MARINE BIOTECHNOLOGY

Population Structure and Stock Identification of Eulachon (*Thaleichthys pacificus*), an Anadromous Smelt, in the Pacific Northwest

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Abstract

The genetic structure of eulachon (*Thaleichthys pacificus*) populations was examined in an analysis of variation of 14 microsatellite loci representing approximately 1900 fish from 9 sites between the Columbia River and Cook Inlet, Alaska. Significant genetic differentiation occurred among the putative populations. The mean F_{ST} for all loci was 0.0046, and there was a significant correlation between population genetic differentiation (F_{ST}) and geographic distance. Simulated mixed-stock samples comprising populations from different regions suggested that variation at microsatellite loci provided reasonably accurate estimates of stock composition for potential fishery samples. Marine sampling indicated that immature eulachons from different rivers, during the 2 to 3 years of prespawning life in offshore marine waters, do not mix thoroughly. For eulachons captured incidentally in offshore trawl fisheries, there was a clear geographic cline in relative abundance of eulachons from different geographic areas. The sample from northern British Columbia was dominated by northern and central coastal populations of British Columbia, the sample from central British Columbia was composed of eulachons from all regions, and the sample from southern British Columbia was dominated by Columbia River and Fraser River populations. These results have implications for the management of trawl fisheries and conservation of spawning populations in some rivers where abundance is at historically low levels.

Key words: eulachon — microsatellite variation — mixed-stock fisheries — population structure — stock identification

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Introduction

Eulachons (*Thaleichthys pacificus*) are an anadromous semelparous smelt (Osmeridae) whose distribution is confined to eastern North America between northern California and the eastern Bering Sea (Hay and McCarter, 2000). Within this range they spawn only in a limited number of rivers, mainly those that have a pronounced spring runoff. Within rivers the duration of the spawning period may be several weeks. Spawning usually begins in January or February in southern rivers such as the Columbia River, and extends into June in northern Alaskan rivers. There is some unexplained temporal variation within this range. For example, the Fraser River population in southern British Columbia spawns mainly in April, later than most northern populations, such as those in the Nass and Skeena rivers, which spawn mainly in March. Although the ecologic basis of spawning times is not clear, it appears that within specific river drainages, eulachons generally have a characteristic timing of spawning. During spawning mature fish spawn a short distance upriver, with the eggs adhering to a sandy substrate or other debris. The larvae generally hatch within 2 to 4 weeks and then are washed downstream, where they may remain in lower-salinity estuarine waters for several weeks. They then move to nearshore waters, where they remain until they become sexually mature, which is thought to be primarily at 3 years of age (Hay and McCarter, 2000). They return to spawn in freshwater, after which they probably die. Eulachons are very high in oil content (Payne et al., 1999), so much so that dried eulachons can be burnt as candles (hence one of the common names of candlefish). The oil is unique among fishes in that it is solid at room temperatures, and in British Columbia, Canada, eulachons have enormous social and cultural significance to First Nations (aboriginal) peoples (Kuhnlein et al., 1982).

Eulachon abundance has varied widely, but nearly all eulachon spawning runs have declined from California to southeastern Alaska in the past 20 years, especially since the mid 1990s (Hay and McCarter, 2000). The cause of these declines remains uncertain. Eulachons are caught as bycatch during shrimp fishing, but in most areas the total bycatch is small, so other factors may be important in the decrease in abundance. The decline in eulachon abundance has prompted fishery managers to limit shrimp fisheries in marine areas adjacent to rivers with poor spawning runs. Although abundance has increased in some British Columbia rivers since 2000, in other rivers, particularly in the central coastal region of British Columbia, levels remain very low.

Effective conservation and management of eulachons require determination of genetic structure of the exploited populations. In some respects eulachon life history is similar to salmonid life history. They return to spawn in freshwater after an extended marine residence. It follows that, as with salmonids, there may be a potential for the development of genetically distinct populations of eulachons. Previous analysis of vertebral number had indicated significant differences among eulachons in different river drainages (Hart and McHugh, 1944). When examined throughout the entire range within British Columbia, the timing of spawning of eulachons in rivers can differ by more than 3 months. Such variation in spawning time is consistent with the hypothesis of local adaptation and genetic differences among populations spawning in different watersheds. Additionally, trends in eulachon population abundance vary among rivers. Since 2000 abundance in some British Columbia rivers, such as the Nass, Skeena, and Fraser, has increased, while in others, such as the Bella Coola in the central coast, it has decreased. These observations support the hypothesis of population differentiation among eulachon spawning in different rivers.

If eulachon spawning runs are distinct, this should be reflected in surveys of genetic variation. In a previous study that centered on surveying genetic variation at the ND5/6 and 12S/16S regions of the mitochondrial genome by restriction fragment length polymorphism analysis, little genetic differentiation was observed among spawning eulachons from the Columbia River in the south to Cook Inlet in central Alaska (McLean et al., 1999). The results of this analysis formed the basis of eulachons in British Columbia and elsewhere being considered as a single stock for assessment and management (Hay and McCarter, 2000). Subsequent analysis of microsatellite variation revealed more differentiation than that observed with mtDNA, yet it was difficult to define distinct demographic units (McLean and Taylor, 2001). The microsatellite loci surveyed in that analysis were characterized by low levels of variation (number of alleles ranged from 3 to 10, heterozygosity from 4% to 64%). In Pacific salmon, loci with such a restricted number of alleles are generally less valuable than more variable loci in detecting population differentiation (Beacham et al., 2002).

Eulachon populations in British Columbia have been a focus of conservation concern for the last 5 years, but a fundamental question about eulachon population structure remains uncertain. Specifically, what is the geographic unit that may be genetically distinct? Are eulachons in the eastern Pacific one genetic stock, as was indicated by the survey of mtDNA variation, or as with anadromous salmonids, is there a level of genetic differentiation among eulachons that is linked to their spawning in rivers or watersheds in the eastern Pacific?

The objective of the current study was to assess the population structure of eulachons within British Columbia, and evaluate whether they were genetically distinct from more distant populations in the Columbia River and Cook Inlet. If some level of genetic differentiation were observed, then we wanted to evaluate the utility of using microsatellite variation for stock identification of mixed-stock samples of eulachons from nearshore marine waters.

Materials and Methods

Collection of DNA Samples and Laboratory Analysis. Putative eulachon populations were genetically characterized with tissue samples obtained from previously collected frozen adult fish sampled from freshwater spawning locations (Table 1). Three mixed-stock samples were also collected and analyzed to determine the origins of eulachons in the samples. The first sample was collected near Nootka Sound, and the second sample was collected near the Goose Island Group during trawl surveys on shrimp abundance aboard the research vessel W. E. Ricker during May 2000 (Figure 1). The final sample was collected from bycatch in research surveys near Chatham Sound during March 2001. DNA was extracted from all samples as described by Withler et al. (2000). Polymerase chain reaction (PCR) products at 14 microsatellite loci, *Tca103, Tca104, Tca111, Tca112, Tca113, Tca114, Tca115, Tca117, Tca118, Tca119. Tca121, Tca122, Tca127*, and *Tca129* (Kaukinen et al., 2004), were size fractionated on denaturing polyacrylamide gels, and allele sizes were determined with the ABI377 automated DNA sequencer. Allele sizes were determined with Genescan 3.1 and Genotyper 2.5 software (PE Biosystems).

Table 1. Population, Sample Collection Years, Number of Eulachon Sampled per Year, and Total Number of Fish Sampled for 9 Sampling Sites

Population	*Years sampled*	*N*	*Total N*
Columbia River	2000	74	74
Cowlitz River	2002	200	200
Fraser River	2000	421	421
Klinaklini River	2002	80	80
Bella Coola River	1997, 1998	55, 108	163
Kemano River	1995, 2001	53, 99	152
Skeena River	2001	367	367
Nass River	1996, 1997, 1998	69, 200, 31	300
Twenty-mile River	2001	101	101
Mixed stock samples			
Nootka Sound	2000	184	184
Goose Island Group	2000	200	200
Chatham Sound	2001	100	100

Data Analysis. Each population at each locus was tested for departure from Hardy-Weinberg equilibrium (HWE) using GDA (Lewis and Zaykin, 2001). Annual samples within populations were tested separately, with 13 tests conducted at each locus. Critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice, 1989). The number of alleles observed per locus was computed with GDA. All annual samples available for a location were combined to estimate population allele frequencies, as recommended by Waples (1990). Weir and Cockerham's (1984) F_{ST} estimates for each locus over all populations were calculated with FSTAT Version 2.9.3.2 (Goudet, 2001), as was the pairwise F_{ST} over all populations. The significance of the multilocus F_{ST} value over all samples was determined by jackknifing over loci. Cavalli-Sforza and Edwards (1967) chord distance (CSE) was used to estimate genetic distances among all populations. An unrooted consensus neighbor-joining tree based on 500 replicate trees was generated with CONSENSE from PHYLIP (Felsenstein, 1993). FSTAT was used to conduct Mantel's (1967) regression of the pairwise F_{ST} values

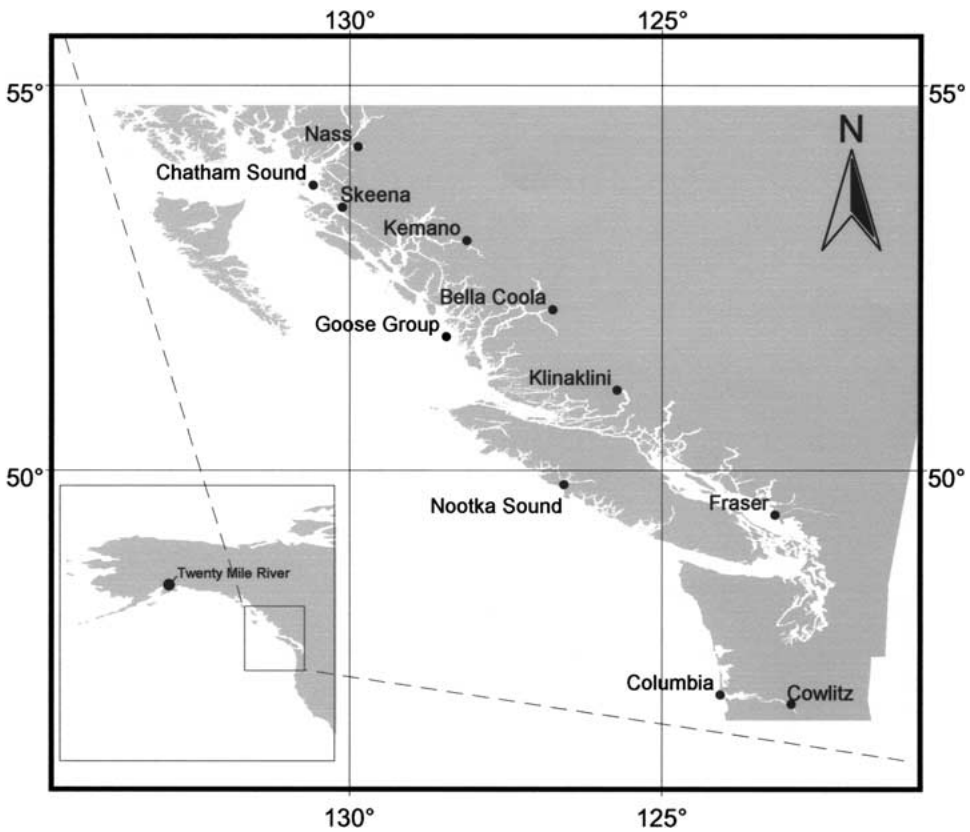

Fig. 1. Map indicating sampling locations of 9 populations and 3 mixed-population samples (Chatham Sound, Goose Group, and Nootka Sound).

Table 2. Number of Alleles, Expected Heterozygosity (H_e), Observed Heterozygosity (H_o), Number of Significant Hardy-Weinberg Equilibrium Tests (N = 13 tests), and F_{ST} Among Nine Eulachon Populations for 14 Microsatellite Loci

Locus	*Alleles*	H_e	H_o	*HWE*	F_{ST} *(SD)*
Tca103	40	0.95	0.91	0	0.0009 (0.0004)[a]
Tca104	52	0.80	0.74	3	0.0080 (0.0064)
Tca111	15	0.78	0.78	0	0.0212 (0.0071)[a]
Tca112	25	0.88	0.86	0	0.0016 (0.0007)[a]
Tca113	32	0.86	0.88	0	0.0015 (0.0009)
Tca114	18	0.86	0.90	2	0.0027 (0.0013)[a]
Tca115	15	0.70	0.74	0	0.0014 (0.0012)
Tca117	29	0.54	0.49	1	0.0081 (0.0032)[a]
Tca118	13	0.58	0.62	1	0.0018 (0.0016)
Tca119	19	0.72	0.65	5	0.0056 (0.0023)[a]
Tca121	62	0.94	0.84	8	0.0027 (0.0013)[a]
Tca122	42	0.90	0.83	4	0.0025 (0.0010)[a]
Tca127	29	0.75	0.73	1	0.0029 (0.0010)[a]
Tca129	32	0.90	0.89	0	0.0034 (0.0011)[a]
All loci	30.2				0.0046 (0.0014)[a]

[a] $P < 0.05$

on geographic distance to test for ''isolation-by-distance'' among populations. Geographic distances were measured as the shortest water distance between populations. Variance components of population differences and annual variation within populations were estimated with GDA. Only populations with 2 or more years of sampling (3 populations, Table 1) were included in the analysis. Negative variance components were set to zero in estimation of relative diversity. (Allele frequencies for all location samples surveyed in this study are available at *http://www.sci.pac.dfo-mpo.gc.ca/mgl/default_e.htm.*)

Estimation of Stock Composition. Genotypic frequencies were determined at each locus in each population, and the Bayesian procedure of estimation of stock composition as outlined by Pella and Masuda (2001) was used in the analysis of the mixed-stock samples. All loci were considered to be in HWE, and expected genotypic frequencies were determined from the observed allele frequencies. Each baseline population was resampled with replacement to simulate random variation involved in the collection of the baseline samples before the estimation of stock composition of each simulated mixture. Simulated mixtures composed of southern populations and mixtures of southern and northern populations were examined to evaluate accuracy and precision of the stock composition estimates. Simulated fishery samples of 150 fish were generated by randomly resampling with replacement the baseline populations in each drainage. Estimated stock composition of a simulated mixture was then determined, and the whole process was repeated 25 times using the program SIMWRITE (M. Masuda, National Marine Fisheries Service, Auke Bay Laboratory, personal Communication) to estimate the mean and standard deviation of the individual stock composition estimates.

Results

Variation Within Populations. All loci surveyed were polymorphic in all of the sampled populations. The number of observed alleles at each locus ranged from 13 to 62, and expected heterozygosity at a locus ranged from 0.54 to 0.95 (Table 2). Maximum heterozygosity was observed at *Tca103*, but it did not have the largest observed number of alleles. Expected heterozygosity was similar among all putative populations, ranging from 0.78 to 0.81. Genotypic frequencies at each locus within sampling location and year generally conformed to those expected under HWE. Possible exceptions were *Tca119, Tca121*, and *Tca122*, for which substantially more of the HWE test results were significant than would be expected by chance (Table 2). More homozgyous fish than expected were observed at these loci. Samples from the major river systems in British Columbia accounted for over 50% of the non-HWE distributions of allele frequencies (Skeena River, 6 tests significant; Nass River, 4 tests; Fraser River, 3 tests). This indicates that those samples may have contained fish from at least 2 separate spawning populations (homozygous excess as a result of the Wahlund effect).

Distribution of Genetic Variation. Gene diversity analysis of the 14 loci surveyed was used to determine the magnitude of annual variation within populations relative to differentiation among 3 putative populations (Nass River, Kemano River,

Table 3. Pairwise F_{ST} Averaged Over 14 Loci for Eulachon from Nine Locations[a]

	Cowlitz	*Fraser*	*Klina*	*Bella*	*Kemano*	*Skeena*	*Nass*	*Twenty*
Columbia	0.0020	0.0022	0.0130	0.0108	0.0083	0.0073	0.0085	0.0068
Cowlitz		0.0016	0.0095	0.0083	0.0066	0.0039	0.0056	0.0048
Fraser			0.0083	0.0062	0.0049	0.0038	0.0051	0.0052
Klinaklini				(0.0014)	(0.0022)	(0.0033)	(0.0037)	0.0091
Bella Coola					0.0019	0.0033	0.0037	0.0071
Kemano						0.0016	0.0028	0.0068
Skeena							0.0035	0.0056
Nass								0.0056

[a]All values were significant ($P < 0.05$) except for those in parentheses.

and Bella Coola River). The amount of variation contained within populations averaged 99.6% for the microsatellite loci. Variation among the 3 putative populations was the largest for *Tca104*, accounting for 1.8% of total observed variation at the locus ($F = 7.62$, $df = 2$ and 3, $0.5 < P < 0.10$). Population differentiation at *Tca117* accounted for 1.4% of the observed variation ($F = 9.19$, $df = 2$ and 3, $0.05 < P < 0.10$). For the remaining loci differentiation among sampling years within populations was similar to the level of differentiation among populations for these 3 putative populations.

Population Structure and Geographic Variation. Significant genetic differentiation was observed among the 9 putative populations of eulachon sampled in our study. The overall F_{ST} for the 14 microsatellite loci surveyed was 0.0046, with individual loci values ranging from 0.0014 at *Tca115* to 0.0212 at *Tca111*, and with 10 of 14 values significantly greater than zero ($P < 0.05$) (Table 2). Differentiation was observed among the putative populations in pairwise comparisons (Table 3). The greatest differentiation was observed between the Columbia River drainage populations and those populations north of the Fraser River. Within the Columbia River drainage, reduced but significant differentiation was observed between samples taken from the Cowlitz River (a tributary that drains into the Columbia) and samples from mainstem Columbia River. At larger geographic scales, regional structuring of population samples was observed in our study. For example, southern populations (Fraser, Columbia, and Cowlitz) clustered together 97% of the time, and central coast populations (Bella Coola, Klinaklini, and Kemano) clustered together 88% of the time (Figure 2).

Is there a relationship between genetic differentiation and geographic separation? The regression of all pairwise F_{ST} values on geographic distance was significant ($r = 0.34$, $P < 0.05$), and geographic distance accounted for 11.6% of the observed variation in F_{ST} values (Figure 3). The significant correlation between genetic and geographic distances for putative eulachon populations was consistent with an isolation-by-distance relationship, but clearly factors other than geographic separation also contributed to the observed genetic variation.

Stock Identification. We examined whether the genetic differentiation observed among the eulachon populations surveyed in our study was sufficient for

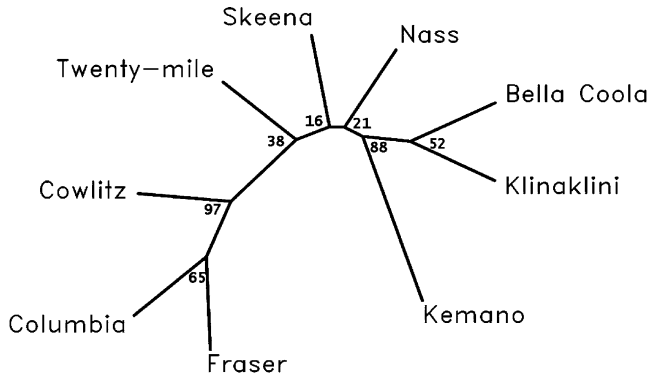

Fig. 2. Unrooted neighbor-joining tree of Cavalli-Sforza and Edwards (1967) chord distance for 9 populations of eulachons surveyed at 14 microsatellite loci. Bootstrap values at the tree nodes indicate the percentage of 500 trees where populations beyond the node clustered together.

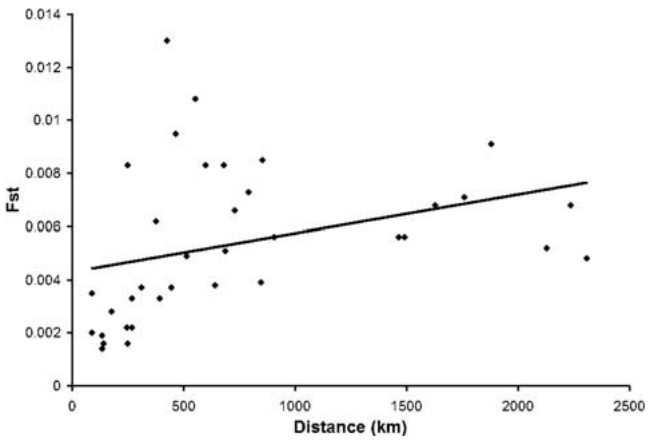

Fig. 3. Pairwise F_{ST} versus geographic distance for 9 populations of eulachons in the Pacific northwest.

Table 4. Estimated Percentage Composition of Two Simulated Mixtures of Eulachons Using Variation at 14 Microsatellite Loci and an Eight-Population Baseline (outlined in Table 1)[a]

Baseline population	*True %*	*Estimated % (SD)*	*True %*	*Estimated % (SD)*
Columbia		1.5 (1.0)	25.0	33.6 (11.5)
Cowlitz	20.0	22.7 (11.5)	25.0	18.3 (11.1)
Σ Columbia	20.0	24.2 (11.6)	50.0	51.9 (11.9)
Fraser	30.0	28.3 (16.6)	50.0	45.4 (12.9)
Klinaklini		1.2 (0.7)		0.4 (0.1)
Bella Coola		1.1 (0.7)		0.6 (0.4)
Kemano	10.0	10.0 (6.2)		0.6 (0.3)
Σ Central mainland	10.0	12.3 (6.4)		1.6 (0.5)
Skeena	30.0	26.4 (13.8)		0.6 (0.3)
Nass	10.0	8.7 (6.5)		0.6 (0.2)

Each mixture of 150 fish was generated 25 times with replacement, and stock compositions of the mixtures were estimated by resampling with replacement each baseline population.

mixed-stock analysis. As the distribution of the Twenty Mile River population in Cook Inlet was likely well outside waters in British Columbia, it would not be expected to occur in fishery samples from British Columbia and so was excluded from the baseline for mixed-stock analysis. Two simulated fishery mixture samples were evaluated for mixtures of eulachons, with one mixture composed of both southern (Fraser River and Columbia River) and northern (rest of British Columbia) populations, and the second mixture composed entirely of southern populations. For the mixture containing a wide geographic range of populations, regional estimated stock compositions were within 4% of actual values. Discrimination among the Columbia River, Fraser River, central coast, Nass River, and Skeena River components was obtained (Table 4). Estimated stock compositions of the second simulated sample, composed equally of Fraser River and Columbia River drainage populations, indicated that estimated stock compositions were again accurate, and that on average only a small percentage of the sample was allocated to populations not present in the mixture. Analysis of the simulated samples suggested that reliable estimates of stock compositions should be available on a regional basis, and likely a population-specific basis.

Stock composition differed substantially in eulachon samples taken as bycatch in shrimp trawl fisheries. The sample from northern British Columbia near Chatham Sound was estimated to be composed almost entirely from northern Nass, Skeena, and central coast populations (Table 5). Given the small regional biases observed in the simulated mixtures, the low estimated stock compositions of the Fraser River and Columbia River components, and that the sample was collected near spawning time, there was probably no significant contribution of either Columbia River or Fraser River populations to eulachons near Chatham Sound. Eulachons in Queen Charlotte Sound (Goose Island) originated from northern British Columbia (Skeena River), the adjacent central coastal populations, as well as from the southern Fraser River and Columbia River drainages. Eulachons sampled off the west coast of Vancouver Island were estimated to have originated primarily from the Columbia River drainage and also

Table 5. Estimated Percentage Stock Compositions of Eulachon as Bycatch in a Shrimp Trawl Fishery Near Chatham Sound, British Columbia in March 2001, in Research Shrimp Surveys aboard *W. E. Ricker* in Queen Charlotte Sound, and off the West Coast of Vancouver Island During May 2000

Baseline population	*CHA (n = 100) % (SD)*	*QCS (n = 200) % (SD)*	*WCVI (n = 184) % (SD)*
Columbia	0.6 (1.4)	2.5 (3.3)	15.1 (8.8)
Cowlitz	1.1 (2.2)	22.1 (5.9)	41.5 (9.8)
Σ Columbia	1.7 (2.4)	24.6 (6.1)	56.6 (10.4)
Fraser	2.1 (3.6)	23.9 (6.8)	37.5 (10.1)
Klinaklini	4.0 (6.8)	1.1 (2.2)	2.3 (3.4)
Bella Coola	12.3 (10.9)	1.1 (2.1)	0.6 (1.4)
Kemano	35.3 (12.4)	21.5 (6.1)	0.3 (0.8)
Σ Central mainland	51.6 (13.8)	23.7 (6.3)	3.2 (3.5)
Skeena	7.3 (7.5)	27.1 (6.9)	0.5 (1.2)
Nass	37.4 (10.9)	0.7 (1.7)	2.3 (3.3)

Stock compositions were estimated with an eight-population baseline.
CHA indicates Chatham Sound; QCS, Queen Charlotte Sound; WCVI, West Coast of Vancouver Island.

the Fraser River drainage. The analysis showed some small allocations from northern and central coast populations, but these were small and consistent with errors of estimation as indicated in the simulated mixtures. There was a clear geographic cline in relative abundance of eulachons from different geographic areas. The sample from northern British Columbia was dominated by northern and central coastal populations, the sample from central British Columbia comprised eulachons from all regions, and the sample from southern British Columbia was dominated by southern populations.

Discussion

In a previous survey of variation at 5 microsatellite loci (McLean and Taylor, 2001), the average number of alleles per locus, the size range of the alleles at a locus, and the heterozygosity per locus were significantly lower than observed in other studies directed at fish (e.g., Olsen et al., 2001, 2002). McLean and Taylor (2001) suggested that the low levels of variation observed could be accounted for by the nature of the repeats examined, rather than as a characteristic of eulachons. Our survey of microsatellite variation revealed much higher levels of variation, with a mean of 30 alleles per locus versus 7 alleles reported by McLean and Taylor (2001). In contrast, our results are comparable with the levels observed in other fish species, and we conclude that eulachons do not have lower levels of microsatellite diversity than other species.

Isolation-by-distance has been observed in genetic population structure for a number of marine species (Goodman, 1998; Maes and Volckaert, 2002; Planes and Fauvelot, 2002). Although mtDNA variation has been reported to support an isolation-by-distance pattern in eulachon population structure (McLean et al., 1999), McLean and Taylor (2001) found no such relationship in microsatellite variation. In our survey, encompassing approximately the same geographic area, an isolation-by-distance relationship was observed. It is possible that the larger number of alleles observed, the greater number of loci surveyed, and the increased population sample sizes in our study contributed to the difference in results between the two studies. Clearly factors other than geographic separation also contributed to the observed eulachon genetic variation.

The geographic identification of population structure in marine species is an important component of fisheries management. Effective management of eulachons to maintain abundance and conserve existing biodiversity requires an understanding of the genetic structure of the species. Like Pacific salmon, eulachons are anadromous, returning to spawn in freshwater after an extended marine residence. Unlike salmon, however, eulachon eggs and larvae reside in freshwater for a short time (2 to 4 weeks) before the hatched larvae are flushed into marine waters. The limited residence in freshwater may also limit the time available for imprinting on natal streams (Hay and McCarter, 2000). As a consequence the level of genetic differentiation may be less than that observed in most salmon species. For example, the genetic differentiation observed among steelhead spawning in different rivers was approximately 10 times greater than that observed among eulachons in the current study (Beacham et al., 2004a), suggestive of less precise homing to natal spawning sites by eulachons than by salmonids. The weaker population differentiation is more like the pattern typically observed in marine species (Shaklee and Bentzen, 1998; Waples, 1998). However, there may be more gene flow among spawning sites in eulachons than in salmonids, and the level of gene flow may not be sufficient to mask genetic differentiation among eulachons in the different spawning sites.

An important question in eulachon management and conservation is the appropriate conservation or management unit. A survey of variation in mtDNA of eulachons ranging from the Columbia River to the Bering Sea provided little evidence of population structure (McLean et al., 1999). Based only on these mtDNA analyses, a conclusion was drawn that eulachons are a single panmictic population, characterized by high rates of gene flow among spawning sites. This conclusion remained tentative, however, because of pronounced differences in spawning times and meristic characters among rivers (Hay and McCarter, 2000). Later surveys of variation at microsatellite loci (McLean and Taylor, 2001) showed a low level of genetic variation that indicated some level of population differentiation, particularly between the Columbia River populations and those farther north. It was still unclear, however, what the appropriate fisheries management unit should entail for eulachons. In the present study, our surveys of eulachon population structure, incorporating a larger and more polymorphic set of microsatellite loci, indicated a finer-scale population structure than observed previously. Our results are consistent with ecologic and biological eulachon differences found among rivers, such as differences in spawning time, size at maturity, and fecundity (Hay and McCarter, 2000). The present study showed genetic differentiation between most putative populations of eulachons spawning in different rivers. Therefore, similar to salmonids, the appropriate management unit would appear at this time to be based on a river drainage basis.

Although our analyses indicate that a river drainage is the basic geographic structural unit for eulachon populations, some important questions remain unresolved. Hay and McCarter (2000) identified a total of 33 rivers or streams in British Columbia where eulachons have been documented as spawning at least once, but only 13 rivers where annual spawning runs occur regularly. Some of these 13 rivers share the same estuary, and McCarter and Hay (1999) suggested that where river drainages are close to each other, such as 3 rivers draining into Gardener Canal in northern British Columbia, gene flow between the drainages may be substantial. If so, the estuary may represent a single population, and therefore the estuary may be the most appropriate management unit of eulachons. Similar questions remain for eulachons that spawn in the lower reaches of large river drainages such as the Columbia, Fraser, and Skeena. Eulachons in the Columbia River migrate upstream and spawn in the Cowlitz River, a tributary of the Columbia River. However, spawning distributions within the Columbia River vary annually, and it appears that in some years no spawning occurs in the Cowlitz tributary (Status Report, 1993). In the Fraser River, they can migrate up to 80 km upstream. Are eulachons spawning within these large river drainages a single panmictic population? On the one hand, the fact that the main spawning sites vary within rivers would indicate that there is extensive mixing among spawning fish. On the other hand, we cannot rule out the possibility that within each river there are different spawning segments that may spawn at slightly different times. In the case of the Columbia River drainage, previous work found evidence of significant differentiation between samples from the Cowlitz River and Columbia River mainstem, both for mtDNA (McLean et al., 1999) and for microsatellites (McLean and Taylor, 2001). Our survey of microsatellite variation again has found differentiation between Cowlitz River and mainstem-spawning Columbia River eulachons, and also provided some indirect evidence (HWE disequilibrium) of possible eulachon differentiation within large river drainages within British Columbia. A more refined sampling regimen would be required to resolve important issues of eulachon population structure within estuaries and large rivers.

Analysis of simulated mixtures of eulachons suggested that microsatellite variation provided a practical means to estimate stock compositions of mixed-stock samples of eulachons from marine waters. Accurate estimates of regional contributions were observed, and in many cases provided reliable estimates of individual populations in the simulated mixtures. Maximum errors for individual populations were about 4%. Although the level of differentiation among populations was modest, it was sufficient to allow reliable determination of the origin of eulachons in mixed-stock samples.

The marine distribution of eulachons originating from the different spawning sites is uncertain. However, analysis of the mixed-fishery samples indicated that eulachons from both the Columbia River and Fraser River occur in the same marine areas off the west coast of Vancouver Island. Mixed-stock analyses indicated that in May 2000 the relative abundance of Columbia River eulachons was about 50% greater than that of Fraser River eulachons. Eulachons from both of these rivers, which represent two of the largest known eulachon runs in the world (Hay et al., 1997; Hay and McCarter, 2000), were also found off the central coast of British Columbia, and in this area the Columbia River and Fraser River populations were relatively equal in abundance. This suggests that Columbia River eulachons were less likely to migrate north of Vancouver Island than were eulachons of Fraser River origin. Eulachons caught in marine waters in the central coastal region of British Columbia comprised populations from a wide range of geographic areas, from as far south as the Columbia River to as far north as the Skeena River. In March 2001 Columbia River and Fraser River eulachons were not detected in any significant amounts off the north coast of British Columbia, indicating that marine rearing areas for these populations did not extend to northern British Columbia.

In summary, eulachons display genetic differentiation among spawning aggregrations in different river drainages, and indeed there is most likely genetic differentiation within major river drainages like the Fraser River and Columbia River. The differentiation at microsatellite loci is sufficient to enable reliable estimates of stock composition to be obtained when applied to mixed-stock samples. Microsatellites have been demonstrated to be effective in applications with the goal of restricting exploitation on populations of conservation concern while enabling the harvest of abundant populations (Beacham et al., 2004b). Given the level of accuracy and ease of application, stock identification based on microsatellite variation will likely be more widely applied in marine applications in the near future.

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