

MtDNA population structure and gene flow in lingcod (*Ophiodon elongatus*): limited connectivity despite long-lived pelagic larvae

Peter B. Marko · Laura Rogers-Bennett ·
Alice B. Dennis

Received: 6 May 2005 / Accepted: 25 May 2006
© Springer-Verlag 2006

Abstract Lingcod, *Ophiodon elongatus* Girard, have a 3-month pelagic larval stage and are an important recreational and commercial species on the west coast of North America. Cytochrome-c oxidase I sequences from tissue samples were used to characterize population structure and infer patterns of gene flow from California to Alaska. No significant genetic structure was found when estimates of Wright's F_{ST} (i.e., Φ_{ST}) were generated among all populations sampled. Nesting populations within regions, however, indicated that the inner coast of Washington State is distinct, a result corroborating previous allozyme work. Coalescent-based estimates of gene flow indicate that although migration can be high from an evolutionary perspective, nearly

half of all comparisons among populations showed no gene flow in at least one direction. From an ecological perspective, moderate migration rates ($Nm < 10$) among most populations provide surprisingly limited connectivity at large ($\sim 1,000$ km) and small (~ 100 km) spatial scales. Coalescent-based estimates also show that gene flow between the inner and the outer coasts is asymmetric, a result consistent with prevailing surface currents. Because the expected inter-locus variances for coalescent-based estimates of gene flow are likely large, future work will benefit from analyses of nuclear DNA markers. However, limited demographic connectivity on large spatial scales may help explain why stock recovery has been uneven, with greater recovery in the northern (87% rebuilt) than in the southern (24% rebuilt) fishery region, supporting a regional management strategy. These results suggest that despite a 3-month pelagic larval stage, some areas may be effectively closed with respect to both population dynamics and fishery management issues.

Communicated by J.P. Grassle, New Brunswick

P. B. Marko (✉) · A. B. Dennis
Department of Marine Sciences, University of North
Carolina at Chapel Hill, 12-7 Venable Hall, Chapel Hill,
NC 27599-3300, USA
e-mail: pmarko@clemson.edu

L. Rogers-Bennett
California Department of Fish and Game,
University of California, Davis, Bodega Marine Lab, P.O.
Box 247, Bodega Bay, CA 94923-0247, USA

Present Address:
P. B. Marko
Department of Biological Sciences, Clemson University,
132 Long Hall, Clemson, SC 29634-0314, USA

Present Address:
A. B. Dennis
Department of Biological Sciences,
Louisiana State University, 202 Life Sciences Building,
Baton Rouge, LA 70803, USA

Introduction

Many demersal marine fish species possess pelagic larvae that can spend up to several months in the plankton, a life-history trait that creates the potential for long distance dispersal on the order of hundreds to thousands of kilometers (Cowen et al. 2003; Largier 2003). For fisheries focused on such species, effective management depends on whether this potential for high dispersal is routinely realized or if the majority of new recruits are supplied locally by the retention of larvae (Mora and Sale 2002; Botsford et al. 2003). Although knowledge of "source" populations that

supply larvae to neighboring sites is vital for fishery management and the conservation of recovering stocks (Pulliam 1988; Rogers-Bennett et al. 1995; Cowen et al. 2003; Crowder et al. 2000; Dethier et al. 2003), direct identification of source–sink relationships has proven difficult, as has direct assessment of larval dispersal pathways and distances (but see Grosberg 1991; Jones et al. 1999; Swearer et al. 1999; Palumbi 2003; Kinlan and Gaines 2003; Reed et al. 2004).

Indirect methods employing genetic markers are useful for measuring the scope and scale of larval dispersal. For species with relatively long-lived pelagic larvae, such studies generally find widespread genetic homogeneity, indicating high connectivity among populations separated by considerable geographic distances (e.g. Shulman and Bermingham 1995; Seeb 1998; Rocha et al. 2002; for reviews see Burton 1982; Gyllenstein 1985; Palumbi 1994; Ward et al. 1994). Although a handful of recent studies have uncovered unexpectedly high levels of phylogeographic differentiation and possible cryptic species diversity in taxa with pelagic larvae (e.g. Taylor and Hellberg 2003), larval mode is generally a good predictor of relative rates of gene flow (Bohonak 1999; Riginos and Nachman 2001). High genetic connectivity in species with pelagic larvae, however, does not necessarily entail significant ecological connectivity and demographic coupling of population dynamics. Because only a few migrants each generation can drive levels of genetic differentiation down to undetectable levels, widespread genetic homogeneity can potentially obscure demographic independence of populations (Taylor and Dizon 1996; Waples 1998; Palumbi 2003). Distinguishing evolutionarily significant gene flow from demographically relevant migration is thus a critical goal for marine population genetics with important implications for restoration and resource management.

Lingcod, *Ophiodon elongatus* Girard, a top-level predator on the west coast of North America provides a typical example of a fished species whose management may benefit from knowledge of population connectivity, particularly between northern (north of Cape Blanco, Oregon, USA) and southern (south of Cape Blanco) fishery areas. In 1994, northern and southern stocks collectively fell below 10% of their unfished (virgin) biomass, triggering a mandated rebuilding phase under the US Sustainable Fisheries Act. By 2005, northern stock estimates rebounded to 29,416 mt (87% of the unfished estimate) but southern stocks increased only moderately to 4,601 mt (24% of the unfished estimate) (Jagiello and Wallace 2005). In late 2005, lingcod stocks were declared rebuilt given that northern and southern stocks collectively climbed

above 60% of the total unfished biomass estimate. Although the differential recovery of northern and southern stocks is suggestive of some degree of demographic independence between these two fishery areas, historical estimates (1956–2005) of female spawning stock biomass have been consistently greater in the north; fishing pressure is also lower in the north, and stock size uncertainty is greater in the south due to sparse fishery age data (Jagiello and Wallace 2005). Therefore, characterization of the spatial scale of demographically significant larval exchange and determining whether lingcod fishery regions represent open or closed populations is needed to fully understand the population dynamics of both local and regional stocks.

Like most demersal fish, lingcod possess a pelagic larval stage. In the fall, adult mating pairs spawn in nearshore waters with larvae hatching out of benthic egg masses in early March (Hart 1973; Cass et al. 1990; O'Connell 1993). Larvae spend their first 2 weeks in the upper 3 m of inshore waters followed by a lengthy and active offshore epipelagic stage, lasting approximately 2.5 months (Hart 1973; Phillips and Baraclough 1977). By early June, juveniles move inshore and settle onto the benthos where adults take up a demersal life-style (Phillips and Baraclough 1977). Tagging data indicate high adult site fidelity (Starr et al. 2004), with males guarding nests; females move offshore between breeding seasons (Cass et al. 1990; Jagiello 1994, 1995, 1999; Jagiello et al. 1996). Therefore, the approximate 3-month epipelagic stage appears to have the greatest potential for long distance dispersal in this species' life history.

The effects of a lengthy pelagic stage on the population structure of lingcod have been investigated with allozyme markers (Jagiello et al. 1996). Based on samples from throughout the species' range (Pt. San Carlos, Baja California, Mexico to Kodiak Island, Alaska, USA), no genetic differentiation was detected on relatively large spatial scales (Jagiello et al. 1996). Homogeneous allele frequencies were reported from nearly all sites, with the exception of inner coast of Washington State (i.e. Puget Sound), indicating partial isolation from the outer coast. Except for inner Washington coast populations, the results from the allozyme study suggest that management decisions should consider coastal lingcod populations as a single stock, given they are genetically homogenous (Jagiello et al. 1996).

Re-assessment of population connectivity with more sensitive genetic markers and more powerful analytical tools may be warranted for recovering populations of fished species. As recommended (Jagiello et al. 1996), we have re-revisited the population structure of this species using a DNA marker, mitochondrial

cytochrome-c oxidase-1 (CO1). Although first and second codon positions are highly conserved, CO1 third positions evolve rapidly, making this locus a common choice for population genetics and phylogeography (e.g. Sotka et al. 2004; Hare and Weinberg 2005). In addition to the advantages of higher mutation rates and smaller effective population size (as compared to allozymes), mtDNA sequences allow the application of more powerful coalescent-based methods that co-estimate both migration and population size in a genealogical context. Because coalescent-based methods do not assume symmetric migration among populations, they also allow hypothesis tests of asymmetrical gene flow. For lingcod, we investigated whether gene flow between the outer and the inner coast (Puget Sound) is asymmetric (Jagiello et al. 1996), due to the predominant efflux of surface waters from Puget Sound to the outer coast (Ebbesmeyer et al. 1984).

Materials and methods

Sampling

Lingcod samples were collected from recreational and commercial fishers along the west coast of the USA from Monterey, California to Kodiak Island, Alaska (Table 1). Specific information about the catch location was recorded using California Department of Fish and Game grids and Washington Department of Fish and Wildlife Management Areas. Most of the fish were caught by a small number of fishers operating < 10 km from port. The Kodiak Island samples were gathered at one site 5 km south of port by a single angler. For all samples, a small section of muscle tissue was cut from the fin ray of each fish and stored in 70% ethanol and stored frozen.

DNA extraction, amplification, and sequencing

Tissue was incubated between 4 and 15 h at 55°C in 500 µl 2× CTAB (hexadecyltrimethylammonium bro-

mid) with 3 µl of proteinase K (Qiagen) and 0.5 µl of β-mercaptoethanol. DNA was isolated with two extractions using chloroform:isoamyl alcohol (24:1) followed by precipitation with ethanol. The DNA was washed twice with ethanol, dried, and resuspended in water.

Partial CO1 sequences were amplified with Taq Extenders™ (Stratagene) and the primers CO1a [5'-AGT ATA AGC GTC TGG GTA GTC] and CO1f [5'-CCT GCA GGA GGA GGA GAY CC] from Palumbi et al. (1991). Reactions were subjected to 94°C for 2 min followed by 40 cycles of 94°C for 40 s, 40–45°C for 1 min, and 72°C for 1–2 min with a final extension at 72°C for 7 min. Amplification products were gel-isolated and purified with a kit (Qiagen). PCR products were sequenced with both primers on an automated sequencer (Applied Biosystems, Inc).

Sequence analysis

Sequences were aligned easily by eye. We then calculated Tajima's *D* for each sample using ARLEQUIN v.2.001 (Schneider et al. 1997). The significance of the *D* statistic was determined by comparison with a table of the β distribution. We next used TCS v.1.13 (Clement et al. 2000) to build a haplotype network using statistical parsimony (Templeton 1992). Population structure was described with an analysis of variance method (Excoffier et al. 1992) implemented in ARLEQUIN. Significance was determined with 100,000 random permutations of the data. Sequence divergences for AMOVAs were calculated using Kimura's two-parameter model (Kimura 1980) with rate heterogeneity among sites; model parameters were estimated separately with PAUP version 4.0b10 (Swofford 2001). The statistical relationship between geographic distance and genetic differentiation was assessed with a Mantel test; significance was determined by permuting the data 10,000 times in the program IBD (Bohonak 2002).

Because a recent population expansion can obscure patterns of contemporary gene flow, evidence of past bottlenecks on population size was assessed by analysis

Table 1 *Ophiodon elongatus*. Cytochrome-c oxidase I nucleotide diversities and Tajima's *D* among populations

Collection site	Collection dates	<i>N</i>	Nucleotide diversity	Tajima's <i>D</i>	<i>P</i>
Kodiak Island, Alaska (KOD)	12 June 1999	6	0.004 ± 0.003	−0.676	0.312
Point Defiance, Washington (PDE)	8 May 1999–22 May 1999	15	0.004 ± 0.003	−1.562	0.053
Straits of Juan de Fuca, Washington (SJF)	9 May 1999–8 June 1999	16	0.001 ± 0.001	0.326	0.371
San Juan Islands, Washington (SJI)	2 May 1999–13 June 1999	20	0.002 ± 0.002	−1.570	0.052
Eureka, California (EUR)	2 June 2000–7 August 2000	40	0.003 ± 0.002	−1.522	0.058
Bodega Bay, California (BOD)	5 June 2000–11 July 2000	47	0.003 ± 0.002	−1.021	0.162
San Francisco, California (SFR)	4 May 2000–18 July 2000	30	0.003 ± 0.002	−0.447	0.348
Monterey Bay, California (MON)	8 May 2000–4 August 2000	12	0.004 ± 0.003	−1.294	0.105

of pairwise distributions of differences between haplotypes (Rogers and Harpending 1993). Goodness of fit tests of the observed to the estimated mismatch distributions, estimates of Θ ($2N_f\mu$, where N_f = female effective population size and μ = the per site per generation mutation rate), and the time since expansion (τ) in units of $1/2u$ generations ($\tau = 2ut$, where t = number of generations since expansion and u = the per haplotype per generation mutation rate) were computed with ARLEQUIN. Time since expansion in generations was then calculated using an mtDNA third position rate of nucleotide substitution for fishes established using pairs of species isolated on either side of the Isthmus of Panama and other estimates of mtDNA nucleotide divergence rates (Donaldson and Wilson 1999; Hickerson and Ross 2001).

We also used the Metropolis–Hastings sampling procedure in the program FLUCTUATE (Kuhner et al. 1998) to obtain maximum-likelihood estimates of both current and ancestral population size. This method uses a sampling of gene trees to jointly estimate Θ and an exponential growth parameter (g). Estimates of these parameters were then used to generate the ancestral value of theta with the expression $\Theta_t = \Theta e^{(-gt)}$, where Θ_t corresponds to Θ at any time t (in generations) in the past. Model parameters (transition to transversion ratio and the gamma shape parameter) were first estimated with PAUP; lingcod generation time is four years (Hart 1973; Phillips and Baraclough 1977). Both the mismatch and FLUCTUATE analyses ignore the possibility of geographic genetic structure, an assumption that is likely violated by most population genetic data sets.

Maximum-likelihood estimates of gene flow were obtained with MIGRATE version 1.67 (Beerli and Felsenstein 1999, 2001). For mtDNA sequences, MIGRATE calculates the likelihood of observed genetic data in terms of Θ , migration, and mutation through an expansion of coalescent theory (Kingman 1982a, b; Hudson 1990; Nath and Griffiths 1993; Notohara 1990). MIGRATE assumes that populations are at equilibrium with respect to drift and migration and that population sizes and migration rates have remained constant over the coalescent period ($\sim 4N_f$ generations). Unlike estimates of Nm from F -statistics and their analogs, MIGRATE allows asymmetrical migration and unequal population sizes. We used default search settings (10 short chains of 10,000 sampled, 500 recorded, followed by 3 long chains of 100,000 sampled, 5,000 recorded) but also employed four-chain heating (Metropolis-coupled Markov chain Monte Carlo). For maternally inherited, haploid mtDNA, MIGRATE yields estimates of $2N_fm_t$, or two times the

number of female migrants entering a population per generation; we therefore report values of Nm for females only.

Results

Patterns of polymorphism

DNA sequences (548 base pairs) were obtained from a total of 186 individuals. Across all collection sites, 19 nucleotide sites were polymorphic, yielding 20 unique haplotypes. One common haplotype (haplotype 3) was present at all sites and was designated ancestral by TCS. Surprisingly, private haplotypes (haplotypes unique to a site) were common, found at sites in Washington, California, and Alaska (Figs. 1, 2). Compared to haplotype diversity, nucleotide diversity was low in lingcod ($\pi = 0.00195$). Although Tajima's D was negative at most localities, the statistic was not significant at any site (Table 1).

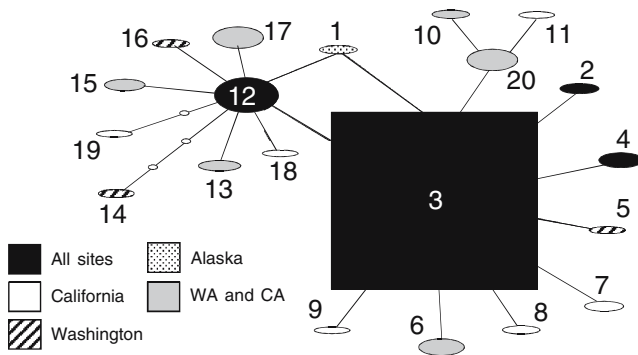
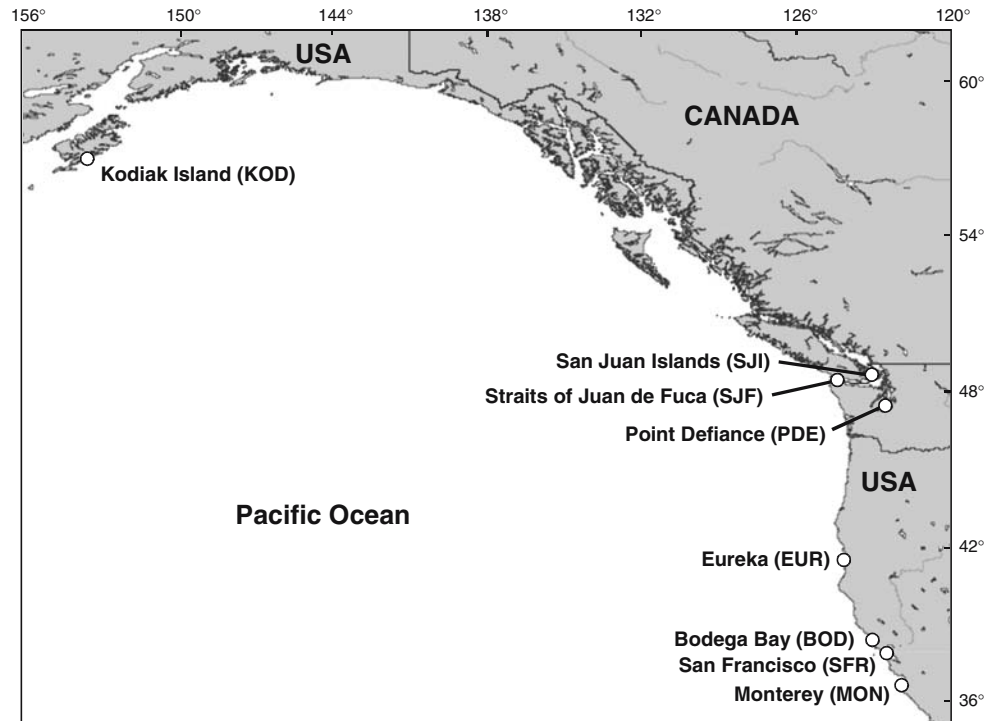
Population structure

We found no significant genetic subdivision across all sites, whether Φ_{ST} was based on haplotype frequencies or sequence divergences (Table 2). Mantel tests also showed no significant relationship between genetic differentiation and geographic distance for either haplotype frequencies ($r = 0.0877$, $P = 0.23$) or sequence divergences ($r = -0.1324$, $P = 0.72$) indicating no evidence of isolation by distance. Exclusion of the smaller samples (Kodiak Island and Monterey) had no effect on the outcome of this analysis (not shown).

Grouping collection sites into the three regions (Alaska, Washington, and California) revealed a small but significant amount of subdivision among regions when Φ_{CT} was estimated from haplotype frequencies (Table 3). Exclusion of the two sites with relatively small sample sizes (Kodiak Island and Monterey) increased the significance of the result (Table 4) but also indicated the presence of significant differentiation between Washington and California populations. No significant differences among regions were detected when an AMOVA was conducted on sequence divergences (Tables 3, 4) indicating that genetic differentiation between regions was limited to differences in haplotype frequencies.

Historical demography

The observed distribution of pairwise differences among haplotypes exhibited positive skew with respect

Fig. 1 *Ophiodon elongatus*. Collection sites**Fig. 2** *Ophiodon elongatus*. Statistical parsimony cladogram for cytochrome-c oxidase I haplotypes. Size of individual ovals in the diagram is proportional to number of individuals possessing each haplotype (the smallest oval size, such as for haplotype 1, represents a single individual). Branches correspond to single mutations and additional circles on branches represent additional inferred mutations. Haplotype #3 (rectangle) is the inferred ancestral haplotype

to the expected equilibrium distribution (Fig. 3). The observed distribution was not ragged, however, and goodness of fit statistics were not significant (Harpending's raggedness index = 0.0431, $P = 0.82$; sum of squared deviations = 0.0027, $P = 0.55$) indicating that the sudden expansion model cannot be rejected for lingcod. The estimated values of Θ before ($\Theta_0 = 0.0$, 95% CI = 0.0–0.891) and after the expansion ($\Theta_1 = 6.09$, 95% CI = 0.824–5061.1) differed, but the 95% confidence intervals do overlap slightly. The value

of τ (time to expansion) was not small ($\tau = 1.26$, 95% CI = 0.286–2.755), yielding 95% confidence limits for the start of a population expansion between 185,000 years and 1.8 million years ago.

Coalescent-based analyses depend fundamentally on the assumption of a molecular clock. Therefore, we limited the FLUCTUATE analysis to third codon positions because a Poisson distributed clock could not be rejected ($2\Delta\ln = 7.42$, $df = 182$, $P > 0.5$). The estimate of the population growth parameter from FLUCTUATE was positive ($g = 409.7$, $SD = 14.8$) but not nearly as large as reported for other northern hemisphere marine species that expanded out of post-glacial refugia (Hickerson and Ross 2001; Wares and Cunningham 2001; Marko 2004). Estimates of ancestral population size therefore indicated relatively modest growth following the last glacial maximum: the 95% confidence interval for the effective female population size 20,000 years ago (5,000 generations) was 96.3–96.8% of the current population size. The 95% confidence interval for the population size at the time of the start of the expansion inferred from the mismatch analysis (using the point estimate of $\tau = 812,903$ years ago) was 22.0–26.9% of the current population size.

Likelihood estimates of migration

Because small sample sizes produce large variances for parameter estimates (Beerli and Felsenstein 1999), we

Table 2 *Ophiodon elongatus*. Population structure based on haplotype frequencies (conventional F -statistics) and DNA sequence divergences

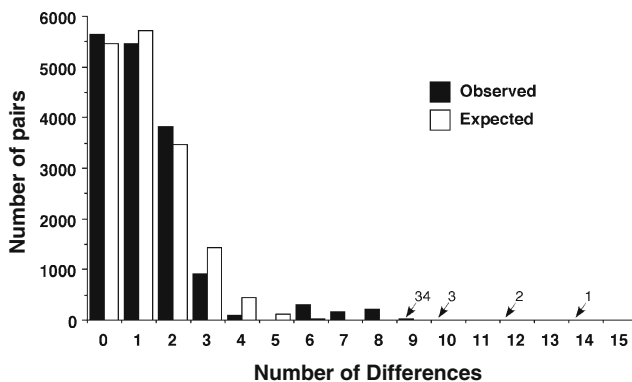
	Source of variation	df	Sum of squares	Variance components	% of variation	P -value
Haplotype frequencies	Among populations	7	2.52	0.001	0.44	0.298
	Within populations	175	57.5	0.329	99.56	
Sequence divergences	Among populations	7	4.13	-0.004	-0.57	0.640
	Within populations	175	117.9	0.673	100.57	

Table 3 *Ophiodon elongatus*. Population structure based on partitioning of collection sites into three geographic regions (Alaska, Washington, and California)

	Source of variation	df	Sum of squares	Variance component	% of variation	P -value
Haplotype frequencies	Among regions	2	1.248	0.008	2.50	0.0470
	Among populations, within regions	5	1.273	-0.003	-0.91	
Sequence divergences	Among regions	2	1.389	0.002	0.36	0.448
	Among populations, within regions	5	2.74	-0.005	-0.77	

Table 4 *Ophiodon elongatus*. Population structure based on partitioning of collection sites into two geographic regions (Washington and California)

	Source of variation	df	Sum of squares	Variance component	% of variation	P -value
Haplotype frequencies	Among regions	1	0.833	0.008	2.50	0.024
	Among populations, within regions	5	1.273	-0.003	-0.88	
Sequence divergences	Among regions	1	0.987	0.006	0.90	0.115
	Among populations, within regions	5	2.74	-0.005	-0.72	

**Fig. 3** *Ophiodon elongatus*. Observed and expected mismatch distribution for cytochrome-c oxidase I haplotypes

focused our analysis on the six sites in Washington and California where sample sizes were clearly appropriate for analysis ($n \geq 15$) with MIGRATE (Pluznikov and Donnelly 1996; Beerli 2004). Estimates of Nm were highly consistent among runs employing different search strategies (long chains, chain heating, multiple replicates), showing none of the characteristic prob-

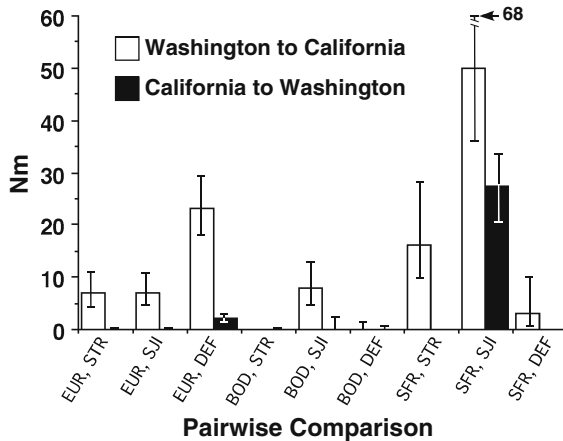
lems associated with low divergences and relatively flat likelihood surfaces (Abdo et al. 2004; Tarjuelo et al. 2004).

Migration rates varied among population comparisons (Table 5). Although a few pairwise comparisons indicated very high rates of gene flow ($Nm > 50$), nearly one-half (14 of 30) of the comparisons in Table 5 had values of $Nm \ll 1$ and the 95% confidence intervals for eleven of these comparisons included zero. Summation of immigration (summing across rows in Table 5) showed that individual sites receive between 0.55 and 61 female migrants each generation, or every 4 years. However, nearly half (47%) of all immigrants in Table 5 originate from the San Francisco population, with the majority (67%) of those immigrants dispersing to the other two sites in California.

The overall geographic pattern of migration between the inner coast of Washington and the outer coast of California was distinctly asymmetric (Fig. 4). Among all pairwise comparisons between sites in these two regions, seven of nine yielded significantly greater southward migration (i.e. Washington to California)

Table 5 *Ophiodon elongatus*. Point estimates and 95% confidence intervals for joint estimates of Θ and migration (Nm) in lingcod from MIGRATE. See Table 1 or Fig. 1 for site abbreviations

Nm (x = Receiving Population)							
Population (x)	Θ	PDE, x	SJF, x	SJI, x	EUR, x	BOD, x	SFR, x
PDE	0.0064 (0.0051, 0.0081)	–	0 (0, 0.2)	0.3 (0.08, 0.9)	2 (1, 3)	0 (0, 0.4)	0 (0, 0.43)
SJF	7×10^{-5} (5×10^{-5} , 0.0001)	0 (0, 0.05)	–	0.5 (0.3, 0.7)	0.05 (0.01, 0.2)	0 (0, 0.1)	0 (0, 0.097)
SJI	0.0002 (0.0001, 0.0005)	7 (4, 10)	4 (2, 7)	–	0 (0, 0.5)	0 (0, 3)	27 (20, 34)
EUR	0.0022 (0.0016, 0.0035)	23 (17, 30)	7 (4, 11)	7 (4, 11)	–	9 (4, 15)	9 (4, 15)
BOD	0.0004 (0.0002, 0.0009)	0 (0, 2)	0 (0, 0)	8 (4, 13)	7 (4, 13)	–	46 (31, 63)
SFR	0.0056 (0.0025, 0.0156)	3 (0.5, 10)	16 (9, 28)	50 (36, 68)	107 (82, 134)	0 (0, 6)	–

**Fig. 4** *Ophiodon elongatus*. Likelihood estimates for female migration (Nm) between sites in California and Washington. Error bars represent 95% confidence intervals. See Table 1 or Fig. 1 for site abbreviations

and very few northward migrants. In the two pairwise comparisons in which this asymmetry was absent, migration was essentially zero in either direction.

Discussion

An extended pelagic larval stage in lingcod suggests a high potential for long-distance transport along the west coast of North America, and the general pattern we found of high mtDNA homogeneity over large spatial scales (hundreds to thousands of kilometers) is broadly consistent with this life history characteristic. Some private haplotypes were found at individual sites, but no obvious phylogeographic structure or clustering of haplotypes with respect to geography was evident in the haplotype network. Across all collection sites, there was no significant subdivision at the level of individual populations. Although the largest values of Φ_{ST} among pairwise comparisons were observed between geographically distant samples, there was no significant pattern of isolation by distance in the data. Therefore, analyses of mtDNA from lingcod indicate that gene

flow is sufficiently high to prevent significant genetic subdivision (as measured by Φ -statistics) across all populations.

An apparent absence of genetic structure, however, can be caused by factors other than high levels of contemporary gene flow. Although a recent population expansion could potentially obscure a pattern of limited gene flow (if Φ_{ST} is far from equilibrium), estimates of ancestral population size indicate that any population bottleneck was likely ancient, and that population growth over the last 20,000 years has been negligible. Genetically based inferences about the demographic histories of populations are also sensitive to error in estimation of mutation rates. The mutation rate that we used was derived primarily from geminate species pairs of fish found on either side of the Isthmus of Panama (Donaldson and Wilson 1999). Although widely used to calibrate rates of molecular evolution, transisthmian calibrations tend to yield upwardly biased estimates of mutation if sister-species isolated on either side of the isthmus split long before the time of final seaway closure (Marko 2002). However, this bias makes our analysis conservative with respect to our conclusions because a smaller mutation rate would push coalescence events further back into the past, yielding greater estimates of the time since the start of any population expansion. A recent selective sweep could also explain widespread genetic homogeneity, but this hypothesis is not consistent with allozyme data from lingcod, which also indicate no genetic differentiation throughout most of the species' range (Jagiello et al. 1996; but see Sotka et al. 2004).

A more powerful analysis of variance with sites nested within geographically disjunct regions does reveal a marginally significant amount of differentiation between Washington and California when Φ_{CT} is calculated from haplotype frequencies. Although it is often problematic to use F -statistics and their analogs to make precise estimates of migration, the presence of significant genetic differentiation likely means that gene flow is curtailed. Using island model assumptions,

$\Phi_{CT} = 0.025$ for mtDNA corresponds to only ~ 20 female migrants exchanged between regions each generation, or every 4 years. However, the assumptions required for the theoretical relationship between F -statistics and Nm to hold are often considered unrealistic (Waples 1998; Whitlock and McCauley 1999; Neigel 2002; but see Bohonak 2002), particularly in marine systems where current-mediated dispersal is likely asymmetrical. Of greater importance, perhaps, is the possibility that additional information about migration between populations could be obscured when structure is characterized by a single global estimate of a Φ_{CT} (Neigel 2002).

Coalescent-based methods for inferring gene flow should overcome some problems associated with migration estimates from F -statistics (Neigel 2002). For lingcod, likelihood estimates of Nm indicate that gene flow is often low: approximately half of the comparisons in Table 5 indicate very low gene flow ($Nm \ll 1$), with most (24 of 30) comparisons involving values of $Nm < 10$. Therefore, while gene flow may be great enough to prevent the accumulation of large genetic differences measurable with Φ -statistics, migration rates may be too small to cause significant demographic coupling of population dynamics. The geographic proximity of some populations also indicates that long-lived lingcod larvae do not routinely travel tens to hundreds of kilometers in large numbers, as is thought to be the case for many demersal marine fishes (Kinlan and Gaines 2003). For example, between the Straits of Juan de Fuca and the San Juan Islands, the two nearest localities on the inner coast of Washington (separated by < 100 km), the 95% confidence intervals for Nm indicate that seven or fewer migrants successfully disperse from the Straits to the San Juans each generation (every 4 years) and no more than 0.7 move in the opposite direction. Among the two nearest outer coast populations (Bodega Bay and San Francisco, also < 100 km apart), no more than 6 females travel from north to south and 63 or fewer migrants move from south to north each generation.

At the regional level, where Φ_{CT} indicates that as few as 20 migrants successfully disperse between Washington and California each generation, MIGRATE indicates that gene flow is larger, but only about an order of magnitude greater. From an evolutionary perspective, this discrepancy is considerable, but from an ecological or fishery perspective, even a few hundred immigrants every four years likely provides little short-term fishery relevant connectivity between California and Washington stocks given larval mortality is likely high. Although both Φ -statistic and coalescent-based analyses agree that gene flow between these regions

may not be high enough to provide significant coupling of lingcod stocks, the results from either analysis must be viewed with caution given that they are based on only mtDNA. Whether our results reflect the idiosyncratic features of a single locus or some degree of local demographic autonomy awaits additional analyses of nuclear markers.

How reliable are gene flow estimates from MIGRATE? Few simulation studies for coalescent-based estimators have been conducted but available analyses (Beerli and Felsenstein 1999, 2001; Abdo et al. 2004) show that MIGRATE yields biased estimates of Nm when genetic diversity is extremely low ($\Theta \leq 0.00025$), migration rates are exceedingly small ($Nm \leq 0.00625$), and only a single locus is employed. Our estimates of Θ for some populations are small, but under these conditions MIGRATE will over-estimate Nm (Abdo et al. 2004), suggesting our conclusions are conservative. Similarly, the addition of more samples could improve our results, but having sampled too few populations generally results in overestimation of Θ and therefore overestimation of Nm , which is the product of migration rate (M) and Θ (Beerli 2004). Therefore, the presence of an unsampled (“ghost”) source population that supplies large numbers of migrants to sampled populations is expected to inflate estimates of Nm between sampled populations (Beerli 2004). Therefore, if our pairwise estimates of Nm are biased, all available evidence suggests they are likely biased upwards. Our data cannot address the possibility that our sampled populations each receive large numbers of migrants from a unique source population, but again, under these circumstances, Θ and Nm for sampled populations will be biased upwards.

Nearly all pairwise estimates of gene flow between Washington and California show statistically significant asymmetry, with most migrants moving from north to south. Given the locations of our samples in Washington, two hypotheses can potentially explain the observed asymmetry in gene flow. First, the equatorward flowing California Current (Strub and James 2000) may predominantly carry lingcod larvae south along the outer coast. The timing of the intensification of the California Current in the late spring to summer coincides with the period during which lingcod larvae hatch from benthic nests, so larvae could be entrained in this current. However, among outer coast samples (in California) there is no obvious asymmetry (Fig. 5). Alternatively, the asymmetry in migration between Washington and California could be explained by the fact that all three of our samples in Washington are from the inner coast and the Straits of Juan de Fuca. Surface currents originating in Puget Sound and the

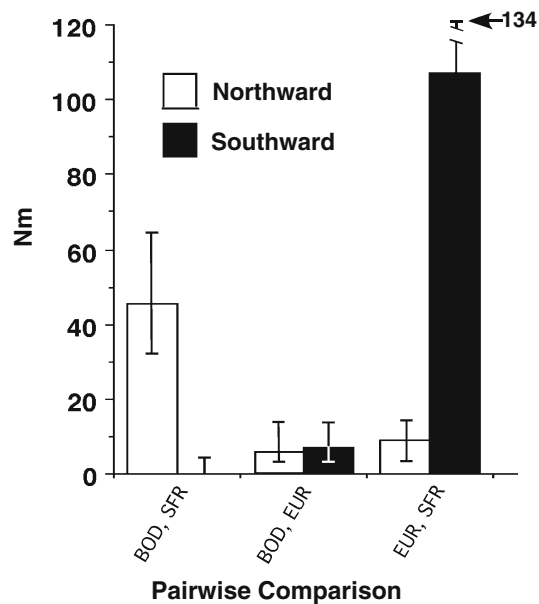


Fig. 5 *Ophiodon elongatus*. Likelihood estimates for female migration (N_m) among sites in California only. Error bars represent 95% confidence intervals for each estimate. See Table 1 or Fig. 1 for site abbreviations

Straits of Georgia flow north and south, respectively, converging and flowing out of the Straits of Juan de Fuca in a net westward direction (Ebbesmeyer 1984). Therefore, if surface currents play a major role in determining the dispersal pathways of epipelagic lingcod larvae, larval dispersal is expected to be asymmetric, with individuals more likely to be transported from the inner coast to the outer coast than vice versa. However, the existence of patterns of gene flow within a single species that are both consistent (inner to outer coast) and inconsistent (California Current) with the movements of major offshore surface currents suggests that the relationships among larval behavior, oceanography, benthic currents and dispersal are likely complex, probably requiring more detailed knowledge of nearshore oceanography (Collin 2003).

Deep phylogeographic breaks have been described in some marine species with moderate to long-lived pelagic larvae (e.g. Taylor and Hellberg 2003; Sotka et al. 2004), demonstrating a complete absence of gene flow within what were believed to be single biological species. Although deep phylogeographic divisions reveal the existence of Evolutionary Significant Units (ESUs) that are important to the goal of biodiversity preservation (Moritz 1994; Fraser and Bernatchez 2001; Avise 2004), relatively shallow patterns of genetic structure may reflect recent restrictions on gene flow (Bohnanak 1999) and therefore be more relevant for understanding larval dispersal in the context of fisheries management (Collin 2003; Avise 2004). In lingcod, we

have found fairly weak patterns of mtDNA differentiation and therefore no evidence of cryptic ESUs. However, both our mtDNA analyses and previous studies of allozyme electromorphs reveal modest yet significant genetic differentiation between inner Puget Sound of Washington and the outer coast. Our likelihood estimates of gene flow also indicate that lingcod are more likely to disperse from Puget Sound to the outer coast, strongly suggesting that the persistence of inner coast stocks relies on local larval production. To prevent overfishing of these isolated stocks, this mild genetic distinction should be taken into consideration, particularly because Puget Sound population densities are currently low (Jagiello et al. 1996) and the fishery is open. On the outer coast of California, our estimates of migration indicate that while gene flow is great enough to nearly genetically homogenize populations, larval transport may limit the immediate recovery of local populations following overfishing. This inference is consistent with the recent observed recovery of spawning stock biomass in the southern assessment area, which has recovered more slowly than the northern assessment region. Therefore, intense fishing in one region could have lasting impacts with long-distance larval transport doing little to rebuild local populations.

On the spatial scale of the sites sampled in this study, our data suggest that some recruits entering lingcod populations may be supplied locally. Similarly, direct observations of larval dispersal in other marine fishes provide evidence for local retention despite lengthy pelagic larval periods (Jones et al. 1999; Swearer et al. 1999). This contrasts with the alternative hypothesis that populations from one site routinely supply larvae to neighboring sites, as has been suggested for some coral reef fishes (Roberts 1997). In this scenario, fishing “downstream” of the source population would have little impact on local stock numbers, as they would be replenished from “upstream” sources. For newly rebuilt lingcod stocks, our gene flow analyses indicate that rates of successful larval exchange could be too low to be relevant for fishery recovery plans, suggesting some populations may be effectively closed over ecological time scales.

Acknowledgments We thank the California Department of Fish and Game (CDFG), Eric Larson, and Patty Wolf for their support. Sport Fish Restoration Funds (Fund F-50-R-13 Project 18, Job 6.) from the CDFG, the University of North Carolina and the Friday Harbor Laboratories (University of Washington) helped fund this study. We thank port samplers in California, Washington and Alaska for tissue samples and Brian Allen and Brenda Erwin for coordinating the sampling in California. This is contribution Number 2312 Bodega Marine Laboratory, University of California, Davis and a contribution of Clemson University Public Service and Agriculture.

References

- Abdo Z, Crandall K A, Joyce P (2004) Evaluating the performance of likelihood methods for detecting population structure and migration. *Mol Ecol* 13:837–851
- Avise JC (2004) Molecular markers, natural history, and evolution, 2nd edn. Sinauer, Sunderland
- Beerli P (2004) Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. *Mol Ecol* 13:827–836
- Beerli P, Felsenstein J (1999) Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics* 152:763–773
- Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proc Natl Acad Sci USA* 98:4563–4568
- Bohonak AJ (1999) Dispersal, gene flow, and population structure. *Q Rev Biol* 74:21–45
- Bohonak AJ (2002) IBD (isolation by distance): a program for analyses of isolation by distance. *J Hered* 93:153–154
- Botsford LW, Micheli F, Hastings A (2003) Principles for the design of marine reserves. *Ecol Appl* 13:S25–S31
- Burton RS (1982) Protein polymorphisms and genetic differentiation of marine invertebrate populations. *Mar Biol Lett* 4:193–206
- Cass AJ, Beamish RJ, McFarlane GA (1990) Lingcod (*Ophiodon elongatus*). *Can Spec Publ Fish Aquat Sci* 109:40
- Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Mol Ecol* 9:1657–1660
- Collin PL (2003) Larvae retention: genes or oceanography? *Science* 300:1657–1658
- Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2003) Connectivity of marine populations: open or closed? *Science* 287:857–859
- Crowder LB, Lyman SJ, Figueira WF, Priddy J (2000) Source–sink population dynamics and the problem of siting marine reserves. *Bull Mar Sci* 66:799–820
- Dethier MN, McDonald K, Strathmann RR (2003) Colonization and connectivity of habitat patches for coastal marine species distant from source populations. *Conserv Biol* 17:1024–1035
- Donaldson KA, Wilson RR (1999) Amphi-panamic geminates of snook (Percoidei: Centropomidae) provide a calibration of the divergence rate in the mitochondrial DNA central region of fishes. *Mol Phylogenet Evol* 13:208–213
- Ebbesmeyer CC et al (1984) Synthesis of current measurements in Puget Sound, Washington, vol 3. Circulation in Puget Sound: an interpretation based on historical records of currents. NOAA (National Oceanographic and Atmospheric Administration) Technical Memorandum NOS (National Ocean Service), OMS-5, Rockville
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes—application to human mitochondrial DNA restriction data. *Genetics* 131:479–491
- Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Mol Ecol* 10:2741–2752
- Grosberg RK (1991) Sperm-mediated gene flow and the genetic structure of a population of the colonial ascidian *Botryllus schlosseri*. *Evolution* 45:130–142
- Gyllenstein U (1985) The genetic structure of fish: differences in the intraspecific distribution of biochemical variation between marine anadromous, and freshwater species. *J Fish Biol* 26:691–700
- Hare MP, Weinberg JR (2005) Phylogeography of surfclams, *Spisula solidissima*, in the western North Atlantic based on mitochondrial and nuclear DNA sequences. *Mar Biol* 146:707–716
- Hart JL (1973) Pacific fishes of Canada. *Bull Fish Res Bd Can* 180:740
- Hickerson MJ, Ross JRP (2001) Post-glacial population history and genetic structure of the northern clingfish (*Gobiosox maeandricus*), revealed from mtDNA analysis. *Mar Biol* 138:407–419
- Hudson RR (1990) Gene genealogies and the coalescent process. *Oxf Surv Evol Biol* 7:1–44
- Jagiello TH (1994) Assessment of lingcod (*Ophiodon elongatus*) in the area north of 45°46' (Cape Falcon) and south of 49°00' N in 1994. Appendix I, In: Status of the Pacific groundfish fishery through 1994 and recommended biological catches for 1995: stock assessment and fishery evaluation, Pacific Fishery Management Council, Portland
- Jagiello TH (1995) Abundance and survival of lingcod at Cape Flattery, Washington. *Trans Am Fish Soc* 124:170–183
- Jagiello TH (1999) Movement, mortality, and size selectivity of sport- and trawl-caught lingcod off Washington. *Trans Am Fish Soc* 128:31–48
- Jagiello TH, Wallace FR (2005) Assessment of Lingcod, *Ophiodon elongatus*, for the Pacific Fishery Management Council in 2005, Report
- Jagiello TH, LeClair LL, Vorderstrasse BA (1996) Genetic variation and population structure of lingcod. *Trans Am Fish Soc* 125:372–386
- Jones GP, Milicich MJ, Emslie MJ, Lunow C (1999) Self-recruitment in a coral reef fish population. *Nature* 402:802–804
- Kimura M (1980) A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120
- Kingman JFC (1982a) The coalescent. *Stoch Process Appl* 13:235–248
- Kingman JFC (1982b) Essays in statistical science: on the genealogy of large populations. *J Appl Prob* 19A:27–43
- Kinlan BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial communities: a community perspective. *Ecology* 84:2007–2020
- Kuhner MK, Yamato J, Felsenstein J (1998) Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics* 149:429–434
- Largier JL (2003) Considerations in estimating larval dispersal distances from oceanographic data. *Ecol Appl* 13:S71–S89
- Marko PB (2002) Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Mol Biol Evol* 19:2005–2021
- Marko PB (2004) 'What's larvae got to do with it?' Patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. *Mol Ecol* 13:597–611
- Mora C, Sale PF (2002) Are populations of coral reef fishes open or closed? *Trends Ecol Evol* 17:422–428
- Moritz C (1994) Defining 'Evolutionary Significant Units' for conservation. *Trends Ecol Evol* 9:373–375
- Nath H, Griffiths R (1993) The coalescent in two colonies with symmetric migration. *J Math Biol* 31:841–851
- Neigel JE (2002) Is F_{ST} obsolete? *Conserv Genet* 3:167–173
- Notohara M (1990) The coalescent and the genealogical process in geographically structured population. *J Math Biol* 29:59–75
- O'Connell VM (1993) Submersible observations on lingcod, *Ophiodon elongatus*, nesting below 30 m off Sitka, Alaska. *US Natl Mar Fish Serv Mar Fish Rev* 55:19–24

- Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annu Rev Ecol Syst* 25:547–572
- Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecol Appl* 13:S146–S158
- Palumbi SR, Martin AP, Romano SL, McMillan WOD, Stice L, Grabowski G (1991) The simple fool's guide to PCR. Version 2.0. University of Hawaii, Honolulu
- Phillips AC, Barraclough WE (1977) On the early life history of lingcod (*Ophiodon elongatus*). *Can Fish Mar Serv Tech Rep* 756, Nanaimo, 35 pp
- Pluzhnikov A, Donnelly (1996) Optimal sequencing strategies for surveying molecular genetic diversity. *Genetics* 144:1247–1262
- Pulliam HR (1988) Sources, sinks, and population regulation. *Am Nat* 132:652–661
- Reed DC, Schroeter SC, Raimondi PT (2004) Spore supply and habitat availability as sources of recruitment limitation in the giant kelp *Macrocystis pyrifera* (Phaeophyceae). *J Phycol* 40:275–284
- Riginos C, Nachman MW (2001) Population subdivision in marine environments: the contributions of isolation by distance, discontinuous habitat, and biogeography to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Mol Ecol* 10:1439–1453
- Roberts CM (1997) Connectivity and management of Caribbean coral reefs. *Science* 278:1454–1457
- Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Mol Ecol* 11:243–252
- Rogers AR, Harpending HC (1993) Population growth makes waves in the distribution of pairwise genetic differences. *Mol Biol Evol* 9:552–569
- Rogers-Bennett L, Bennett WA, Fastenau HC, Dewees CM (1995) Spatial variation in red sea urchin reproduction and morphology: implications for harvest refugia. *Ecol Appl* 5:1171–1180
- Schneider S, Kueffer J, Roessli D, Excoffier L (1997) Arlequin version 1.1: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland
- Seeb LK (1998) Gene flow and introgression among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. *J Hered* 89:393–403
- Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. *Evolution* 49:897–910
- Sotka EE, Wares JP, Barth JA, Grosberg RK, Palumbi SR (2004) Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Mol Ecol* 13:2143–2156
- Starr RM, O'Connell V, Ralston S (2004) Movements of lingcod (*Ophiodon elongatus*) in southeast Alaska: potential for increased conservation and yield from marine reserves. *Can J Fish Aquat Sci* 61:1083–1094
- Strub PT, James C (2000) Altimeter-derived variability of surface velocities in the California current system: 2. Seasonal circulation and eddy statistics. *Deep-Sea Res Part II: Topical Stud Oceanogr* 47:831–870
- Swofford DL (2001) PAUP*. Phylogenetic analysis using parsimony (*and other methods), Version 4. Sinauer Associates, Sunderland
- Swearer SE, Caselle JE, Lea DW, Warner RR (1999) Larval retention and recruitment in an island population of a coral-reef fish. *Nature* 402:799–802
- Tarjuelo I, Posada D, Crandall KA (2004) Phylogeography and speciation of colour morphs in the colonial ascidian *Pseudodistoma crucigaster*. *Mol Ecol* 13:3125–3136
- Taylor BL, Dizon AE (1996) The need to estimate power to link genetics and demography for conservation. *Conserv Biol* 2:661–664
- Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. *Science* 299:107–109
- Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132:619–633
- Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *J Hered* 89:438–450
- Ward RD, Woodrark M, Skibinski DOF (1994) A comparison of genetic diversity levels in marine freshwater, and anadromous fishes. *J Fish Biol* 44:213–232
- Wares JP, Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution* 55:2455–2469
- Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity* 82:117–125