard mortality during this time period comes from the limited-entry trawl fishery north of 34° 27' N. latitude (NWFSC, 2010). The establishment of coastwide Rockfish Conservation Areas and Cowcod Conservation Areas south of Point Conception (34° 27' N. latitude) has been effective at minimizing cowcod bycatch.

Table 1. Total mortality (mt) of cowcod by year and area. Commercial mortality estimates are from the West Coast Groundfish Observer Program and recreational estimates are from RecFIN (catch types A and B1).

YEAR	COMMERCIAL		RECREATIONAL		TOTAL	ABC	OY
	North of 34° 27'	South of 34° 27'	North of 34° 27'	South of 34° 27'			
2002	2.90	0.00	0.09	0.49	3.47	24	4.8
2003	0.26	0.00	--	--	0.26	24	4.8
2004	0.74	0.02	--	0.45	1.21	24	4.8
2005	0.59	0.00	--	0.15	0.74	24	4.2
2006	0.87	0.00	--	0.07	0.94	24	4.2
2007	2.72	0.00	0.19	0.11	3.02	36	4
2008	0.20	0.00	--	0.25	0.44	36	4
2009	0.45	0.00	--	0.11	0.56	13	4
Grand Total	8.74	0.02	0.28	1.61	10.66		

Retention of cowcod was prohibited from 2000-2010 in all fisheries and estimates of total mortality from that time period are essentially estimates of discarded catch. The precision of discard estimates for this species is often low due to small sample size and infrequent encounters. Beginning in 2011, the rationalized trawl fleet is permitted to retain cowcod associated with Individual Fishing Quotas. Mandatory onboard observers record estimates of retained and discarded catch. Observer data from the rationalized trawl fleet has potential to provide important information for future assessments, particularly for the region north of Point Conception. Retention of cowcod remains prohibited in fisheries that are not subject to the requirement for 100% observer coverage, i.e. non-IFQ commercial fleets and the recreational fishery.

Authors of previous cowcod stock assessments made the assumption that the population in the SCB was an isolated, independent stock (Butler et al., 1999; Piner et al, 2005; Dick et al., 2009). No methods were available to directly estimate OFL for the region north of Point Conception, and abundance of the stock in Mexican waters remains unknown. Recent (and ongoing) investigations using genetics and otolith microchemistry have identified evidence of stock structure in cowcod (Attachment A). This information may help inform future Council decisions regarding management.

The procedure for calculating the cowcod OFL was revised for the 2011-2012 management cycle. The Council's Scientific and Statistical Committee classified the stock assessment for cowcod in the SCB as

a Category 2 (data-moderate) assessment. Sustainable yield from Point Conception to Cape Mendocino was estimated using a new Category 3 (data-poor) method, Depletion-Based Stock Reduction Analysis or DB-SRA. The 2011-2012 OFL for the combined stock south of 40° 10' N. latitude is now the sum of the OFLs from these two models (Table 2). To account for scientific uncertainty, the Acceptable Biological Catch (ABC) was derived from the Council's ABC control rule (Table 2). The ACL calculation followed the convention from previous management cycles, and was set equal to twice the ACL associated with the SCB substock.

Table 2. Components of the calculations used to establish 2011-2012 OFLs, ABCs, and ACLs for cowcod in the combined Monterey-Conception Area (U.S. waters south of 40° 10' N. latitude).

	Area		
	South of 34°27' N. Lat.	34°27' N. Lat. - 40°10' N. Lat.	Monterey + Conception (South of 40°10' N. Lat.)
Model	Stock Synthesis	DB-SRA	--
OFL [mt]¹	6.4	6.8	13.2
Stock Category	2(c)	3(d)	--
Assessment Uncertainty (σ)	0.72	1.44	--
P*	0.4	0.4	--
% Reduction in OFL	16.7%	30.6%	--
ABC [mt]¹	5.3	4.8	10.1
2011-2012 ACL [mt]²	1.5	1.5	3.0

¹ The Council does not specify area-specific OFLs or ABCs for cowcod. Values shown illustrate the steps taken to calculate the combined Monterey-Conception OFL and ABC

² The ACL for cowcod is based on results of a rebuilding analysis. The ACL for the Monterey and Conception areas (combined) is currently defined as twice the ACL from the area south of 34° 27' N. Lat.

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Cowcod Species of Concern 2009 Grant: Final Progress Report

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In 2009 we were awarded funding from the Species of Concern Internal Grant Program. Our project focused on the use of genetic and otolith tools to evaluate stock structure and population bottlenecks in a depleted rockfish, cowcod (*Sebastes levis*). The objectives of this project were to investigate whether A) this species is composed of one continuous stock or are there major differences in stock composition, specifically, between the two marine biogeographic regions separated by Point Conception, and B) determine if cowcod populations experienced a loss of genetic variation due to a population bottleneck (i.e. dramatic change in effective population size). To address these objectives we utilized archived tissue and otoliths from individuals that were collected along the coast of California and Oregon (by NMFSC Hook and Line Survey and their West Coast Groundfish Bottom Trawl Survey) during 2003-2008 (Figure 1).

In summary, from our genetic analysis we detected significant structure that suggested a minimum of two management units for this species. Further, cowcod have low genetic diversity, relative to other rockfishes, yet neither cowcod stock appears to have suffered detectable loss in genetic variation despite declines in abundance. From our otolith chemistry analysis we detected several regions along California's coast that varied significantly in terms of trace element concentrations. However, only marginally significant differences were detected between regions separated by Point Conception when we compared the chemical signature corresponding to an individual's entire life. These otolith chemistry results lead us to speculate that the movement of individuals and/or the yearly fluctuations of the element composition of water prevent any large-scale geographic correlations from emerging.

Results

Genetic analysis:

The microsatellite dataset was analyzed with the program STRUCTURE, and when the number of clusters was set to $K=2$, two spatially segregated groups of individuals were observed with northern and southern distributions (clusters designated in purple and red colors respectively, Figure 1). STRUCTURE analysis revealed a possible third cluster ($K=3$) was also supported and further divided the southern region (third cluster indicated with green color, Figure 1). MtDNA haplotype frequencies were also observed to differ significantly between regions separated by Point Conception (Figure 2).

Kriging analysis performed in ArcMap (ESRI) using the individual STRUCTURE assignment values (Figure 1) indicated the spatial boundary between STRUCTURE populations is not located exactly at Point Conception (boundary A, Figure 1), but appears to be located further south near the Channel Islands (boundary B, Figure 1).

The location of boundary B as a more accurate division between distinct genetic lineages is supported using F -statistics. The microsatellite pairwise F_{ST} value was found to be higher between regions divided by boundary B ($F_{ST} = 0.092$) compared to those divided by boundary A ($F_{ST} = 0.066$, Table 1). In contrast, the mitochondrial pairwise F_{ST} values were nearly equivalent when comparing regional divisions using either boundary A or B (Table 1).

The cowcod regions divided at boundary B were found to have higher mtDNA gene diversity in the southern region versus northern (Table 1). This trend was not as dramatic when comparing microsatellite gene diversity, moreover, the northern region had slightly higher microsatellite allelic richness (Table 1). Compared to other rockfishes, cowcod are relatively low in genetic diversity; they were found either in the bottom third (mtDNA gene diversity; Figure 3) or the least genetically diverse (msat gene diversity; Figure 4).

Among the four tests we conducted to obtain evidence for population bottlenecks, two tests using BOTTLENECK and M-ratio analyses were found not to be significant (Table 2). However, our estimates of effective population size using LDNe indicated infinite and relatively small effective sizes for the northern and southern regions, respectively (Table 2). In addition, calculation of Tajima's D using the mtDNA sequences showed significantly negative values for both northern and southern regions which suggests rapid demographic expansion has occurred (Table 2).

Otolith chemical analysis:

The chemistry at the edge of the otolith was used to assess the extent to which spatial variability existed in trace elemental concentrations. Since we were interested in assessing spatial variability we focused on fish collected within a year so as to prevent any temporal variability in trace elemental signatures from confounding our analysis.

Using quadratic discriminant function analysis (a multivariate approach to assessing the extent to which individuals can be correctly assigned to a region from which they were collected) to analyze otolith edge concentrations, we detected significant differences among three regions in 2004 and 2005 (see below and see Figure 5 A, B, D, & E). Interestingly, the spatial pattern of elemental differences varied between the two

years of data. Unfortunately, given that fish were collected from different locations in 2004 and 2005 we are unable to speculate as to whether the temporal differences are due to circulation patterns (i.e., location, strength, and/or duration of ocean currents) or are a result of sampling different areas.

Otoliths of fish collected in 2004 (Wilks Lambda = 0.60; Approx F (10,78) = 2.68 $p = 0.02$) showed barium to be positively correlated with discriminant function 1 (Figure 5D). For discriminant function 2 magnesium and manganese were positively correlated while lead was negatively correlated. Fish collected in 2005 (Wilks Lambda = 0.66; Approx F(10, 88) = 1.99; $p < 0.05$) showed differentiation along discriminant function 1 for which strontium and barium were negatively correlated and magnesium was positively associated. Manganese and lead were positively associated with discriminant function 2, while magnesium was negatively correlated (Figure 5E).

Jack-knife classification (wherein one individual at a time is removed from the dataset, treated as an unknown sample, and reclassified to assess the accuracy of the differentiation) revealed moderate levels of correct classification; for fish collected in 2004 jack-knife classification was 63%, while 67% for those collected in 2005.

As otoliths grow throughout the life of an individual they continually record the environment in which the fish reside. This environmental record is the result of ions of trace elements found in the water being absorbed by a fish and deposited onto the otolith. By analyzing the chemistry of the entire otolith will therefore provide information corresponding to the entire life of the fish. For our purposes we were interested in determining the extent to which fish from different populations (north and south of our genetic boundaries; see Figure 1) varied in terms of their trace elemental concentrations. Our analysis indicated that there was marginal significant differences between fish collected north and south of Point Conception (Wilks Lambda = 0.89; Approx F(5,90) = 2.149; $p = 0.06$), and that 72% of the individuals could be correctly classified to the region from which they were collected (Figure 5 C & F; magnesium and manganese were positively and negatively associated with discriminant function 1, respectively).

Discussion and Management Implications

Based on the genetic analyses, there are a minimum of two genetically distinct stocks (i.e. lineages) of cowcod with greatest support for separation at boundary B. The precise mechanism responsible for the genetic differentiation observed among these lineages is presently unknown. The presence of these two lineages combined with our finding that there are chemical differences that distinguish the environments inhabited by these lineages, supports the idea of separate ecological compartments as proposed by Longhurst (2007). We expected that if cowcod was shown to represent more than one population, these populations would be separated at Point Conception, because other studies demonstrated this location to be both an inter- and intra-specific biogeographic boundary. However, the location of the genetic boundary between these lineages appears to be further south of Point Conception. Future studies should investigate how stable is the location for boundary B. If boundary B were found to be relatively stable over time, perhaps oceanographic features restrict gene exchange between coastal and island habitats. Although it is possible that Point Conception really does delineate a boundary

between two ecological compartments that are also relevant for cowcod, but shifts in upwelling or stochastic processes in recruitment blur this boundary during particular time periods. If this were the case, it would help explain why whole otolith microchemistry was unable to show a large difference between cowcod samples collected north and south of Point Conception, despite the fact that highly genetically diverged lineages are present.

Compared to other rockfishes, cowcod has relatively low genetic diversity, but it is difficult to say whether a bottleneck event occurred and is the primary cause of this low genetic diversity. From stock assessments, we are certain that the abundance of cowcod has declined at least to 3.4% - 16.3% of historical biomass (Dick et al. 2007). However, it is possible that even this severe decline would present a challenge for genetic detection given the fact that the effective population size of marine fishes can be disproportionately higher than abundance estimates. The only tests that may support a bottleneck event are the LDNe estimates which showed a remarkably low effective size of the southern population. In addition, the Tajima's D statistics showed that both the northern and southern populations may have experienced rapid demographic expansion, presumably following a severe reduction.

The low effective population size estimate of the southern population seems strange given the fact that we found generally higher genetic diversity of the southern stock compared to its northern counterpart. The way that LDNe estimates effective population size is related to linkage disequilibrium which is not only influenced by effective population size but also cryptic population structure (i.e. Wahlund effects). Since we found that the southern region could be further divided into two groups using STRUCTURE, the low effective size estimate may simply be due to this Wahlund effect. It is interesting though that only the southern region could be further resolved into two STRUCTURE groups. This may be due to multiple sweepstakes-type of recruitment events that occurred in this region, and could be tested by evaluating age structure among these groups. We would expect one group to be generally composed of older age classes than the other.

Our study supports managing cowcod as two separate stocks. The challenge to managers will be in demarcating these stocks. For simplicity, Point Conception may be a useful proxy for the boundary between the major genetic lineages; however, a biologically more accurate boundary would require a higher resolution parsing of these regions to reflect boundary B. Our analyses support that these lineages occupy different environments and so are not likely ecologically interchangeable. Overfishing may not be the sole reason for the low genetic diversity observed in this species, but care should be taken to prevent any further loss of the level of diversity that is present.

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Attachment A

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Table 1. Genetic diversity measures and F -statistics for regions separated by either boundary A or B.

Boundary	Population	N	Pairwise F_{ST}		Gene diversity		Allelic richness	# of haplotypes	F_{IS}
			Microsatellite	Mitochondrial	Microsatellite	Mitochondrial			
A	North	141	0.066	0.348	0.331 ± 0.059	0.546 ± 0.045	4.836	11	0.060
	South	153			0.358 ± 0.064	0.753 ± 0.033	4.218	24	0.147
B	North	168	0.092	0.343	0.328 ± 0.059	0.516 ± 0.043	4.871	11	0.061
	South	126			0.357 ± 0.063	0.785 ± 0.032	4.265	22	0.136

Table 2. Tests for evidence of bottlenecks. The analyses BOTTLENECK, M-ratio, and LDNe were performed using the microsatellite data. Tajima's D was estimated using the mitochondrial data.

Boundary B Population	BOTTLENECK (Two-phase mutation model)			M-ratio		LDNe (95%CI)	Tajima's D	Tajima's D P
	# Loci with Het. Deficit	Excess	Wilcoxon Test 1-tail P H-excess	M-ratio	P			
North	17	6	1.000	0.794	0.337	Infinite	-1.424	0.048
South	6	10	0.372	0.791	0.319	16.0 (11.8-21.2)	-1.411	0.046

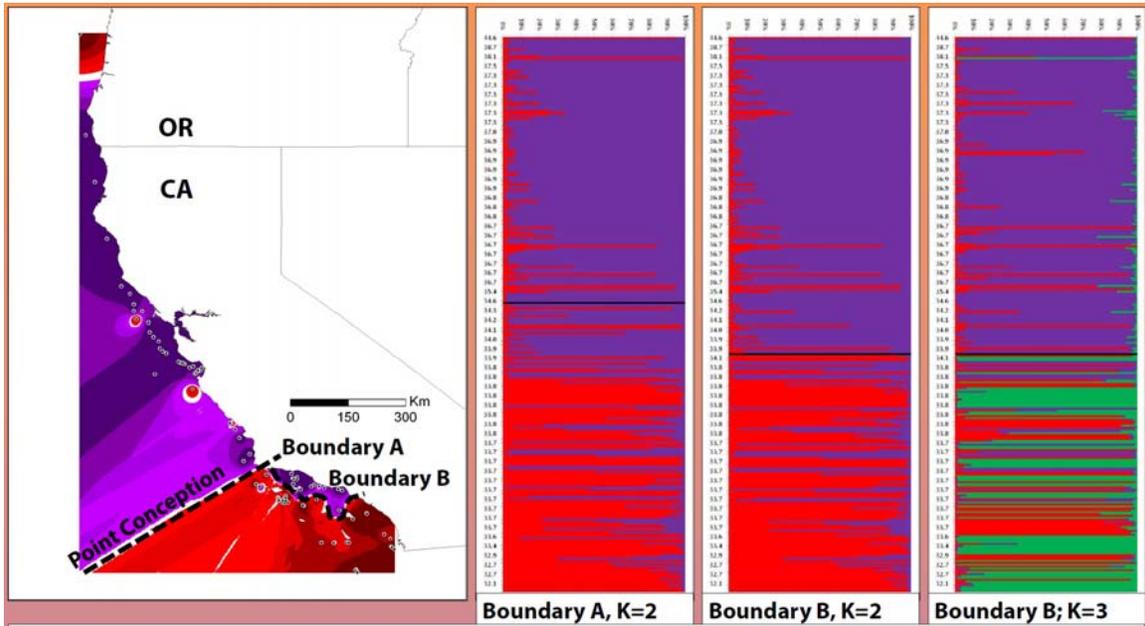


Figure 1. Map of cowcod samples. The circles indicate locations where cowcod were collected, and shading in two colors corresponds to areas of high averages of individual assignment ($Q=55-100\%$) to one of two STRUCTURE clusters. The clusters come into contact in a region of the Channel Islands and mainland coast south of Point Conception (boundary B). Vertical STRUCTURE plots show all individual Q scores by latitude, for either boundary A (Point Conception) or boundary B, and either two or three clusters (K).

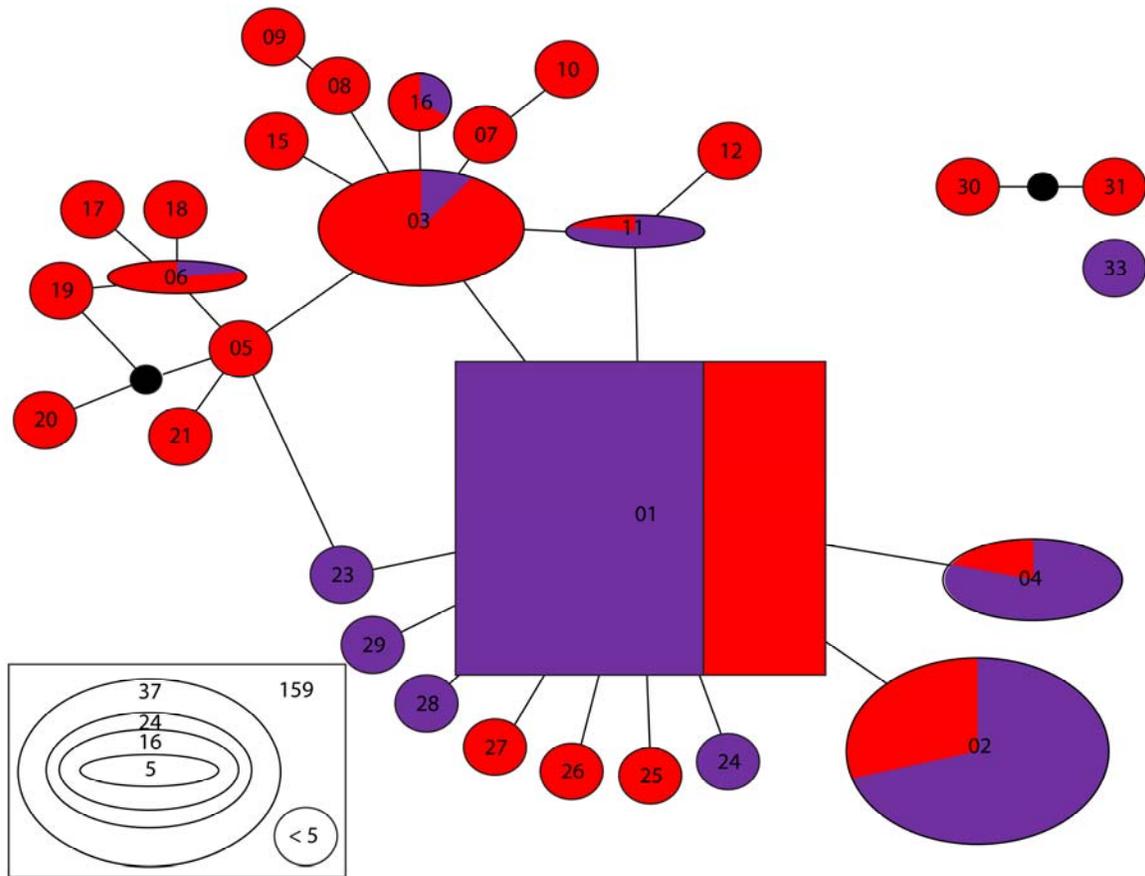


Figure 2. Mitochondrial sequence network and haplotype frequencies for two cowcod populations. Each shape in the network represents a unique sequence, solid dots are unobserved haplotypes, and lines represent a single mutation. Size of the shape is scaled to the overall frequency (legend of sample sizes; bottom left). Pie charts indicate haplotype frequencies for two regions separated by boundary B (Fig. 1). Purple and red colors indicate proportions of individuals from the northern and southern region, respectively. Haplotypes 30, 31 and 33 represent large in-dels that cannot be connected to main network.

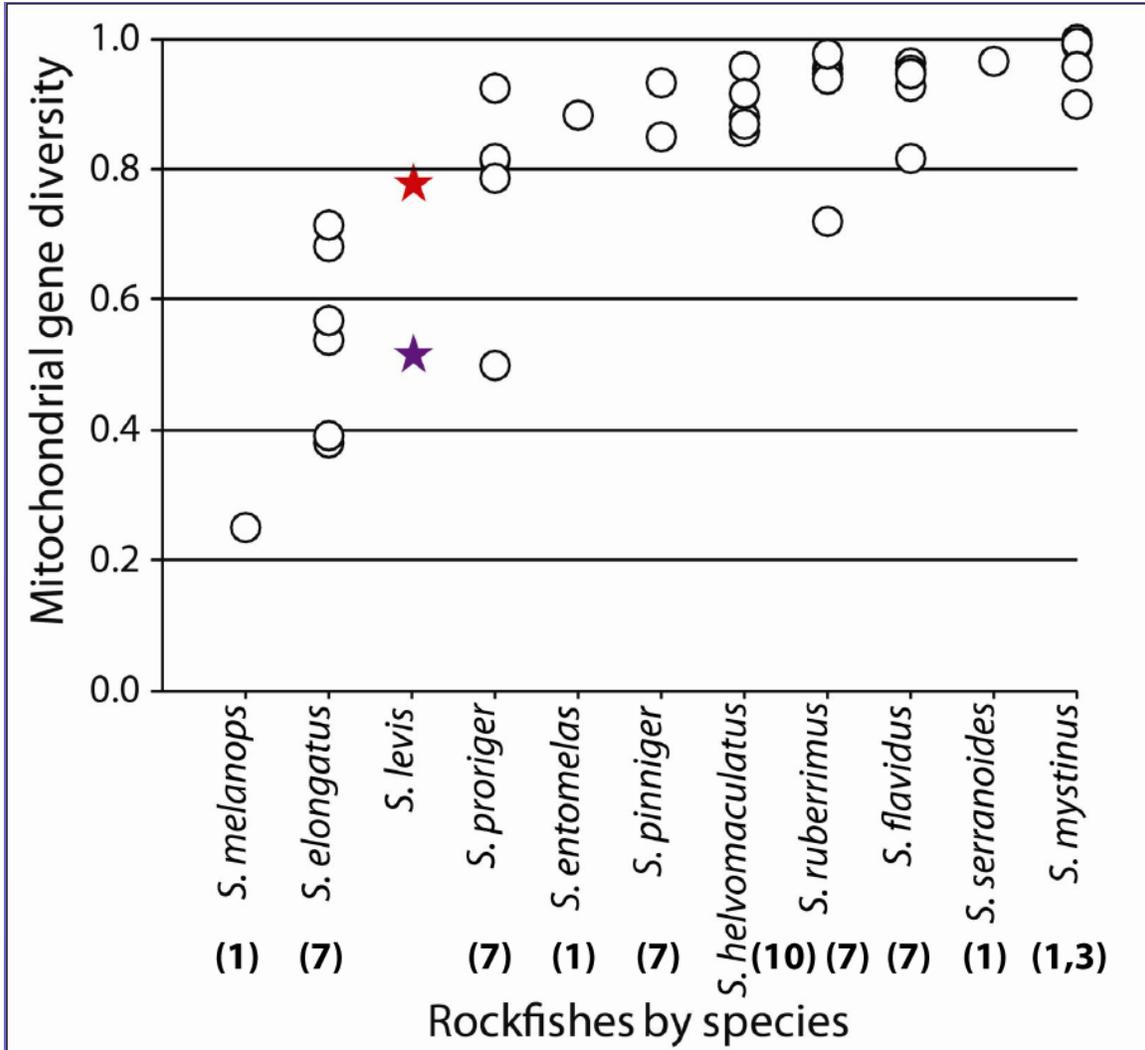


Figure 3. Comparison of mitochondrial d-loop gene diversity among rockfishes. Data from eleven different rockfish species were compared to cowcod populations north and south of boundary B (purple and red stars, respectively). Each data point is based on individual collections from both published and unpublished studies (#'s= citations).

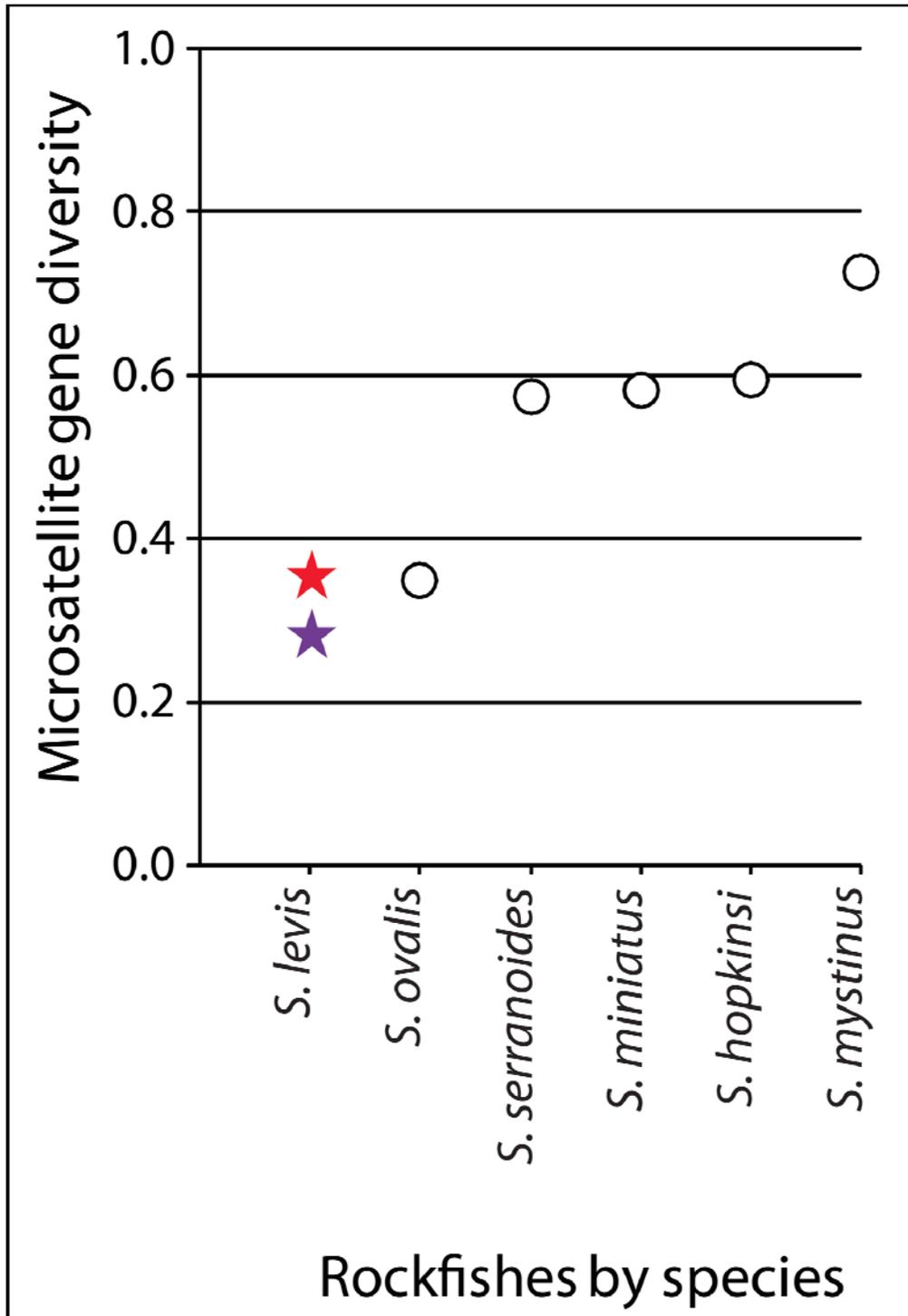


Figure 4. Comparison of microsatellite gene diversity among rockfishes. Data from five rockfish species genotyped with the same 24 loci are compared to cowcod populations north and south of boundary B (purple and red stars, respectively). None of the loci were discovered in these species (i.e. no ascertainment bias). All collections came from Central or Southern California (Gilbert-Horvath, L. and Garza, J.C.; unpublished data).

Attachment A

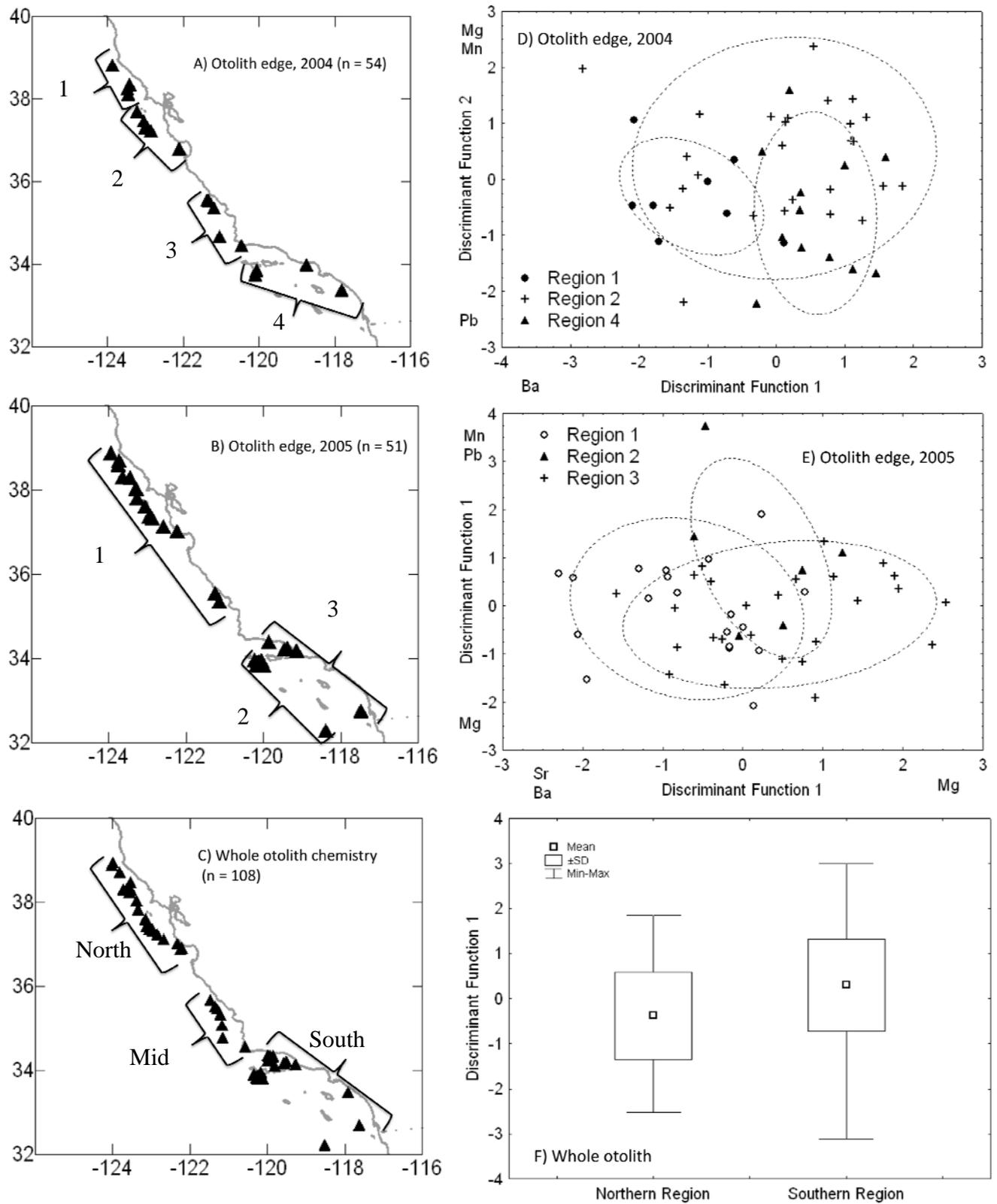


Figure 5: Locations (triangles) from which fish were collected, and used for otolith edge analysis (2004, A; 2005 B) and whole otolith analysis (C). Factor scores produced from discriminant function analyses (DFA) of chemical signatures derived from otolith edges

(2004, D; 2005, E) and whole otoliths (F) provide a visual representation of the the degree of overlap in trace element concentrations. DFA indicated significant differences (Wilks lambda $p < 0.05$; jack-knife classification of 63%) in otolith edge concentrations among fish (collected in 2004, A & D) from regions 1, 2, and 4. A DFA of fish collected in 2005 (B & E) indicated that three regions were significantly different (Wilks lambda $p < 0.05$; jack-knife classification of 67%) also in terms of edge concentrations. Finally, marginal significance (Wilks lambda $p = 0.06$; jack-knife classification of 72%) was detected from the DFA of whole otolith trace elemental concentrations (C & F)

Attachment A

Genetic and otolith related tasks that were completed using Species of Concern Grant funding.

Location of work	Collaborators	Purpose	Task
NWFSC	Anna Elz & Jon Hess	Genetic data collection	-Tissue samples (n=204) were successfully extracted. -Following tissue exchange with SWFSC, 320 individuals were sequenced for 500bp of the control region (d-loop) of the mitochondrial genome.
NWFSC	Jon Hess	Microsatellites analysis	-16 loci were found to be polymorphic (more than 1 allele present) in both stocks north and south of Point Conception. -Basic diversity statistics were conducted and a STRUCTURE analysis, and heuristic spatial interpolation were performed (Figure 1).
NWFSC	Jon Hess	Mitochondrial sequence analysis	-33 haplotypes (unique sequences) found among 320 individuals. -Calculated frequencies of haplotypes in the northern and southern cowcod populations (Figure 2).
SWFSC	Libby Gilbert-Horvath and John Carlos Garza	Genetic data collection	-122 tissue samples were successfully extracted. -24 microsatellite loci were optimized for genotyping. -We used the following published loci:Sme4, Spi14, Spi6, Sra15-8, Sal3, Seb25, Sme11, Sme3, Sme5, Spi4, Sra16-5, Sal4, Sra7-2, Ssc51, Sth45, Seb9, Sme13, Sra1523, Sth37, Sal2, Seb37, Spi12, Sra6-52, and Ssc1 (Westerman et al 2005, Gomez-Uchida et al. 2003, Miller et al. 2000, Seeb unpublished). -After tissue exchange with NWFSC, 294 individuals were successfully genotyped for at least 16 loci out of a total possible 24 loci available.
NWFSC	Paul Chittaro	Otolith microstructure and chemistry data collection	-200 otoliths were polished and digital images taken. -Microstructural analysis conducted at NWFSC. Estimates obtained of daily somatic growth and annual somatic growth. -Microchemical analysis using LA-ICPMS (GeoAnalytical Lab, Washington State University) was used to acquire trace elemental (magnesium, manganese, strontium, barium, and lead) concentrations along a transect from the otolith core to its edge. -Multivariate analyses conducted to investigate spatial and temporal variability in trace elemental concentrations and population structure.

Dissemination of project results

The genetics and otolith chemistry aspects of this project have been disseminated separately. Below are the corresponding abstracts submitted to AFS 2010, Pittsburgh.

National American Fisheries Society September 13, 2010; Pittsburgh, PA

Poster presentation entitled “Genetic Evaluation of Stock Structure and Population Bottlenecks in the Severely Depleted Cowcod”

Co-authored by- Jon E. Hess, Paul Chittaro, Anna Elz, , Libby Gilbert-Horvath, Victor Simon and John Carlos Garza

Abstract:

Cowcod (*Sebastes levis*) range from Oregon to Baja California and are currently assumed to be one continuous population. Since 2004, it has been on the National Marine Fisheries Service “Species of Concern” list due to its dramatic decline in abundance (<3.4% - 16.3% of historical biomass). We address the following questions: 1) Is there population subdivision within the species, specifically, between two marine biogeographic regions separated by Point Conception, and 2) Have cowcod populations experienced loss of genetic variation due to a reduction in population size? Sixteen variable microsatellite loci were genotyped and ~500 bp of the mitochondrial control region were sequenced for 294 fish distributed throughout the species range. We observed significant structure when fish were grouped into two regions separated by Point Conception ($F_{ST}=0.066$). However, upon further inspection, an individual-based spatial analysis using Bayesian cluster assignment of the microsatellite genotypes localized this genetic break further south ($F_{ST}=0.092$), corresponding to separation between a Channel Islands region versus the mainland. These results indicate a minimum of two management units in this species. In general, cowcod shows low genetic diversity compared to other rockfishes. However, neither cowcod stock appears to have suffered detectable loss in genetic variation, despite declines in abundance.

National American Fisheries Society September 13, 2010; Pittsburgh, PA

Oral presentation presentation entitled: “Differential Somatic Growth Rates and Population Subdivision of a Region Separated by Point Conception, CA, in a Depleted Rockfish (*Sebastes levis*).”

Co-authored by- Paul Chittaro, Jon E. Hess, Victor Simon, and John Carlos Garza

Abstract:

Cowcod rockfish (*Sebastes levis*), a once commonly harvested species was declared overfished in 2000, and added to the National Marine Fisheries Service Species of Concern list in 2004. To assist in the management of this species, we used otolith chemistry and microstructure to obtain information related to population structure and the spatial variability in daily and annual somatic growth. Using trace element concentrations from the otolith edge (representing less than one year of growth) of fish collected in 2004 and 2005, we observed significant spatial variability that permitted the correct classification (using QDFA) of fish to their location of collection (63% and 75% jack-knife classification). To investigate whether individuals collected from recently

identified genetic populations (separated at Point Conception, CA) occupied chemically distinct environments during their lives, we examined whole otolith elemental signatures (representing the concentration averaged over a fish's entire life). Results indicated that cowcod collected north (n=48) and south (n=48) of Point Conception could be moderately differentiated (jack-knife classification of 72%). Next, we tested how variable otolith chemical profiles were among fish from different locations and different populations yet of the same age and hatch year. Using age-specific barium concentrations, acquired from laser ablations of the otolith edge to core, we observed instances where fish from different locations or populations varied significantly in their concentrations for one or more years of their lives. Finally, using otolith microstructural analyses we detected significantly higher juvenile somatic growth for fish collected south of Point Conception compared to those north, yet no differences were observed in terms of annual increments. Overall, these results suggest that chemical differences in the environment exist that would permit more detailed analyses of population structure and connectedness, although temporal variability in elemental concentrations will need to be considered in future research. Additionally, our observation of growth differences between populations highlights the need for research to examine factors that are responsible for improved growth and whether this results in increased survival.