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Dispersal and population structure of Neotrypaea californiensis

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DISPERSAL AND POPULATION STRUCTURE OF *NEOTRYPAEA CALIFORNIENSIS*

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ABSTRACT

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Highly dispersing larvae of marine invertebrates are expected to have weak population structure along their ranges, but some species do not realize their dispersal potential and can have strong structure. The burrowing shrimp, *Neotrypaea californiensis*, inhabits estuaries of the U.S. Pacific coast. A region of mtDNA from larvae collected in 2005 off the Oregon coast and in Yaquina Bay in 2006 was analyzed in order to determine their population structure and dispersal patterns. Haplotypes were shared among most larvae except Yaquina Bay larvae, which had unique haplotypes. An eddy off the coast of Yaquina Bay caused by water movement around Heceta Bank and the eddy formed from the bi-directional plume of the Columbia River can locally retain larvae and cause larvae from different source populations to recruit into an estuary, increasing genetic diversity in the estuary and the number of haplotypes. However, different ocean conditions can cause interannual recruitment variability.

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Introduction

For several invertebrate taxa with planktonic larvae, dispersal distance is positively correlated with the time spent in the ocean (Shanks *et al.* 2003) and there is a predicted correlation between dispersal potential and the amount of gene flow between populations of a species (Hedgecock 1986). Infauna larvae released from a bay or estuary may spend several weeks, months, or years in the ocean until they settle into their habitat (Hohenlohe 2004) and they can disperse as little as a meter or up to thousands of kilometers.

Larval behavior and coastal oceanography can retain larvae nearshore or near their natal estuary. Decapod crustacean larvae are active swimmers and some species can avoid surface currents by synchronizing their vertical migration through the water column with the flood and ebb tides (Marta-Almeida *et al.* 2006). During flood tides, larvae swim to the upper portion of the water column to retain themselves along the continental shelf. During ebb tides, the larvae descend through the water column, which prevents advection far offshore (Cronin and Forward 1986; Marta-Almeida *et al.* 2006; Olmi 1994; Yannicelli *et al.* 2006). Nocturnal diel vertical migration is a common behavior for crustacean larvae to avoid predators (Bollens and Frost 1989). Larvae migrate to the surface during the night to feed so that they avoid being seen by predators. Physical processes such as taylor columns, frontal zones, Ekman convergence and divergence, and strong eddies can lead to retention along the shelf (Marta-Almeida *et al.* 2006). Retention in the natal region can be beneficial in that it ensures that more larvae

will return to the estuary, however, retention can prevent gene flow to another estuary (Bilton *et al.* 2002) and causes heterogeneity in population structures over time.

The mitochondrial gene, cytochrome c oxidase subunit I (*COI*), is often used as a genetic marker in population genetic studies. *COI* codes for a vital enzyme involved in cellular respiration. The gene mutates at a rapid rate and is inherited maternally, which makes *COI* an ideal genetic marker to detect intraspecific differences among individuals because small changes at the nucleotide level can be identified and analyzed. Species with high dispersal potential are expected to have a homogenous genetic distribution along their dispersal range (Hedgecock 1994; Palumbi 1994), but some species do not achieve their dispersal potential (Knowlton and Keller 1986) and there can be strong genetic structure. Their realized dispersal capability is much less than their potential dispersal capability so population subdivisions can be present within a small geographic region (Barber *et al.* 2002).

Neotrypaea californiensis is a native, burrowing thalassinid shrimp inhabiting the middle to low intertidal zones of estuaries from southeast Alaska to Baja California. Females are ovigerous from April to August and the hatched larvae leave the estuary with the tides. They develop through five planktonic larval stages in six to eight weeks in the nearshore coastal ocean. The postlarvae recruit into estuaries during nocturnal flood spring tides between August and October (Dumbauld *et al.* 1996). The shrimp settle onto mudflats and remain there throughout their four to five year lifespan, although some may live up to seven years (Dumbauld *et al.* unpublished). Although the ecology of the adult

population has been studied, their dispersal and population structure has not been studied in detail.

The California Current is the primary transport mechanism for *N. californiensis* during their dispersal season. It is an eastern boundary, equatorward surface current of the clockwise-flowing North Pacific Gyre of the Pacific Ocean and is a site of nutrient-rich upwelling events during the spring and summer. It is roughly 1000 km wide that reaches 500 m in depth and travels on average 10 cm/s (Hickey *et al.* 1979). Although the net direction for the California Current is south during the larval release and recruitment, small regions along the current can reverse direction based on daily wind direction variation and relaxation events (Gan and Allen 2005). The upwelling winds can vary on a daily basis and there can be a 100 km variation in coastal topography (Botsford 2001). These variables coupled with larval behavior lead to the advection of infauna larvae, which is a determining factor of recruitment into estuaries (Yannicelli *et al.* 2006).

Gene flow can be impeded by currents in the form of gyres and eddies (Bucklin 1991; Palumbi 1994) and populations can be fragmented by physical barriers such as land masses (Goetze 2005). Upwelling along irregular coastlines and over steep slopes can create a strong eddy field. Populations found within an eddy or current jet can be genetically distinct from those that are found offshore (Miller *et al.* 1999). Genetic breaks can be present between geographically connected populations because these populations were historically isolated during glacial periods (Barber *et al.* 2002; Kelly *et al.* 2006). After thousands of years, this subdivision can remain even though the physical landscape and oceanic conditions favored a reversal of this heterogeneous distribution.

Distinct clades can also be present when there is gene flow between migrants from different, genetically distinct source populations (Bilodeau *et al.* 2005). Along the Oregon coast, there are irregularities that can cause the coastal upwelling jet to diverge from its southward direction. Coastal headlands can cause currents to diverge from their normal paths and change circulation patterns. This can prevent waters north and south of the headland from mixing, which can cause genetic subdivision in a population (Wing *et al.* 1998; Cassone and Boulding 2005).

Rationale for the research

Most studies designed to infer gene flow in marine invertebrates involve analyzing only adult samples. However, larvae should be analyzed in parallel with the adult population to make more accurate assessments about dispersal, recruitment, and population structure. Previous studies of *COI* from adult crustacean populations sampled from Oregon and/or California revealed distinct haplotypes (maternally-inherited genetic makeup of an individual) and population subdivision caused by coastal topography (Cassone and Boulding 2005; Petersen 2007; Sotka *et al.* 2004). A portion of *N. californiensis COI* from larvae collected in 2005 and 2006 was analyzed in order to determine if there is similar genetic structuring along the sampled range. Coastal oceanography and offshore topography along the shrimp's biogeographical range were considered in the interpretation of the results and larval dispersal patterns and estuary recruitment capability were inferred.

Materials and Methods

Samples

During June 2005, coastal ocean plankton were sampled along 11 oceanographic lines from LaPush, WA to Cape Perpetua, OR during the Ocean Survival of Salmonids project funded by the Bonneville Power Authority (BPA) and NOAA-Fisheries. These lines included Tatoosh Island, LaPush, Queets River, Grays Harbor, Willapa Bay, Columbia River (CR), Cape Falcon, Cape Meares (CM), Cascade Head (CH), Newport Hydrographic (NH), and Cape Perpetua. In August 2005, three of these oceanographic lines (CR, CM, NH) were resampled along with the Heceta Head (HH) line (Fig. 1) during a Pacific Coast Ocean Observing System (PaCOOS) cruise funded by NOAA-Fisheries. During these cruises, oblique plankton tows of the upper 20-30 m of the water column were made using a 330 μm mesh bongo net system for each station. Plankton samples were immediately preserved in 95% ethanol. Upon return to the laboratory, samples were re-sieved (300 μm) and stored in fresh 95% ethanol. Samples were subsequently sorted for *N. californiensis* larvae which were measured, staged, and placed in vials with fresh 95% ethanol. A total of 200 larvae sampled off the coast of Oregon were used for genetic analyses from the CR, CM, CH, NH, and HH lines (Fig. 1, Table 1). This sample set also includes 19 larvae collected within Yaquina River estuary, Newport, OR (YB) in July 2006. For the YB larvae, daily sampling of 100-120 m^3 of water from the main tidal channel in Yaquina Bay was done using a centrifugal plankton pump constructed and positioned off a dock at the Hatfield Marine Science Center (HMSC). Zooplankton were captured in a 350 μm mesh plankton net. These samples

were preserved and sorted using the methods described above. There were also four samples from a *N. californiensis* larval rearing project at HMSC. These larvae were raised from brooding adult female shrimp collected from Yaquina Bay intertidal habitats. These were used as reference samples (i.e., larvae known to be produced from the YB adult shrimp population) during the genetic analyses.

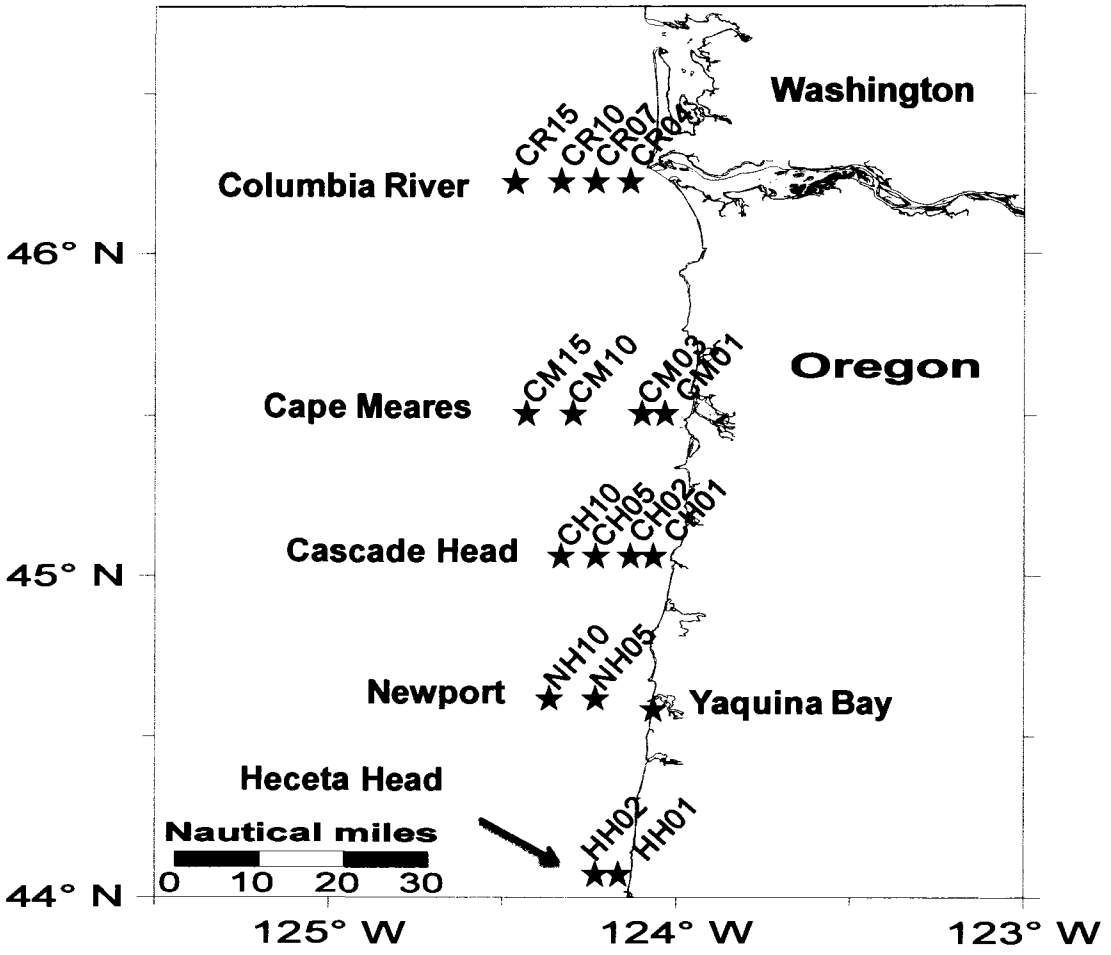

Fig. 1 Sampling locations along the Oregon coast. CR = Columbia River, CM = Cape Meares, CH = Cascade Head, NH = Newport Hydrographic, HH = Heceta Head. The numbers next to each site name indicate the distance from shore in nautical miles where each group of larvae was collected from.

Table 1 Sample collection dates, sizes, and locations

Date collected	Site	n	Nautical miles from shore	Latitude N	Longitude W
6/19/05	Columbia River	5	4	46° 10.0'	124° 4.6'
6/19/05	Columbia River	13	7	46° 10.0'	124° 9.5'
8/30/05	Columbia River	19	7	46° 10.0'	124° 9.5'
8/30/05	Columbia River	34	10	46° 10.0'	124° 13.1'
8/30/05	Columbia River	6	15	46° 10.0'	124° 20.0'
6/20/05	Cape Meares	2	10	45° 29.0'	124° 12.5'
6/20/05	Cape Meares	2	15	45° 29.0'	124° 19.6'
8/31/05	Cape Meares	10	1	45° 29.0'	124° 0.4'
8/31/05	Cape Meares	6	3	45° 29.0'	124° 2.2'
6/21/05	Cascade Head	33	1	45° 3.0'	124° 2.0'
6/21/05	Cascade Head	8	2	45° 3.0'	124° 4.5'
6/21/05	Cascade Head	5	5	45° 3.0'	124° 8.0'
6/21/05	Cascade Head	14	10	45° 3.0'	124° 14.0'
8/29/05	Newport	6	5	44° 40.0'	124° 10.5'
8/29/05	Newport	4	10	44° 40.0'	124° 17.2'
7/14/06	Yaquina Bay	6	-	44° 37.2'	124° 0.4'
7/19/06	Yaquina Bay	2	-	44° 37.2'	124° 0.4'
7/26/06	Yaquina Bay	11	-	44° 37.2'	124° 0.4'
2006	Yaquina Bay hatchery	4	-	44° 37.2'	124° 0.0'
8/21/05	Heceta Head	5	1	44° 0.0'	124° 12.0'
8/28/05	Heceta Head	5	2	44° 0.0'	124° 24.0'
		200			

DNA extraction

Genomic DNA was extracted from each larva by adding a mixture of 300 µl lysis buffer (0.5% SDS, 100 mM NaCl, 100 mM Tris [pH 8.0], 25 mM EDTA) and 100 µg of proteinase K (Fisher Scientific) to each sample. Samples were incubated at 65°C for 0.5-2 hrs until the tissue was fully digested. Samples were incubated for an additional 15 min at 37°C upon the addition of 8 µg RNase (Fisher Scientific). Proteins were precipitated with 7.5M ammonium acetate and the DNA was isolated with 100% isopropanol

following the removal of proteins. Due to the low expected yield of DNA, 10 μg of glycogen (Gentra Systems) was used to pull down the DNA from solution. The DNA was washed with 70% ethanol, air-dried, then resuspended in 30 μl of TE buffer (10 mM Tris [pH 8.0] and 1 mM EDTA). The DNA was allowed to rehydrate overnight and stored at 4°C.

DNA amplification

A series of polymerase chain reactions (PCR) were used to amplify regions of *COI*. Custom primers were designed using Primer3 v.0.3.0 software (Rozen and Skaletsky 2003). The first PCR reaction was used to amplify a 900 bp region of *COI*. This was performed in a 25 μl reaction volume containing a buffered solution of 50mM KCl, 10 mM Tris [pH 8.3], 0.2 mM dNTPs (Fisher), 0.2 μM forward primer (SCOIFB 5' TGGGGCAATTACAATGTT 3'), 0.2 μM reverse primer (SCOIRB 5' ATCAGCAGGAGGATAAGGAT 3'), 0.4 mg/ml bovine serum albumin (BSA), 4 mM $MgCl_2$, 1 unit Taq DNA polymerase (AllStar), and 10-20 ng larval DNA. Sterile water instead of DNA was added to one of the reaction tubes as a negative control. The reaction was performed on a Personal Thermal Mastercycler (Eppendorf) with the following parameters: initial denaturation for 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C for denaturation, 45 sec at 53-58°C for primer annealing, and 1 min at 72°C for DNA strand extension. This was followed by a final extension step for 10 min at 72°C. Specificity, size, and quality of the amplicons were verified on a 2% agarose gel pre-stained with 1% ethidium bromide alongside a 100 bp DNA size marker. The gel

was run at 150-160 V for 45-60 min and visualized under ultraviolet light on a Bio-Rad Gel Doc unit.

The second PCR reaction was a nested PCR amplifying a 700 bp region within the 900 bp region amplified in the first reaction. The nested PCR was performed in a 25 µl reaction containing 0.2 mM dNTPs, 0.2 µM forward primer (SCOIFmore 5' TTTTGATCCAGCAGGAGGAG 3'), 0.2 µM reverse primer (SCOIRmore 5' GACCCTATAGAAGAAACCACATTTC 3'), 2 mM $MgCl_2$, and 0.5 unit Taq DNA polymerase. Amplicon from the first PCR was diluted 10 to 100-fold with water and 1 µl of this dilution was used as the template. The concentration of DNA was estimated visually from the gel based on the DNA marker that was run on the same gel. If there was little or no visible amplicon on the agarose gel after the first PCR, then 0.5 µl of undiluted amplicon from the first PCR was used as the template. Thermal cycler parameters were as follows: 5 min at 94°C, followed by 30 cycles of 30 sec at 94°C, 30 sec at 58-62°C, and 1 min at 72°C with a final extension at 72°C for 10 min.

A second nested PCR amplifying a 591 bp region within the 700 bp region amplified in the first nested PCR was done in order to obtain more pure amplicons in sufficient quantities that would be suitable for sequencing. Thermal cycler conditions were the same as the first nested reaction except the forward primer SCOIFnew 5' CCTGGGTTTGGTATAATTTCTCA 3' and the reverse primer SCOIRnew 5' ATCGGGGTAATCTGAATATCG 3' were used instead. It was also necessary to dilute the amplicon as much as 1000-fold in order to prevent nonspecific amplicons from being synthesized during the reaction.

DNA sequencing

Samples that contained only the specific amplicon of interest and had concentrations estimated to be between 10 ng/μl and 40 ng/μl were selected for sequencing. Any excess dNTP's, primers, and single-stranded amplicons were removed by adding 2 μl ExoSAP-IT (USB) to 15 μl sample. Samples were incubated for 30 min at 37°C and 15 min at 80°C to deactivate the enzyme. The forward primer used for sequencing, COIFnew, was diluted two-fold to 5 μM. Samples were sent to Geneway Research (Hayward, CA) for sequencing. Chromatogram sequences were obtained through the use of the ABI Prism 3700 DNA Analyzer automated sequencer (Applied Biosystems) using Big Dye terminator chemistry.

Data analysis

A 548 bp region of the DNA was edited visually and aligned using the ClustalW multiple alignment algorithm in BioEdit software v7.0.5.2 (Hall 1999). DnaSP v4.20.2 (Rozas *et al.* 2003) was used to determine haplotype frequencies and polymorphisms (silent and replacement mutations) in each sequence as well as haplotype and nucleotide diversity indices. The population parameters, Θ_π and Θ_S, were estimated by Arlequin v3.11 (Excoffier *et al.* 2005). Theta S is based on the number of polymorphic sites and Θ_π is based on the mean number of pairwise nucleotide differences. Theta π is not influenced by any variation in sample size so it was included in this analysis. Cladograms of the larval haplotypes and the entire sample set of larvae were generated using the neighbor joining method with 10,000 bootstrap replicates and a Kimura two-

parameter distance model with MEGA v3.1 (Kumar *et al.* 2004). Three major haplotypes representing adult *N. californiensis* collected in 2005-2006 from Washington and Oregon estuaries (including Yaquina Bay) that were previously analyzed from our lab were also included in the larval haplotype cladogram. Three adult *N. gigas* from our lab were used as outgroups in the cladogram of all the sampled larvae. DnaSP was used to obtain Fu and Li's D, D*, F, and F* test statistics using an alpha of 0.05. For Fu and Li's D and F tests, a *N. affinis* sequence was used as the outgroup. Arlequin was used to obtain Tajima's D statistic and Fu's Fs test statistic. An alpha of 0.05 was used to determine significant Tajima's D values and an alpha of 0.02 was used to determine significant Fu's Fs test values. A value of zero indicates that the mutations are neutral and do not affect an individual's reproductive fitness. The observed mutations would therefore be attributed to genetic drift instead of natural selection. A negative value for a neutrality test is an indication that there is an excess of rare polymorphisms. This may be due to purifying selection, a bottleneck, and/or a recent population expansion. A positive value indicates a low number of high and low frequency polymorphisms that can be a result of balancing selection and a historically stable population (Fu 1997; Fu and Li 1993; Tajima 1989). Populations with significant negative neutrality test values were further analyzed by a mismatch distribution using DnaSP to confirm the population's evolutionary history.

To understand overall population structure and connectivity, a Mantel test and pairwise F_{ST} estimates of genetic differentiation were performed. The Mantel test was used to determine whether there was a correlation between geographic distance and genetic distance using 1,000 permutations with Alleles in Space v1.0 (Miller 2005). A

value closer to negative one indicates a negative correlation while a value closer to one indicates a positive correlation. Pairwise F_{ST} values were calculated based on standard F-statistics and the frequency of haplotypes using Arlequin with 10,000 permutations. An alpha of 0.05 was used to determine significant differences between populations. Each of the 21 sampled sites was treated and analyzed as a separate population due to the uncertainty of the source of each population. F_{ST} values between 0.000 and 0.035 indicated strong gene flow (Wright 1965), any value between 0.035 and 0.050 was considered moderate gene flow, and any value above 0.05 was considered weak gene flow. Any negative F_{ST} values that were calculated as a result of the corrections for unequal sample sizes used in the algorithm were treated as a zero value and therefore the populations would be considered to have strong gene flow between each other.

Results

There was high haplotype and nucleotide diversity when all the samples were analyzed together ($h = 0.958$, $\pi = 0.045$, Table 2). When the individual sampling sites were analyzed, only one of the sampled groups (YB 7/19/06) had low haplotype ($h < 0.5$) and low nucleotide ($\pi < 0.005$) diversity indices. This group had two larvae that shared the same haplotype, indicating that they are from the same maternal lineage. There was a mean nucleotide difference of 23.238 based on pairwise sequence comparisons of the entire sample set (Table 2), indicating that there were a lot of mutations at this locus. A broad range of the amount of pairwise nucleotide differences was present when the populations were analyzed individually (4.400-25.337 nucleotide differences). For CR larvae, there were fewer nucleotide differences between individuals collected further away from shore. This pattern was evident for NH and HH larvae, although only two sites were sampled along each line. There were 81 variable sites among 115 total haplotypes that were identified and all the mutations between haplotypes were substitutions (transitions or transversions). Seventy-seven of the 81 variable sites consisted of silent substitutions and four variable sites were replacement substitutions (Table 2). Replacement substitutions occurred in larvae sampled from CH, YB, and HH.

The number of haplotypes at a particular sampled site was highly correlated with the number of samples from each of those sites ($r = 0.954$). There were five dominant haplotypes (H1, H4, H16, H27, and H30) that represented 42% of the total sampled larvae (Table 3), which means that larvae were related to each other. Each of these haplotypes represented 11 or more individuals. H16 was the most widespread larval

haplotype and was shared with 33 larvae from CR, CM, CH, NH, and HH. H1 was shared with 15 larvae from CH and YB, H4 was shared with 13 larvae from CR and CH, H27 was shared with 11 larvae from CR, CM, and CH, and H30 was shared with 12 larvae from CR, CM, and CH. Six haplotypes represented two individuals each and there were 104 unique haplotypes (designated by the term 'singletons'). The presence of many singletons shows the genetic diversity of this species. Universal haplotypes (haplotypes present in every sampled site) were not present (Fig. 3). H16, H27, and H30 were in one clade (Fig. 2) and H1 and H4 were in a second clade, indicating that these two haplotypes were more genetically distant than those in the first clade. The second clade (Clade II, Table 3) consisted of 33 haplotypes with the majority of them from CH and YB. There was only one YB and one CM larva in Clade I, one HH larva in Clade II, and all NH larvae were in Clade I.

The three major adult shrimp haplotypes (aH1, aH2, and aH5) were identical to the major larval haplotypes from Clade I (Fig. 2). H16 was identical to aH1 and H27 and H30 were identical to aH2 and aH5, respectively. H1 and H4 did not have any genetic similarity to the major adult haplotypes and any of the 219 minor haplotypes associated with adults collected from various estuaries in Washington and Oregon even though H1 represented YB larvae. YB larvae seem to be more genetically distant than any other group of larvae (Fig. 5). It is the only group that did not share a common major haplotype, H16, with all the other sampled regions (Figure 3, Table 3).

Table 2 Haplotype (h) and nucleotide (π) diversity indices and genetic variability indices for each of the sites and a site composite. n = number of samples, H(n) = number of haplotypes, sm = number of silent mutations, rm = number of replacement mutations, Θ_S = population parameter estimate based on the number of segregating sites (number of polymorphic nucleotide sites), Θ_π = population parameter estimate based on mean number of pairwise nucleotide differences

Site	h	π	n	H(n)	sm	rm	Θ_S	Θ_π
CR 6/19/05, 4 nm	0.900	0.045	5	4	44	0	20.640	23.600
CR 6/19/05, 7 nm	0.872	0.030	13	9	46	0	14.501	15.718
CR 8/30/05, 7 nm	0.982	0.025	19	17	41	0	11.731	13.111
CR 8/30/05, 10 nm	0.906	0.021	34	18	39	0	9.538	10.898
CR 8/30/05, 15 nm	0.800	0.014	6	4	20	0	8.759	7.467
CM 6/20/05, 10 nm	1.000	0.025	2	2	13	0	13.000	13.000
CM 6/20/05, 15 nm	1.000	0.010	2	2	5	0	5.000	5.000
CM 8/31/05, 1 nm	0.978	0.035	10	9	39	0	13.786	18.111
CM 8/31/05, 3 nm	1.000	0.042	6	6	47	0	20.146	22.067
CH 6/21/05, 1 nm	0.941	0.049	33	21	61	3	15.523	25.337
CH 6/21/05, 2 nm	1.000	0.025	8	8	36	0	13.884	12.893
CH 6/21/05, 5 nm	0.700	0.008	5	3	10	0	4.800	4.400
CH 6/21/05, 10 nm	0.967	0.047	14	12	65	2	21.697	24.538
NH 8/29/05, 5 nm	0.933	0.021	6	5	30	0	13.139	11.067
NH 8/29/05, 10 nm	1.000	0.013	4	4	13	0	7.091	6.833
YB 7/14/06, pump	0.933	0.014	6	5	15	0	6.569	7.467
YB 7/19/06, pump	0.000	0.000	2	1		no polymorphisms		
YB 7/26/06, pump	0.727	0.031	11	6	32	3	11.950	16.145
YB hatchery	0.833	0.032	4	3	23	3	14.182	16.667
HH 8/21/05, 1 nm	0.700	0.033	5	3	35	2	17.760	17.200
HH 8/28/05, 2 nm	1.000	0.009	5	5	10	0	4.800	4.600
All sites	0.958	0.045	200	115	77	4	13.622	23.238

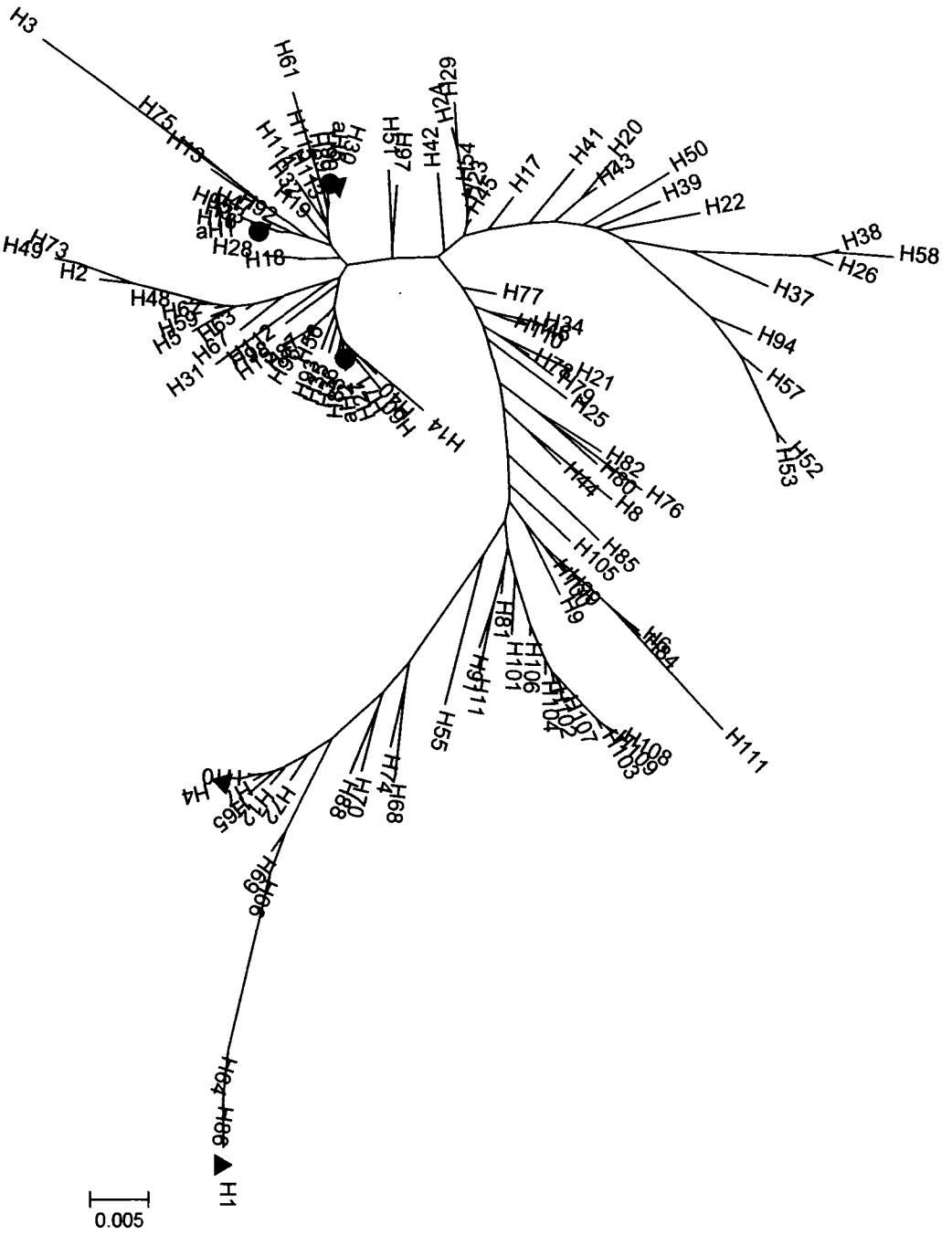

Fig. 2 Unrooted cladogram of the 115 larval haplotypes. Solid triangles represent the five major haplotypes and the solid circles represent the three major adult haplotypes.

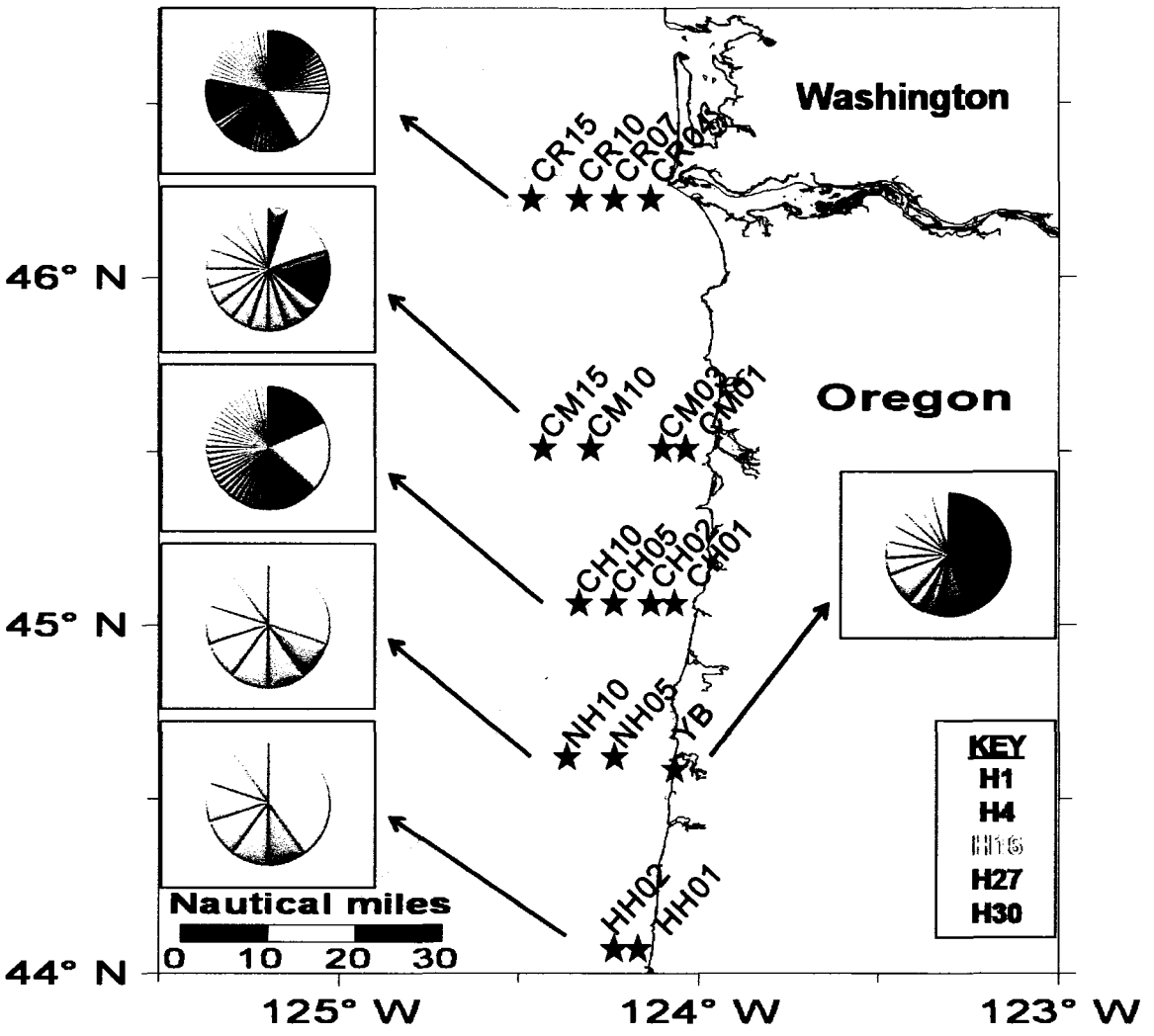

Fig. 3 Distribution of haplotypes along the Oregon coastline. CR = Columbia River (n = 77), CM = Cape Meares (n = 20), CH = Cascade Head (n = 60), NH = Newport Hydrographic (n = 10), YB = Yaquina Bay (n = 23), HH = Heceta Head (n = 10)

Table 3 Haplotype composition and number of larvae representing each haplotype. CR = Columbia River (n = 77), CM = Cape Meares (n = 20), CH = Cascade Head (n = 60), NH = Newport Hydrographic (n = 10), YB = Yaquina Bay (n = 23), HH = Heceta Head (n = 10)

	Clade I Population							Clade I Population							Clade II Population					
Haplotype	**CR**	**CM**	**CH**	**NH**	**YB**	**HH**	**Haplotype**	**CR**	**CM**	**CH**	**NH**	**YB**	**HH**	**Haplotype**	**CR**	**CM**	**CH**	**NH**	**YB**	**HH**
2	1	1					50		1					1			5		10	
3	1						51		1					4	7		6			
5	1						52		2					6	1					
8	1						53		1					7	1					
13	1						54		1					9	1					
14	1						56		1					10	1					
15	1						57		1					11	1					
16	12	3	11	3		4	58		1					12	1					
17	1						59			1				55		1				
18	1						60			1				64			1			
19	1		1				61			1				65			1			
20	1						62			1				66			1			
21	1						63			1				68			1			
22	1						67			1				69			1			
23	1						71			1				70			1			
24	1						73			1				72			1			
25	1						75			1				74			1			
26	1						76			1				81			1			
27	7	2	2				77			1				84			1			
28	1						78			1				86			1			
29	1						79			1				88			1			
30	9	1	2				80			1				91			1			
31	1						82			1				99					2	
32	1						83			1				100					1	
33	1	1					85			1				101					1	
34	1						87			1				102					2	
35	1						89			1				103					1	
36	1						90			1				104					1	
37	1						92				1			106					1	
38	1						93				1			107					1	
39	1						94				1			108					1	
40	1						95				1			109					1	
41	1						96				1			111						1
42	1						97				1			**Total**	**13**	**1**	**24**	**0**	**22**	**1**
43	1						98				1									
44	1						105					1								
45	1						110						1							
46	1						112						1							
47	1						113						1							
48		1					114						1							
49		1					115						1							
							Total	**64**	**19**	**36**	**10**	**1**	**9**							

Table 4 Tests of neutrality statistics. Bold values indicate significant values ($p < 0.02$ for Fu's F_S statistic and $p < 0.05$ for Tajima's D and Fu and Li's tests). CR = Columbia River (n = 77), CM = Cape Meares (n = 20), CH = Cascade Head (n = 60), NH = Newport Hydrographic (n = 10), YB = Yaquina Bay (n = 23), HH = Heceta Head (n = 10). nd = no data due to the lack of the minimum number of samples needed for the analysis

		Fu and Li's tests of neutrality				
Site	**Tajima's D**	**D**	**F**	**D***	**F***	**Fu's Fs**
CR 6/19/05, 4 nm	1.080	1.140	1.292	0.956	1.019	3.060
CR 6/19/05, 7 nm	0.375	0.696	0.658	0.006	0.088	1.352
CR 8/30/05, 7 nm	0.475	0.743	0.804	0.052	0.206	-4.771
CR 8/30/05, 10 nm	0.514	**1.823**	1.661	1.261	1.195	-1.038
CR 8/30/05, 15 nm	-0.922	-0.055	-0.320	-0.886	-0.973	1.967
CM 6/20/05, 10 nm	nd	nd	nd	nd	nd	2.565
CM 6/20/05, 15 nm	nd	nd	nd	nd	nd	1.609
CM 8/31/05, 1 nm	1.522	**1.654**	**1.957**	**1.522**	**1.618**	-0.359
CM 8/31/05, 3 nm	0.610	0.401	0.487	0.260	0.332	0.132
CH 6/21/05, 1 nm	2.344	**2.039**	**2.584**	**1.777**	**2.294**	0.587
CH 6/21/05, 2 nm	-0.381	0.702	0.498	-0.424	-0.462	-1.669
CH 6/21/05, 5 nm	-0.596	-0.486	-0.606	-0.596	-0.629	2.055
CH 6/21/05, 10 nm	0.580	**1.551**	1.520	1.111	1.086	-0.168
NH 8/29/05, 5 nm	-0.999	0.718	0.369	-1.007	-1.099	0.986
NH 8/29/05, 10 nm	-0.367	-0.299	-0.366	-0.367	-0.373	-0.124
YB 7/14/06, pump	0.842	0.895	1.017	0.824	0.899	0.332
YB 7/19/06, pump		no polymorphisms				
YB 7/26/06, pump	1.638	**1.928**	**2.311**	0.996	1.317	4.320
YB hatchery	1.807	2.122	2.430	1.807	1.889	3.504
HH 8/21/05, 1 nm	-0.237	0.639	0.545	-0.237	-0.256	5.126
HH 8/28/05, 2 nm	-0.298	-0.081	-0.152	-0.298	-0.314	-1.481
All sites	2.163	**1.918**	**2.309**	1.340	**1.927**	**-23.681**

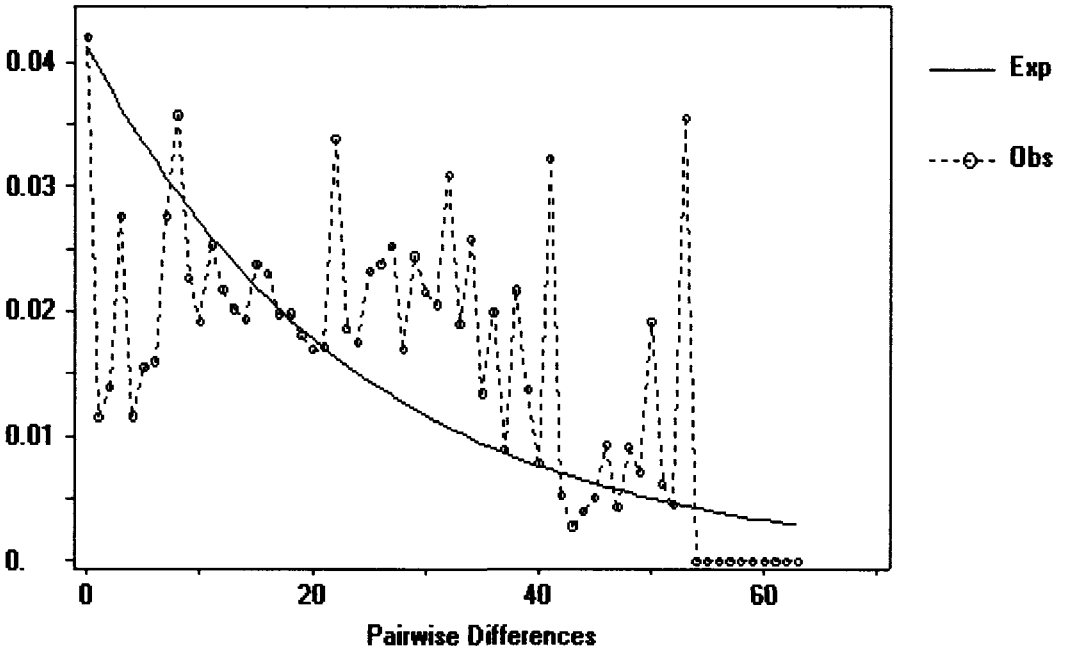

Fig. 4 Mismatch analysis of larval *COI* sequences showing the frequency (y-axis) of the number of pairwise nucleotide differences (x-axis) for each sample. The smooth curve represents a classic model of a recent population expansion.

When all the larvae were analyzed as a single population, there were significant positive values ($p < 0.05$) for Fu and Li's D, F, and F* test statistics (1.918, 2.309, and 1.927, respectively), indicating that there was balancing selection at the *COI* locus. However, there was a significantly negative value for Fu's Fs test statistic (-23.681, $p < 0.01$, Table 4), indicating that there could be purifying selection at the same locus and that a bottleneck followed by a recent population expansion may have occurred. The observed mismatch distribution deviated from the expected unimodal distribution, suggesting that there was no recent population expansion (Fig. 4). This result validated the positive values of the neutrality test statistics. All Fu and Li's tests statistics were significant and positive for August CM01 and June CH01 populations. There were

positive Fu and Li's D (1.928) and F (2.311) test statistics for one of the YB populations. There were no significant Tajima's D values for any population (Table 4).

Population connectivity and gene flow along the Washington and Oregon coast

Geographic distance and genetic distance were poorly correlated ($r = 0.071$, $p = 0.999$) suggesting that there was no isolation by distance effect. Most of the significant genetic differences occurred between YB or June CR and the rest of the larvae, but the differences were not enough to be considered a genetic break or subdivision. There was no apparent relationship between the amount of gene flow and the distance from shore each larva was collected, except for several of the CR larvae. A summary of the key observations from Table 5 is shown below based on the pairwise F_{ST} estimates of genetic differentiation:

Columbia River: There was weak gene flow between June 2005 CR larvae and most of the other sampled groups ($F_{ST} = 0.061 - 0.312$), except CH01 ($F_{ST} = 0.003 - 0.023$). CR larvae collected 4 nm and 7 nm from the shore were more genetically similar to each other than those collected 10 nm and 15 nm from the shore regardless of the month they were collected ($F_{ST} = 0.000$-0.159), indicating there was a lot of diversity among individuals collected from the same region. August CR15 larvae had stronger gene flow with HH01 than August CR07 did, suggesting that the distance from shore can have a profound effect on dispersal capability and recruitment.

Cape Meares: Larvae along the CM line were similar to each other at all sampled distances from shore ($F_{ST} = 0.000 - 0.016$). However, there was weak gene flow between

these groups and YB larvae (F_{ST} = 0.149 – 0.268). There was high gene flow between CM larvae and all the other groups.

Cascade Head: There was weak gene flow between June CH01/CH02 populations and June CH05 larvae (F_{ST} = 0.069 – 0.134) and August CR10 larvae (F_{ST} = 0.047 – 0.053). There was no genetic subdivision in this region, but the larvae were from different maternal lineages. Similar to the CR larvae, more distant CH larvae (June CH05 and CH10) had stronger gene flow with HH01.

Yaquina Bay and Newport: Weak gene flow existed between YB larvae and almost all the other sampled populations. Most of the significant F_{ST} values representing genetic differences were associated with YB populations. Weak gene flow was present between July 14 and July 26 YB larvae (F_{ST} = 0.183), which indicates that there are from diverse maternal lineages. NH larvae, which were sampled adjacent to YB, had strong gene flow with most larvae except YB (F_{ST} = 0.183). Strong gene flow existed between NH and HH larvae (F_{ST} = 0.000 – 0.010).

Heceta Head: There was high gene flow (F_{ST} = 0.034) between the HH01 and HH02 larvae, suggesting that these groups of larvae may have come from the same source population. There were significant differences between HH01 and the following populations: June CR07 (F_{ST} = 0.197), June CH01 (F_{ST} = 0.069), June CH02 (F_{ST} = 0.134), and all YB larvae (F_{ST} = 0.177 – 0.483). HH02 larvae had high gene flow with most of the other populations except June 2005 CR larvae (F_{ST} = 0.050 – 0.074) and several YB larvae (F_{ST} = 0.286), suggesting that there is heterogeneity along the sampled

region even though gene flow between some individuals from the two most distant sampled regions (CR and HH) was high.

Adult shrimp: The adults collected during 2005 and 2006 in Washington and Oregon had high gene flow with most of the sampled sites ($F_{ST} \leq 0.000$), suggesting that most of the sampled larvae are originating from those estuaries. There was weak gene flow between these adults and June 2005 CR larvae ($F_{ST} = 0.058 - 0.084$). Gene flow with YB larvae ranged from moderate to weak ($F_{ST} = 0.040 - 0.368$). This pattern is consistent with the observed haplotype clades in the cladogram.

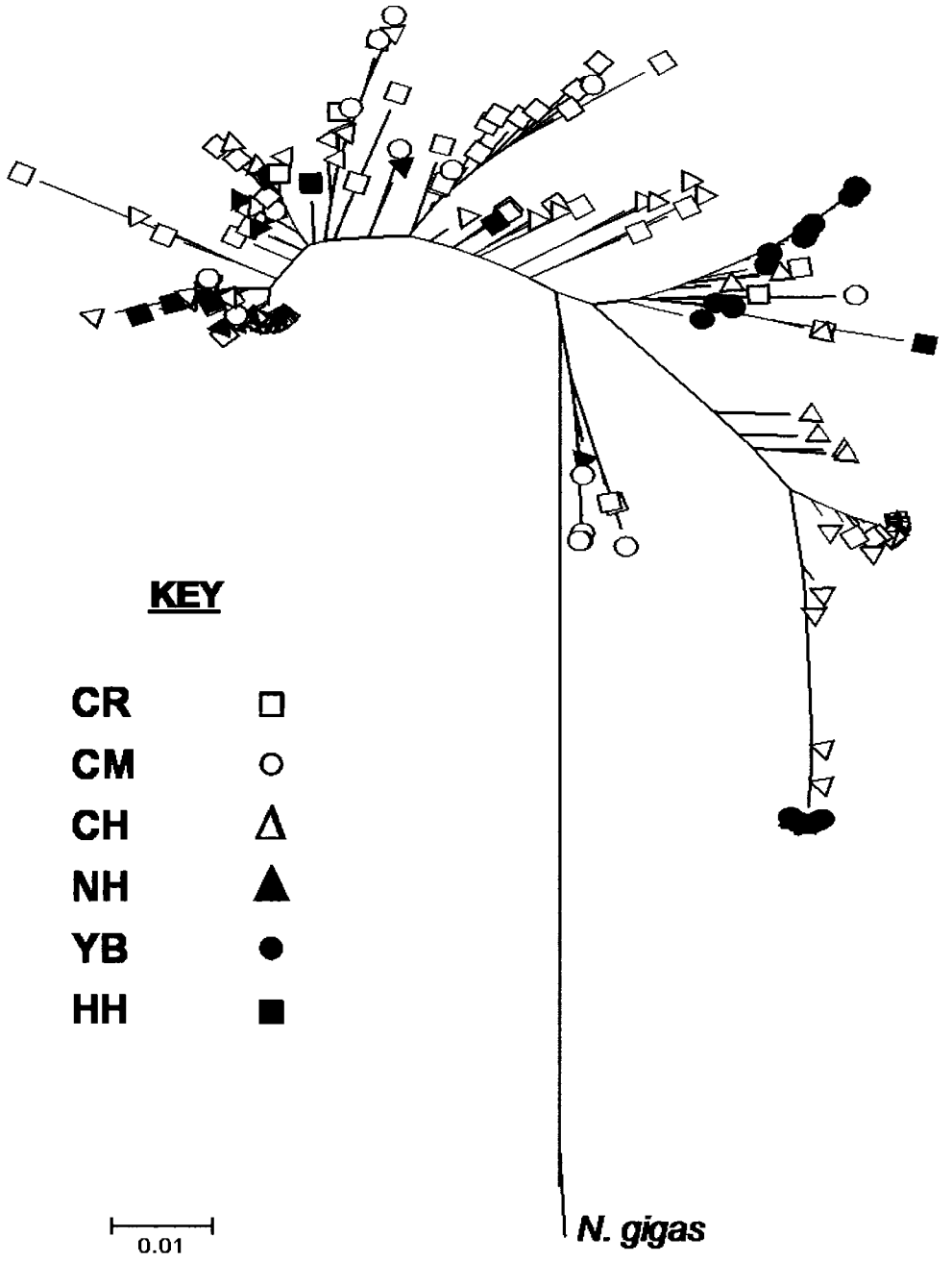

Fig. 5 Rooted cladogram of all larvae samples labeled by sampled region. CR = Columbia River (n = 77), CM = Cape Meares (n = 20), CH = Cascade Head (n = 60), NH = Newport Hydrographic (n = 10), YB = Yaquina Bay (n = 23), HH = Heceta Head (n = 10). *N. gigas* was used as the outgroup.

Table 5 Pairwise F_{ST} estimates between samples. Bold values indicate significant differences between populations. CR = Columbia River (n = 77), CM = Cape Meares (n = 20), CH = Cascade Head (n = 60), NH = Newport Hydrographic (n = 10), YB = Yaquina Bay (n = 23), HH = Heceta Head (n = 10)

	CR 6/19/05, 4 nm	CR 6/19/05, 7 nm	CR 8/30/05, 7 nm	CR 8/30/05, 10 nm	CR 8/30/05, 15 nm	CM 6/20/05, 10 nm	CM 6/20/05, 15 nm
CR 6/19/05, 4 nm							
CR 6/19/05, 7 nm	-0.045						
CR 8/30/05, 7 nm	**0.051**	**0.071**					
CR 8/30/05, 10 nm	**0.097**	**0.110**	0.009				
CR 8/30/05, 15 nm	0.152	**0.159**	0.011	0.029			
CM 6/20/05, 10 nm	0.069	0.096	-0.072	-0.020	-0.154		
CM 6/20/05, 15 nm	-0.036	0.096	0.013	0.072	0.143	0.000	
CM 8/31/05, 1 nm	0.056	**0.077**	-0.007	0.002	0.040	-0.036	0.016
CM 8/31/05, 3 nm	0.048	0.072	-0.026	0.000	-0.013	-0.091	0.000
CH 6/21/05, 1 nm	0.003	0.023	0.012	**0.047**	0.040	-0.035	0.044
CH 6/21/05, 2 nm	0.045	0.068	0.009	**0.053**	0.094	0.000	0.000
CH 6/21/05, 5 nm	0.200	**0.197**	0.033	0.029	-0.074	-0.145	0.212
CH 6/21/05, 10 nm	**0.061**	**0.080**	-0.017	0.000	-0.012	-0.094	0.024
NH 8/29/05, 5 nm	0.083	**0.101**	-0.014	0.025	-0.040	-0.146	0.047
NH 8/29/05, 10 nm	0.053	0.078	-0.030	0.015	-0.018	-0.143	0.000
YB 7/14/06, pump	0.083	**0.101**	**0.039**	**0.083**	0.133	0.047	0.047
YB 7/19/06, pump	0.350	**0.312**	**0.230**	**0.272**	**0.400**	0.500	0.500
YB 7/26/06, pump	**0.202**	**0.198**	**0.136**	**0.171**	**0.242**	0.208	0.208
YB hatchery	0.131	**0.143**	**0.074**	**0.120**	0.185	0.111	0.111
HH 8/21/05, 1 nm	0.200	**0.197**	0.043	0.077	-0.074	-0.145	0.212
HH 8/28/05, 2 nm	0.050	0.074	-0.022	0.022	0.004	-0.111	0.000
2005/2006 adults	0.058	0.084	-0.084	-0.158	-0.137	-0.200	0.000

Table 5, cont'd. Pairwise F_{ST} estimates between samples. Bold values indicate significant differences between populations. CR = Columbia River (n = 77), CM = Cape Meares (n = 20), CH = Cascade Head (n = 60), NH = Newport Hydrographic (n = 10), YB = Yaquina Bay (n = 23), HH = Heceta Head (n = 10)

	CM 8/31/05, 1 nm	CM 8/31/05, 3 nm	CH 6/21/05, 1 nm	CH 6/21/05, 2 nm	CH 6/21/05, 5 nm	CH 6/21/05, 10 nm	NH 8/29/05, 5 nm
CM 8/31/05, 3 nm	-0.022						
CH 6/21/05, 1 nm	0.024	0.003					
CH 6/21/05, 2 nm	0.011	0.000	0.033				
CH 6/21/05, 5 nm	0.069	0.049	**0.069**	**0.134**			
CH 6/21/05, 10 nm	-0.008	-0.031	0.003	0.017	0.006		
NH 8/29/05, 5 nm	0.010	-0.024	0.012	0.032	-0.026	-0.025	
NH 8/29/05, 10 nm	-0.012	-0.043	-0.002	0.000	0.010	-0.036	-0.051
YB 7/14/06, pump	0.043	0.033	**0.062**	0.032	**0.177**	0.048	0.067
YB 7/19/06, pump	**0.252**	**0.268**	0.165	**0.248**	**0.483**	**0.199**	0.311
YB 7/26/06, pump	**0.149**	**0.153**	**0.095**	**0.144**	**0.284**	**0.114**	**0.183**
YB hatchery	0.081	0.075	0.041	0.070	0.239	0.052	0.111
HH 8/21/05, 1 um	0.088	0.049	**0.069**	**0.134**	-0.094	0.021	-0.026
HH 8/28/05, 2 nm	-0.008	-0.034	0.004	0.000	0.034	-0.025	-0.034
2005/2006 adults	-0.096	-0.125	-0.024	0.000	-0.132	-0.112	-0.081

Table 5, cont'd. Pairwise F_{ST} estimates between samples. Bold values indicate significant differences between populations. CR = Columbia River (n = 77), CM = Cape Meares (n = 20), CH = Cascade Head (n = 60), NH = Newport Hydrographic (n = 10), YB = Yaquina Bay (n = 23), HH = Heceta Head (n = 10)

	NH 8/29/05, 10 nm	YB 7/14/06, pump	YB 7/19/06, pump	YB 7/26/06, pump	YB hatchery	HH 8/21/05, 1 nm	HH 8/28/05, 2 nm
YB 7/14/06, pump	0.037						
YB 7/19/06, pump	0.314	0.311					
YB 7/26/06, pump	0.168	**0.183**	-0.121				
YB hatchery	0.083	0.073	-0.081	-0.062			
HH 8/21/05, 1 nm	0.010	**0.177**	**0.483**	**0.284**	0.239		
HH 8/28/05, 2 nm	-0.053	0.035	**0.286**	0.159	0.078	0.034	
2005/2006 adults	-0.091	0.040	0.368	0.182	0.092	-0.034	-0.071

Discussion

It was hypothesized that there would be weak population structure along the sampled range because *N. californiensis* larvae can potentially travel southward nearly 500 km, which is based on an eight week dispersal period and 10 cm/s average flow rate of the California Current. This is a much farther distance than the sampled range of roughly 300 km. However, there were several assumptions when this hypothesis was tested: 1) larvae passively drift along the California Current 2) larvae do not migrate vertically through the water column 3) there are no dispersal barriers and 4) oceanic conditions are consistent from year to year. *N. californiensis* larvae were less abundant past 10 nm from shore during the collection period, suggesting that the larvae were retained nearshore either by ocean circulation forces, larval behavior, or a combination of both factors. There were also barriers limiting or impeding the gene flow of populations within close proximity of each other, but the barriers were not strong enough to cause genetic discontinuity along the range and the entire sampled population was historically stable. Heterogeneous patches existed along the sampled range, suggesting that some estuaries have more diverse populations of *N. californiensis* than others.

Barth *et al.* (2005) found that Heceta Bank, which is a seamount offshore of Heceta Head, caused the upwelling jet to go around the bank. Water velocities north and south of the bank were higher than those found inshore of the bank. The lower water velocities caused water retention over the bank, leading to higher amounts of phytoplankton and zooplankton (Ressler *et al.* 2005). *N. californiensis* would thrive with the amount of food available in this area and the larvae originating north of this bank

could be retained over the bank and recruit into Yaquina Bay during relaxation events. YB larvae had unique haplotypes, unlike the YB adults collected in 2005. These adults shared haplotypes with adults collected from Washington estuaries in the same year, but the YB adults also had many more haplotypes than other estuaries sampled from Washington and Oregon. There could be two reasons for the inconsistency between the larvae and adult genetic data: 1) the larvae sample size was much less than the sample size of adults so the number of larval haplotypes was lower as a result and 2) there is interannual recruitment variability. Given that the number of samples was highly correlated with the number of observed haplotypes, it is likely that a larger sample size from YB would result in a larger number of observable haplotypes. Since the YB adults that were collected were most likely a mixture of different age classes, this would mean that depending on the year, new recruits to YB would consist of either retained YB larvae released the same year and/or larvae from other estuaries. The genetic differences observed between different groups of YB larvae reflect this ($F_{ST} = 0.183$). If Yaquina Bay is within a retention zone, then it makes sense that there is increased diversity there. Larvae from more northern areas would be retained within this region, increasing the size of the gene pool. The larvae that would be able to recruit into YB during flood tides would increase the total genetic diversity in YB. The retention zone at YB could act as a depository of larvae from CR, CM, CH, and NH and a barrier to areas south of the zone, although there can be some genetic leakage. In estuaries that are not near a retention zone, only a subsample of the total larvae that are in a retention zone would be able to recruit there. In this study, three groups of YB larvae were analyzed, each collected

roughly a week apart. The 19 larvae analyzed were most likely a mixture of offspring from different mothers. The July 14 population had high haplotype diversity ($h = 0.933$), the July 19 population had no diversity probably due to the small sample size (n = 2), and the July 26 population was not as diverse as any other sampled larvae along the Oregon coast. The haplotype diversity could be the consequence of different pulses of larvae being released from different adult mothers of various age classes. Comparing the haplotypes of newly settled juveniles from different years with the YB larval haplotypes from this study could reveal shared haplotypes. Any similar haplotypes observed would provide further support for the retention zone hypothesis.

In a recent study published by Petersen (2007), adult shore crabs (*Hemigrapsus oregonensis*) from 2000-2001 in Oregon were analyzed. *Hemigrapsus* has a slightly different life history to that of *N. californiensis* so it makes for an interesting comparison. *Hemigrapsus* populations from Yaquina Bay, Tillamook Bay, and Coos Bay were related to each other and had shared haplotypes ($F_{ST} < 0.01$). Tillamook Bay is the closest estuary to the north of CM so it is likely that some CM larvae came from Tillamook Bay. Overall, the haplotype diversity was high for *N. californiensis* larvae which suggest many maternal lineages are present. Larvae collected at CR, CM, CH, NH, and HH shared haplotypes with adults collected from estuaries of Washington and Oregon in 2005-2006, indicating that there was high gene flow among these regions. However, the genetic heterogeneity observed suggests that larvae from various genetic backgrounds (i.e., more diverse haplotypes) were retained near YB upon release during this particular year, either by coastal oceanography and/or by their natural behavior. There is evidence that the

availability of larvae during a particular season coupled with ideal physical transport mechanisms from a retention zone can cause interannual recruitment variability in several taxa of invertebrate populations (Wing *et al.* 2003). YB larvae shared a major haplotype (H1) with Cascade Head samples, but not with HH larvae. However, HH larvae shared haplotypes with larvae from the other sampled regions and some HH larvae had high gene flow with CH larvae. During some years, the larvae may not be as strongly retained, allowing larvae to continue their southward transport. It is unlikely that *N. californiensis* larvae remain in YB after hatching because the plankton pump used to catch them was placed at the mouth of the estuary, but this can only be concluded for the July 2006 sampling period. YB shrimp would begin to have a more homogenous structure over time if they never left the estuary or the larvae released from YB always recruited back into YB. The diverse haplotypes of the YB larvae and adults supports the hypothesis that recruitment can be variable from year to year. Heceta Bank is about 9 nm wide and 13 nm long. It is possible that larvae at least 10 nm offshore would be able to bypass the retention zone, as evident in the strong amount of gene flow between CR15 larvae and HH01 larvae as well as between CH10 and HH01 larvae.

The weak gene flow observed between June CH01/CH02 larvae and August CR10 and between CR larvae may be the result of the flow characteristics of the Columbia River plume. During the summer, the huge volume of water flowing out of the Columbia River creates a plume that normally flows southwest during upwelling, but also flows north at the same time during downwelling events. This causes an eddy to form near the mouth of the Columbia River, creating a retention zone nearshore (Hickey *et al.*

2005). This would not have affected June CR samples because of the late upwelling season in 2005, but it could have prevented August CR larvae from mixing with other populations during downwelling periods. Genetic diversity is very high in this region and the larvae sampled in this region probably came from multiple northern bays including False Bay, Grays Harbor, and Willapa Bay. Fewer nucleotide differences between CR larvae corresponded with increased sampling distance. The larvae collected at 15 nm from shore were likely to have been advected far offshore because they could not successfully migrate through the water column to retain themselves nearshore. The force of the water coming out of Columbia River would push larvae away from shore and those that have specific haplotypes would be selected for and be able to remain neashore.

Selection

COI was under selective pressure in *N. californiensis*. Approximately 95% of the total mutations in the entire sample set were synonymous substitutions, meaning these mutational mutations would not affect the structure of the enzyme. Fu and Li's D and F neutrality test statistics were positive for several groups of larvae and for the entire sample set combined, suggesting that there was balancing selection and the population was historically stable. This is consistent with the analysis of adult populations from our lab. The high larval haplotype and nucleotide diversity observed corroborates the balancing selection theory. A large effective female population size most likely contributed to the amount of observed haplotype diversity. The mismatch analysis, along with the genetic diversity observed suggests that the sampled shrimp were from a

historically stable population. Balancing selection maintains polymorphisms in the population so the variations of *COI* may allow larvae to oxidize metabolites at a wider temperature range. This can produce more energy so larvae that can prevent themselves from being carried out far into the ocean would have a selective advantage by having access to more food and being able to retain themselves nearshore.

Conclusion

The dispersal patterns of planktonic larvae can be difficult to study for many species because of their size, behavior, and the complex marine environment in which they are in. Analyzing only the adult populations can confound assessments of dispersal and recruitment. Genetic analyses of the dispersing larvae can reveal details of a species' population structure. Since oceanic conditions and processes can be variable from year to year, it seems implausible for larvae to realize their full dispersal potential. This research showed the importance of understanding coastal topography, oceanography, and behavior in order to better understand a marine species' life history and population structure.

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