

*Historical and contemporary forces
shape genetic variation in the Olympic
mudminnow (Novumbra hubbsi), an
endemic fish from Washington State, USA*

**Patrick W. DeHaan, Brice A. Adams,
Roger A. Tabor, Denise K. Hawkins &
Brad Thompson**

Conservation Genetics

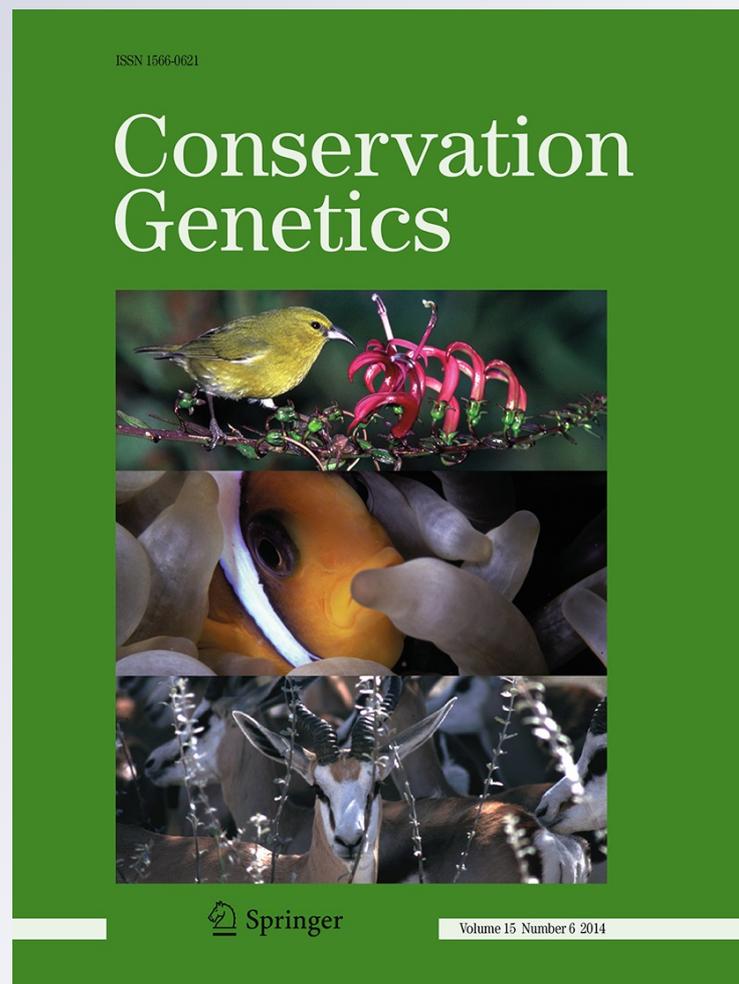
ISSN 1566-0621

Volume 15

Number 6

Conserv Genet (2014) 15:1417-1431

DOI 10.1007/s10592-014-0627-7



Your article is protected by copyright and all rights are held exclusively by Springer Science+Business Media Dordrecht (outside the USA). This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Historical and contemporary forces shape genetic variation in the Olympic mudminnow (*Novumbra hubbsi*), an endemic fish from Washington State, USA

Patrick W. DeHaan · Brice A. Adams ·
Roger A. Tabor · Denise K. Hawkins ·
Brad Thompson

Received: 23 April 2014 / Accepted: 10 June 2014 / Published online: 25 June 2014
© Springer Science+Business Media Dordrecht (outside the USA) 2014

Abstract Genetic data have become increasingly useful for conservation planning when data regarding population status and long-term viability is limited. The Olympic mudminnow is the only fish species endemic to Washington State, USA. The species is an increasing priority for conservation given its limited distribution and increasing habitat loss. Presently, information important for developing conservation plans including population abundance data, knowledge of population boundaries, and estimates of gene flow among populations are limited. We used microsatellite markers to assess the level of genetic variation within and among Olympic mudminnow collections from 23 sites across the species range. Genetic variation within collections ranged widely and was greatest within the Chehalis River Basin, a former glacial refugium. Analysis of population boundaries showed that each collection site represented a unique population with the exception of collections made within two large wetland and stream complexes. Genetic variation among populations

appears to be strongly influenced by glacial history and the species' life history. Populations originating from the Chehalis River glacial refugium clustered together in multiple analyses and populations from the Olympic Coast, which persisted in separate refugia and have limited capacity for dispersal, showed a high level of differentiation. Competing theories existed regarding the origins of disjunct populations in east Puget Sound and genetic data showed that these populations represent undocumented introductions rather than a glacial remnant or historic colonization from the Chehalis refugium. Data presented in this study will help fill important information gaps and advance conservation planning for this species.

Keywords Olympic mudminnow · Microsatellites · Genetic variation · Historic isolation · Introduced populations

Introduction

Genetic data have become increasingly important for managing species of conservation concern. One advantage of incorporating genetic data into conservation plans is that genetic data allow biologists to make inferences regarding species and populations when traditional types of data (e.g., mark-recapture abundance estimates, movement data from physical tags) are unavailable. For example, genetic information can be used to estimate population size when traditional census data is lacking (Kendall et al. 2008; Brinkman et al. 2011), to infer trends in abundance (i.e., increasing, declining) when long term abundance data are limited (Osborne et al. 2010; Charlier et al. 2012), and to infer patterns of movement in the absence of tagging data (Taylor et al. 2011; Homola et al. 2012).

Electronic supplementary material The online version of this article (doi:10.1007/s10592-014-0627-7) contains supplementary material, which is available to authorized users.

P. W. DeHaan (✉) · B. A. Adams · D. K. Hawkins
U.S. Fish and Wildlife Service, Abernathy Fish Technology
Center, 1440 Abernathy Creek Rd., Longview, WA 98632, USA
e-mail: patrick_dehaan@fws.gov

R. A. Tabor · B. Thompson
U.S. Fish and Wildlife Service, Washington Fish and Wildlife
Office, 510 Desmond Dr. SE, Suite 102, Lacey, WA 98503, USA

Present Address:

D. K. Hawkins
U.S. Fish and Wildlife Service, Washington Fish and Wildlife
Office, 510 Desmond Dr. SE, Suite 102, Lacey, WA 98503, USA

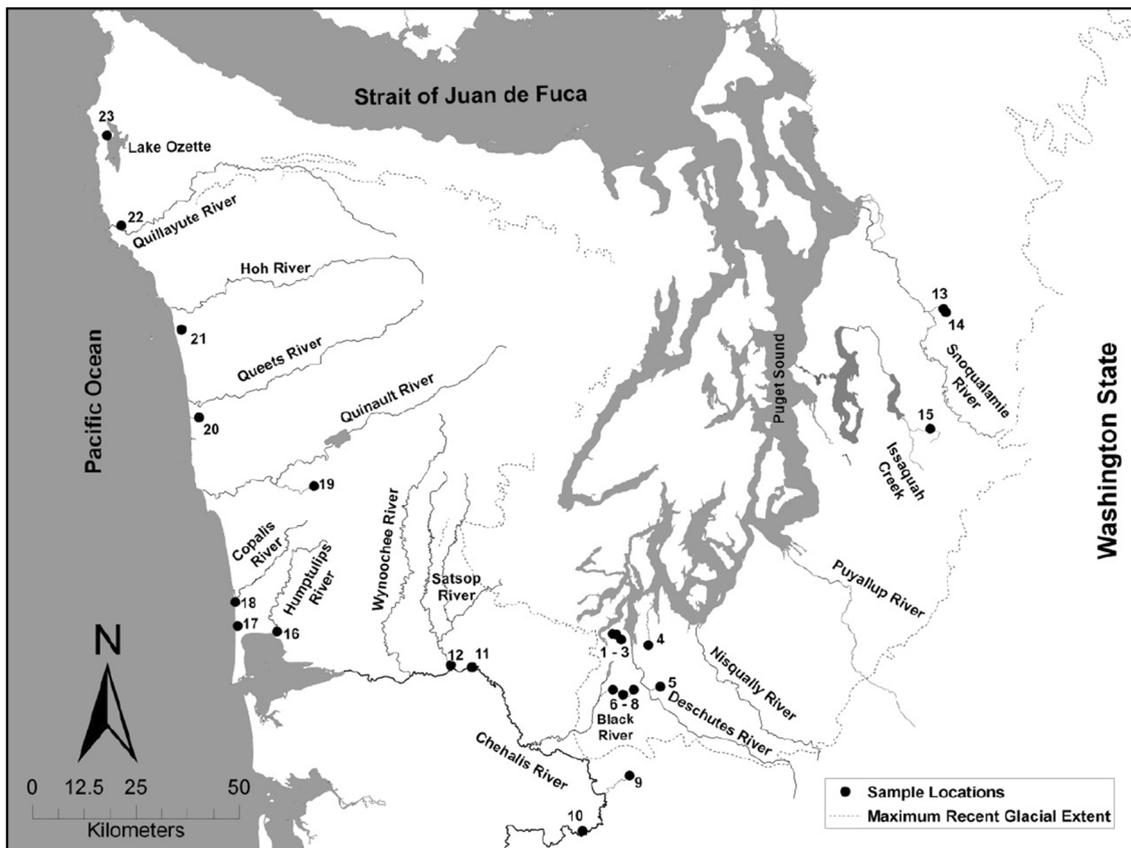


Fig. 1 Locations where Olympic mudminnow were collected for this study. The *dashed line* represents the maximum extent of the Puget Ice Lobe during the most recent glacial period. Sampling location numbers correspond to collection site numbers in Table 1

When genetic data is used for conservation planning, it's important that biologists have an understanding of how historical and contemporary factors influence genetic variation within and among populations. Historical factors that have shaped genetic variation include glacial events, floods, and changing climates (Bernatchez and Wilson 1998; Tilston Smith et al. 2011; Strugnell et al. 2012). Contemporary factors that influence genetic variation include landscape features and aspects of the species biology such as population size and dispersal ability (Whiteley et al. 2004; Gomez-Uchida et al. 2009; Tilston Smith et al. 2011). Contemporary influences may also include anthropogenic factors such as habitat fragmentation, translocations, and population supplementation (Meldgaard et al. 2003; Metcalf et al. 2012). Understanding how different factors influence genetic variation is important for prioritizing populations for conservation. Populations that are highly differentiated and contain unique genetic material as a result of historic isolation may have a higher conservation value than introduced populations that are highly differentiated due to increased genetic drift resulting from low numbers of founders.

The Olympic mudminnow (*Novumbra hubbsi*) provides a good example of a species where genetic data can provide

useful information for conservation planning. Olympic mudminnow are endemic to western Washington State, USA (Fig. 1). The Olympic mudminnow is the only member of the genus *Novumbra* and one of only five small-bodied fishes in the family *Esocidae* termed “mudminnow”. The species inhabits marshy, wetland type habitat with muddy substrate, little to no water velocity, and abundant aquatic vegetation (Meldrim 1968; Harris 1974; Mongillo and Hallock 1999). Olympic mudminnow tolerate a range of environmental conditions but they generally avoid saline waters and swift currents (Meldrim 1968) and they do not often occur when introduced predatory fishes are present (Mongillo and Hallock 1999). Much of the species' habitat has been lost in recent decades due to urbanization and development (Mongillo and Hallock 1999). The species' present distribution consists of four broad geographic areas within Washington State: (1) the Chehalis River Basin; (2) coastal drainages on the Olympic Peninsula (e.g., Lake Ozette, Quinalt River, Copalis River); (3) south Puget Sound; and (4) east Puget Sound (Lake Washington and Snoqualmie River drainages; Fig. 1). Despite the species' limited distribution, Olympic mudminnow have been documented at over 100 distinct

sites (e.g., wetlands, creeks, sloughs; Mongillo and Hallock 1999). Due to habitat loss and the species' limited distribution, Olympic mudminnow are considered a sensitive species and an increasing priority for conservation (Mongillo and Hallock 1999).

The distribution of Olympic mudminnow has been largely influenced by glaciation. At the maximum extent of the Pleistocene glaciation (approximately 15,000 years ago), Puget Sound and a portion of western Washington State were covered by the Puget Lobe of the Cordilleran Ice Sheet. During this period Olympic mudminnow were restricted to refugia in the Chehalis River Basin and coastal river systems on Washington's Olympic Peninsula (McPhail 1967; McPhail and Lindsey 1986). As the Puget Lobe receded, a freshwater lake formed at the southern end of present day Puget Sound, and this lake drained south into the Chehalis River. Olympic mudminnow were able to access newly available freshwater habitat in Puget Sound via the proglacial connections between southern Puget Sound and the Chehalis River (McPhail 1967). Because Olympic mudminnow avoid swift currents and saline waters, they did not disperse beyond drainages in southern Puget Sound.

One notable exception to the pattern of Olympic mudminnow distribution is the species occurrence in drainages of east Puget Sound (Lake Washington and Snoqualmie River drainages; Fig. 1). Mongillo and Hallock (1999) considered these populations introduced based on several lines of evidence including: Olympic mudminnow would likely have avoided the swift flowing waters that connected south Puget Sound to east Puget Sound during glacial recession; Olympic mudminnow have never been found in Puget Sound drainages north of the Nisqually River; no Olympic mudminnow were discovered following a rotenone treatment of a lake downstream of the Cherry Creek population in east Puget Sound; and Olympic mudminnow are found at greater elevations in east Puget Sound than most other sites where they occur naturally. Alternatively, Trotter et al. (2000) proposed that Olympic mudminnow in east Puget Sound may be native and reached these areas via waterways that connected east Puget Sound to south Puget Sound during glacial recession. The authors also suggested that Olympic mudminnow may have persisted at the glacial margins and then recolonized habitat in east Puget Sound following glacial recession (Trotter et al. 2000).

Although there is a basic understanding of Olympic mudminnow biology, life history, and distribution, data important for conservation planning including population abundance, the degree of migration and gene flow among populations, and the long-term viability of populations are limited. Genetic data could provide useful information to help fill these gaps and are important for advancing conservation planning. Based on these needs, our study had four objectives: (1) To describe the level of genetic

variation within Olympic mudminnow collections from throughout the species range; (2) To determine the spatial scale that constitutes a population and to determine the level of genetic variation among populations; (3) To determine the spatial structuring of populations in order to aid with possible designation of management or conservation units; and (4) To determine the origins of Olympic mudminnow in east Puget Sound.

Materials and methods

Sample collection

We collected Olympic mudminnow at 23 different sites throughout the species' range (Fig. 1; Table 1). Collection sites were distributed among four broad geographic areas: south Puget Sound ($n = 5$), the Chehalis River Basin ($n = 7$), east Puget Sound ($n = 3$), and the Olympic Coast ($n = 8$). Collections from Hopkins Ditch, a low-gradient stream in the Chehalis River Basin, and Green Cove, a wetland and low-gradient stream complex in south Puget Sound, included three replicate collection sites spaced approximately 1–2 km apart in Green Cove, and approximately 2.5 km apart in Hopkins Ditch. We made multiple collections at these sites to help determine the spatial scale that defines a population. We collected Olympic mudminnow from 2010 to 2012 by dip netting, baited minnow traps, and electrofishing. Collections targeted 50 individuals per site. We took a small piece of tissue from the caudal fin of each individual and preserved it in 100 % non-denatured ethanol.

Laboratory methods

We extracted DNA from tissue samples using Qiagen DNeasy 96 extraction kits (Qiagen Inc). We genotyped individuals at 13 microsatellite loci (*Nhub01* through *Nhub13*) following the methods outlined in Adams et al. (2013). Forward primers were 5'-end labeled with fluorescent dyes and following PCR, we conducted electrophoresis on an Applied Biosystems 3130xl genetic analyzer using the GeneScan-500LIZ size standard (Life Technologies Co.). We analyzed electropherograms for each individual at each locus with GeneMapper v4.0 software (Life Technologies). To assess genotyping error rate, we re-extracted DNA from 10 % of the individuals analyzed and these fish were then re-genotyped.

Statistical analysis

We grouped mudminnow according to collection site for statistical analysis (Table 1). We tested collections for departures from Hardy–Weinberg equilibrium (HWE)

Table 1 Collection site numbers, collection site names, geographic area of each collection site, number of individuals collected at each site, and measures of genetic diversity for 23 Olympic mudminnow collection sites

Collection site number	Collection site name	Geographic area	Number of individuals	A_T	A_P	A_M	A_R	H_{exp}	H_{obs}	F_{IS}
1	Green Cove 1	S. Puget Sound	45	92	0	7.077	6.529	0.604	0.603	0.002
2	Green Cove 2	S. Puget Sound	50	102	0	7.846	7.187	0.590	0.602	-0.020
3	Green Cove 3	S. Puget Sound	50	96	0	7.385	6.736	0.591	0.597	-0.011
4	Woodard Creek	S. Puget Sound	50	70	0	5.385	4.920	0.562	0.550	0.022
5	Spurgeon Creek	S. Puget Sound	50	96	0	7.385	6.808	0.653	0.651	0.003
6	Hopkins Ditch 1	Chehalis River	47	127	0	9.769	8.906	0.711	0.715	-0.006
7	Hopkins Ditch 2	Chehalis River	50	126	0	9.692	8.708	0.725	0.729	-0.006
8	Hopkins Ditch 3	Chehalis River	50	121	0	9.308	8.570	0.706	0.675	0.044
9	S. Hanaford Creek	Chehalis River	34	111	1	8.538	8.413	0.732	0.740	-0.011
10	Adna Wetland	Chehalis River	46	41	0	3.154	2.859	0.423	0.428	-0.014
11	Chehalis Oxbow Lake	Chehalis River	43	160	12	12.308	11.125	0.708	0.688	0.028
12	Satsop Slough	Chehalis River	50	170	15	13.077	11.322	0.729	0.728	0.002
13	Peoples Creek	E. Puget Sound	50	81	0	6.231	5.658	0.605	0.603	0.004
14	Cherry Creek	E. Puget Sound	50	93	0	7.154	6.623	0.658	0.669	-0.017
15	E.F. Issaquah Creek	E. Puget Sound	50	57	0	4.385	4.159	0.530	0.518	0.022
16	Gillis Slough Pond	Olympic Coast	43	123	4	9.462	8.711	0.703	0.678	0.035
17	Conner Creek	Olympic Coast	50	147	3	11.308	9.976	0.703	0.717	-0.019
18	Ditch along Hwy 109	Olympic Coast	47	132	2	10.154	9.119	0.751	0.755	-0.004
19	Upper Cook Creek	Olympic Coast	49	52	0	4.000	3.699	0.414	0.388	0.063
20	N.F. Whale Creek	Olympic Coast	44	68	4	5.231	4.926	0.408	0.426	-0.046
21	Steamboat Creek Bog	Olympic Coast	33	45	5	3.462	3.418	0.384	0.394	-0.027
22	James Pond	Olympic Coast	50	51	0	3.923	3.774	0.373	0.380	-0.018
23	Lake Ozette Pond	Olympic Coast	50	75	9	5.769	5.339	0.557	0.526	0.057

Measures of genetic diversity include: total number of alleles observed at each collection site (A_T), number of private alleles observed at each collection site (A_P), mean number of alleles per locus (A_M), allelic richness (A_R), expected heterozygosity (H_{exp}), observed heterozygosity (H_{obs}), and F_{IS} . Collection site numbers correspond to map locations in Fig. 1

expectations using exact tests implemented in the program GENEPOP v4.0.7 (Raymond and Rousset 1995). We also used GENEPOP to test collections for evidence of linkage disequilibrium (LD). We adjusted significance values for HWE and LD tests for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989). We used the program CONVERT v1.31 (Glaubitz 2004) to estimate allele frequencies for each collection site. We then determined the total number of alleles observed (A_T) and the number of private alleles observed (A_P) for each collection site.

We used the program GDA (Lewis and Zaykin 2001) to estimate measures of genetic variation within each collection including the mean number of alleles per locus (A_M), expected heterozygosity (H_{exp}), observed heterozygosity (H_{obs}), and F_{IS} . Additionally, we used the program HP-Rare v1.0 (Kalinowski 2005) to estimate allelic richness (A_R) for each collection based on a minimum sample size of 58 genes. We grouped collections according to their geographic areas (east Puget Sound, south Puget Sound, Chehalis River Basin, Olympic Coast) to determine if there

were significant differences in measures of genetic diversity among geographic areas. We used permutation tests (1,000 permutations) implemented in the program FSTAT v2.9.3.2 (Goudet 2001) to determine if there were significant differences in H_{obs} , H_{exp} , A_R , and F_{IS} among geographic areas.

We used two methods to test each population for evidence of a genetic bottleneck. We first tested each collection site for evidence of a recent genetic bottleneck (within the past 0.2–4.0 generations) using the program BOTTLENECK (Piry et al. 1999). This program tests for an excess of heterozygotes relative to the frequency of alleles in a population (Cornuet and Luikart 1996). We assumed a two-phased model of mutation with 90 % stepwise mutations and 10 % variance in non-stepwise mutations. We evaluated the significance of genetic bottleneck tests using a one-sided Wilcoxon test. We also tested each collection site for evidence of a genetic bottleneck using the M-ratio method (Garza and Williamson 2001). This method compares the distribution of alleles to the overall range of allele size. This method is able to detect a genetic bottleneck that

has occurred upwards of 100 generations ago and can detect the signature of a genetic bottleneck even after the population has made a demographic recovery (Williamson-Natesan 2005). We calculated the M -ratio for each collection site using the program `M_P_VAL.exe` (available online: <http://swfsc.noaa.gov/textblock.aspx?Division=FED&id=3298>). We assumed 88 % step-wise mutations and 2.8 average sized non step-wise mutations as suggested by Garza and Williamson (2001). This program requires the user to input the population parameter θ , which is equal to $4 \times N_e \times$ mutation rate. The effective size (N_e) of each population was unknown so we tested a range of values (50, 100, 500) and we assumed a microsatellite mutation rate of 5×10^{-4} (Jarne and Lagoda 1996). Two of our loci, *Nhub04* and *Nhub05*, did not have a consistent repeat motif and they were omitted from this analysis. Garza and Williamson (2001) suggest that if the M -ratio falls below a critical value, M_c , the population has undergone a genetic bottleneck. We used the program `Critical_M` (available online: <http://swfsc.noaa.gov/textblock.aspx?Division=FED&id=3298>) to estimate M_c for each value of θ such that $M\text{-ratio} > M_c$ in 95 % of 10,000 simulations.

We employed a hierarchical sampling and analysis strategy involving collections from four geographic areas, multiple collection sites within geographic areas, and multiple collections within two sites (Green Cove and Hopkins Ditch), to determine the spatial scale that constitutes a population. We first estimated the overall level of genetic variation among all collection sites (global F_{ST}) and the associated 95 % confidence interval based on 1,000 bootstrap replicates using the program `FSTAT`. We also calculated estimates of F_{ST} for each of the four geographic areas and conducted permutation tests with `FSTAT` (1,000 permutations) to determine if there was a significant difference in the level of genetic variation among geographic areas. We then used `FSTAT` to estimate the level of genetic variation among all pairs of collection sites (pairwise F_{ST}). We used `GENEPOP` to perform tests of allele frequency heterogeneity to determine if there were significant differences in allele frequencies among all collection sites. Significance values for allele frequency heterogeneity tests were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989).

We used two methods to determine the spatial genetic relationship among Olympic mudminnow collection sites. First we conducted a multivariate discriminant analysis of principal components (DAPC; Jombart et al. 2010) of our allele frequency data using the *adegenet* package (Jombart 2008) for the R statistical environment (R Development Core Team 2013). DAPC is similar to principle component analysis (PCA) but unlike PCA, which maximizes the total variation in the dataset, DAPC maximizes the variation among different groups and minimizes variation within

groups (Jombart et al. 2010). We also constructed a consensus neighbor-joining tree (NJ-tree) using the program `PHYLIP v3.6` (Felsenstein 1993). We generated 1,000 replicate datasets using a bootstrap procedure and then estimated Cavalli-Sforza and Edwards' (1967) chord distances among collection locations and we constructed a consensus NJ tree based on these values.

We conducted an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to determine how genetic variation was partitioned among collections and among the different geographic areas. We conducted four AMOVAs with collection sites organized into two, three, four, and five different groups. Collection sites were organized into groups based on geography and genetic similarities observed from the DAPC and NJ-tree analyses. The AMOVA with two groups included a coastal group (sites 16–23) and a Puget Sound and Chehalis River group (sites 1–15). The AMOVA with three groups included three groups identified in the DAPC and NJ-tree analyses: (1) south Puget Sound and Chehalis River (sites 1–12); (2) south Olympic Coast and east Puget Sound (sites 13–19); and (3) north Olympic Coast (sites 20–23). The AMOVA with four groups organized collection sites according to the four geographic areas outlined in Table 1. The AMOVA with five groups organized collection sites into five groups observed from the DAPC and NJ-tree analyses: (1) south Puget Sound and Chehalis River (sites 1–12); (2) south Olympic Coast and east Puget Sound (sites 13–19); (3) N.F. Whale Creek and James Pond (sites 20 and 22); (4) Lake Ozette (site 23); (5) Steamboat Creek Bog (site 21). We conducted AMOVA using the program `ARLEQUIN v3.5.1.2` (Excoffier et al. 2005). Significance tests for the different variance components were based on 10,010 permutations.

We compared the level of variation among collection sites based on allele identity (F_{ST}) to the level of variation based on allele size (R_{ST}) to infer the relative divergence time among collection sites. If populations have diverged relatively recently, differences among populations would be due primarily to genetic drift and estimates of F_{ST} would be similar to estimates of R_{ST} . If populations were historically diverged from one another, differentiation would also be the result of stepwise mutations and estimates of R_{ST} would presumably be greater than estimates of F_{ST} (Hardy et al. 2003). We were particularly interested in the comparisons involving collection sites from east Puget Sound given the competing theories regarding the origins of Olympic mudminnow in east Puget Sound. If mudminnow historically inhabited east Puget Sound, we would expect estimates of R_{ST} to be significantly greater than estimates of F_{ST} . Alternatively, if Olympic mudminnow in east Puget Sound were the result of recent translocations, we would expect estimates of F_{ST} to be similar

Table 2 Mean estimates of genetic variation for the four geographic areas samples were collected from

Geographic area	n	A_T	A_R	H_{exp}	H_{obs}
Chehalis River Basin	7	122.286	8.558	0.676	0.671
Olympic Coast	8	86.625	6.120	0.541	0.537
South Puget Sound	5	91.200	6.436	0.600	0.600
East Puget Sound	3	77.000	5.480	0.598	0.597

n number of collection sites within the geographic area, A_T total number of alleles, A_R allelic richness, H_{exp} expected heterozygosity, H_{obs} observed heterozygosity

to estimates of R_{ST} for comparisons between east Puget Sound and potential source populations. We calculated pairwise estimates of R_{ST} among all collection sites using the program SPAGeDi v1.2 (Hardy and Vekemans 2002) and we used a permutation test (1,000 permutations) implemented in SPAGeDi to determine if there were significant differences among pairwise estimates of F_{ST} and R_{ST} .

Results

Genetic variation within collections

Our estimated genotyping error rate was 0.2 % based on samples we re-extracted and re-genotyped. Fifteen of the 23 collection sites had at least one locus that was fixed for a single allele and there were also several instances where a collection site had a locus with an allele at a frequency of 0.9 or greater (Supplemental Table). Prior to Bonferroni correction, we observed 11 deviations from HWE out of 265 total tests. Following Bonferroni corrections we observed no loci that deviated from HWE expectations at any collection site. We observed 11 locus pairs out of 1,452 comparisons that showed evidence of linkage. There was no clear pattern of linkage among collection sites or pairs of loci.

Estimates of genetic variation ranged widely among the 23 collection sites. The total number of alleles observed at a site ranged from 41 at Adna Wetland to 170 at Satsop Slough (Table 1). We observed private alleles in nine of the 23 collection sites. The number of private alleles observed ranged from one in S. Hanaford Creek to 15 in Satsop Slough (Table 1). Six of the collection sites with private alleles were on the Olympic Coast and the other three sites were within the Chehalis River Basin. The mean number of alleles per locus and allelic richness were both lowest in Adna Wetland (3.154 and 2.859, respectively; Table 1) and greatest in Satsop Slough (13.077 and 11.322, respectively; Table 1). Expected heterozygosity and observed heterozygosity were both lowest in James Pond

(0.373 and 0.380, respectively; Table 1) and greatest in the collection from the ditch along Highway 109 (0.751 and 0.755, respectively; Table 1). Comparisons of genetic diversity among geographic areas showed that all estimates of genetic diversity were greatest in the Chehalis River Basin (Table 2) and permutation tests showed that observed and expected heterozygosity were significantly greater in the Chehalis River Basin compared to the coastal populations ($P < 0.05$). All other comparisons were not significant.

Two collection sites showed evidence of a recent genetic bottleneck based on an excess of heterozygotes; S. Hanaford Creek (Wilcoxon test $P = 0.024$) and James Pond (Wilcoxon test $P = 0.012$; Table 3). Preliminary tests showed that varying N_e when estimating the parameter θ did not change the M -ratio for any of the collection sites. We observed 11 instances where the M -ratio for a collection site was less than our estimate of M_c , suggesting the collection came from a population which had undergone a genetic bottleneck (Table 3). Seven of these 11 collection sites showed evidence of a genetic bottleneck when we assumed a value of θ equal to 0.1 or 0.2 ($N_e = 50$ and 100, respectively) and the other four collections showed evidence of a genetic bottleneck at all three values of θ (Table 3).

Analysis of population boundaries

The overall level of genetic variation among collection sites (i.e., global F_{ST}) was 0.273 (95 % CI 0.200–0.361). Estimates of F_{ST} among collections within the four geographic areas were as follows: Chehalis River Basin $F_{ST} = 0.101$; south Puget Sound $F_{ST} = 0.167$; east Puget Sound $F_{ST} = 0.089$; and Olympic Coast $F_{ST} = 0.350$. Permutation tests showed that the F_{ST} estimate among collection sites on the Olympic Coast was significantly greater than the other geographic areas and all other comparisons were not significant. Pairwise estimates of F_{ST} ranged from 0.002 for comparisons within the Green Cove and Hopkins Ditch sites to 0.539 for the comparison between James Pond and Upper Cook Creek (Table 4). In general, the greatest pairwise F_{ST} estimates were between collections sites on the Olympic Coast and collection sites from other geographic areas. Tests of allele frequency heterogeneity showed that there were significant differences in allele frequencies among all collection sites with the exception of the comparisons among the Hopkins Ditch sites and the comparisons among the Green Cove sites.

Spatial structure of populations

The first variance component (x-axis) on the DAPC plot separated the four collection sites from the northern

Table 3 Genetic bottleneck test results for 23 Olympic mudminnow collection sites

Collection site number	Collection site name	Het excess	<i>M</i> -ratio	<i>M_c</i>		
				θ = 0.1	θ = 0.2	θ = 1.0
1	Green Cove 1	0.830	0.860	0.856	0.848	0.813
2	Green Cove 2	0.974	0.916	0.856	0.851	0.811
3	Green Cove 3	0.661	0.899	0.856	0.851	0.811
4	Woodard Creek	0.765	0.824	0.856	0.851	0.811
5	Spurgeon Creek	0.182	0.906	0.856	0.851	0.811
6	Hopkins Ditch 1	0.318	0.938	0.855	0.850	0.813
7	Hopkins Ditch 2	0.249	0.955	0.856	0.851	0.811
8	Hopkins Ditch 3	0.170	0.943	0.856	0.851	0.811
9	S. Hanaford Creek	0.024	0.908	0.856	0.847	0.810
10	Adna Wetland	0.246	0.695	0.856	0.847	0.812
11	Chehalis Oxbow Lake	0.966	0.778	0.856	0.848	0.810
12	Satsop Slough	0.968	0.816	0.856	0.851	0.811
13	Peoples Creek	0.740	0.798	0.856	0.851	0.811
14	Cherry Creek	0.473	0.811	0.856	0.851	0.811
15	E.F. Issaquah Creek	0.259	0.765	0.856	0.851	0.811
16	Gillis Slough Pond	0.793	0.842	0.856	0.848	0.810
17	Conner Creek	0.936	0.832	0.856	0.851	0.811
18	Ditch along Hwy 109	0.318	0.841	0.855	0.850	0.813
19	Upper Cook Creek	0.652	0.955	0.855	0.848	0.816
20	N.F. Whale Creek	0.820	0.986	0.855	0.851	0.813
21	Steamboat Creek Bog	0.288	0.821	0.856	0.848	0.808
22	James Pond	0.012	0.947	0.856	0.851	0.811
23	Lake Ozette Pond	0.382	0.915	0.856	0.851	0.811

Values in the Het excess column represent *P* values for tests of a heterozygote excess. The *M*-ratio represents the ratio of the distribution of alleles to allele size range as described by Garza and Williamson (2001). Critical *M* values (*M_c*) are given for each value of θ (4 × *N_e* × mutation rate) that we tested. Values in bold represent tests that showed evidence of a genetic bottleneck

Olympic Coast (collection sites 20–23) from all other collection sites (Fig. 2). Two of the northern Olympic Coast collection sites, Lake Ozette (site 23) and Steamboat Creek Bog (site 21), grouped independently and the other two sites, N.F. Whale Creek (site 20) and James Pond (site 22), grouped together. The second variance component (*y*-axis) on the DAPC plot separated collection sites from Chehalis River Basin and south Puget Sound (sites 1–12) from collection sites on the southern Olympic Coast and east Puget Sound (sites 13–19; Fig. 2). Woodard Creek (site 4) in south Puget Sound grouped somewhat intermediate to these two clusters. Similar to the DAPC plot, the NJ-tree grouped collection sites into three main groups: (1) south Puget Sound and the Chehalis River Basin; (2) south Olympic Coast and east Puget Sound; and (3) north Olympic Coast (Fig. 3). The majority of the branches on the tree had greater than 50 % bootstrap support. The Woodard Creek and Adna Wetland collections grouped intermediate to the collections from the north Olympic Coast and the south Olympic Coast and east Puget Sound; however bootstrap support for these nodes was <50 % (Fig. 3).

We conducted multiple AMOVAs to examine how variation was partitioned among different groupings of collection sites. We observed the greatest amount of variation among groups (19.74 %) when collection sites were

organized into five groups and the least amount of variation among groups (7.08 %) when collection sites were organized into two groups (Table 5). Significance tests showed the level of variation among groups and among collections sites within groups was significantly different from 0.0 (*P* < 0.0001) for all four AMOVAs but the level of variation among individuals within collection sites was not significantly different from 0.0 for any of the AMOVAs.

History of divergence among collection sites

We compared pairwise estimates of *F_{ST}* and *R_{ST}* to infer the divergence history among collection sites. Permutation tests identified 30 comparisons out of 253 total where pairwise *R_{ST}* was not significantly greater than pairwise *F_{ST}* suggesting the two collection sites being compared had diverged relatively recently (Table 6). Eleven of those 30 comparisons involved collection sites in east Puget Sound. We observed non-significant comparisons among the three east Puget Sound collection sites as well as between the east Puget Sound collection sites and collection sites on the south Olympic Coast. The remaining pairwise comparisons where *R_{ST}* was not significantly greater than *F_{ST}* involved collection sites within the Chehalis River Basin (n = 14; including the comparisons among the Hopkins Ditch sites),

comparisons among the Green Cove sites in south Puget Sound (n = 3), and comparisons among coastal sites (n = 2; Table 6).

Discussion

Genetic variation within collections

Populations with increased genetic variation may have increased fitness, be less susceptible to extirpation, and be

better suited to adapt to future changes in environmental conditions (Quattro and Vrijenhoek 1989; Reed and Frankham 2003; Allendorf and Luikart 2007). Data on trends in abundance for Olympic mudminnow are limited and information on genetic variation within collection sites could also be used to infer population viability. For example, within the Chehalis River Basin, estimates of genetic variation for Adna Wetland were among the lowest we observed, whereas estimates of genetic variation for Chehalis Oxbow and Satsop Slough were 2–4 times greater. Collection sites such as Adna Wetland may

Table 4 Pairwise estimates of genetic variation (F_{ST}) among 23 Olympic mudminnow collection sites

Collection site number	Collection site name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Green Cove 1														
2	Green Cove 2	0.006													
3	Green Cove 3	0.002	0.003												
4	Woodard Creek	0.251	0.261	0.268											
5	Spurgeon Creek	0.170	0.182	0.187	0.210										
6	Hopkins Ditch 1	0.095	0.102	0.103	0.207	0.088									
7	Hopkins Ditch 2	0.094	0.107	0.108	0.186	0.078	0.003								
8	Hopkins Ditch 3	0.100	0.107	0.108	0.202	0.092	0.004	0.002							
9	S. Hanaford Creek	0.110	0.123	0.124	0.167	0.067	0.037	0.023	0.033						
10	Adna Wetland	0.286	0.302	0.296	0.315	0.279	0.248	0.236	0.246	0.235					
11	Chehalis Oxbow Lake	0.136	0.146	0.146	0.228	0.131	0.068	0.073	0.078	0.070	0.212				
12	Satsop Slough	0.134	0.142	0.145	0.216	0.102	0.074	0.071	0.075	0.063	0.226	0.055			
13	Peoples Creek	0.301	0.303	0.300	0.290	0.302	0.268	0.257	0.264	0.242	0.347	0.230	0.243		
14	Cherry Creek	0.262	0.264	0.262	0.282	0.276	0.225	0.215	0.221	0.210	0.308	0.180	0.189	0.061	
15	E.F. Issaquah Creek	0.357	0.354	0.351	0.385	0.369	0.319	0.315	0.316	0.307	0.412	0.277	0.287	0.126	0.080
16	Gillis Slough	0.272	0.281	0.279	0.263	0.230	0.190	0.186	0.193	0.185	0.311	0.162	0.173	0.154	0.096
17	Conner Creek	0.222	0.229	0.230	0.222	0.208	0.167	0.166	0.170	0.159	0.292	0.145	0.145	0.200	0.149
18	Ditch along Hwy 109	0.222	0.229	0.228	0.242	0.220	0.168	0.167	0.173	0.169	0.295	0.150	0.160	0.179	0.139
19	Upper Cook Creek	0.401	0.397	0.398	0.402	0.378	0.339	0.337	0.332	0.347	0.472	0.317	0.322	0.301	0.252
20	N.F. Whale Creek	0.421	0.421	0.424	0.454	0.396	0.369	0.361	0.366	0.377	0.482	0.345	0.336	0.414	0.389
21	Steamboat Creek Bog	0.441	0.445	0.443	0.335	0.394	0.372	0.362	0.371	0.351	0.516	0.370	0.354	0.372	0.366
22	James Pond	0.440	0.441	0.443	0.472	0.414	0.390	0.378	0.386	0.394	0.487	0.365	0.353	0.433	0.409
23	Lake Ozette Pond	0.401	0.410	0.408	0.382	0.362	0.342	0.332	0.343	0.330	0.461	0.330	0.328	0.378	0.350

Table 4 continued

Collection site number	Collection site name	15	16	17	18	19	20	21	22
1	Green Cove 1								
2	Green Cove 2								
3	Green Cove 3								
4	Woodard Creek								
5	Spurgeon Creek								
6	Hopkins Ditch 1								
7	Hopkins Ditch 2								
8	Hopkins Ditch 3								
9	South Hanaford Creek								
10	Adna Wetland								
11	Chehalis Oxbow Lake								
12	Satsop Slough								
13	Peoples Creek								
14	Cherry Creek								
15	EF Issaquah Creek								
16	Gillis Slough	0.198							
17	Conner Creek	0.244	0.138						
18	Ditch along Hwy 109	0.226	0.135	0.040					
19	Upper Cook Creek	0.325	0.235	0.279	0.277				
20	North Fork Whale Creek	0.465	0.364	0.391	0.372	0.515			
21	Steamboat Creek Bog	0.445	0.360	0.358	0.343	0.498	0.500		
22	James Pond	0.485	0.384	0.415	0.398	0.539	0.030	0.529	
23	Lake Ozette Pond	0.420	0.320	0.341	0.314	0.472	0.349	0.393	0.373

Values in bold represent comparisons that did not show significant differences in allele frequencies based on tests of allele frequency heterogeneity

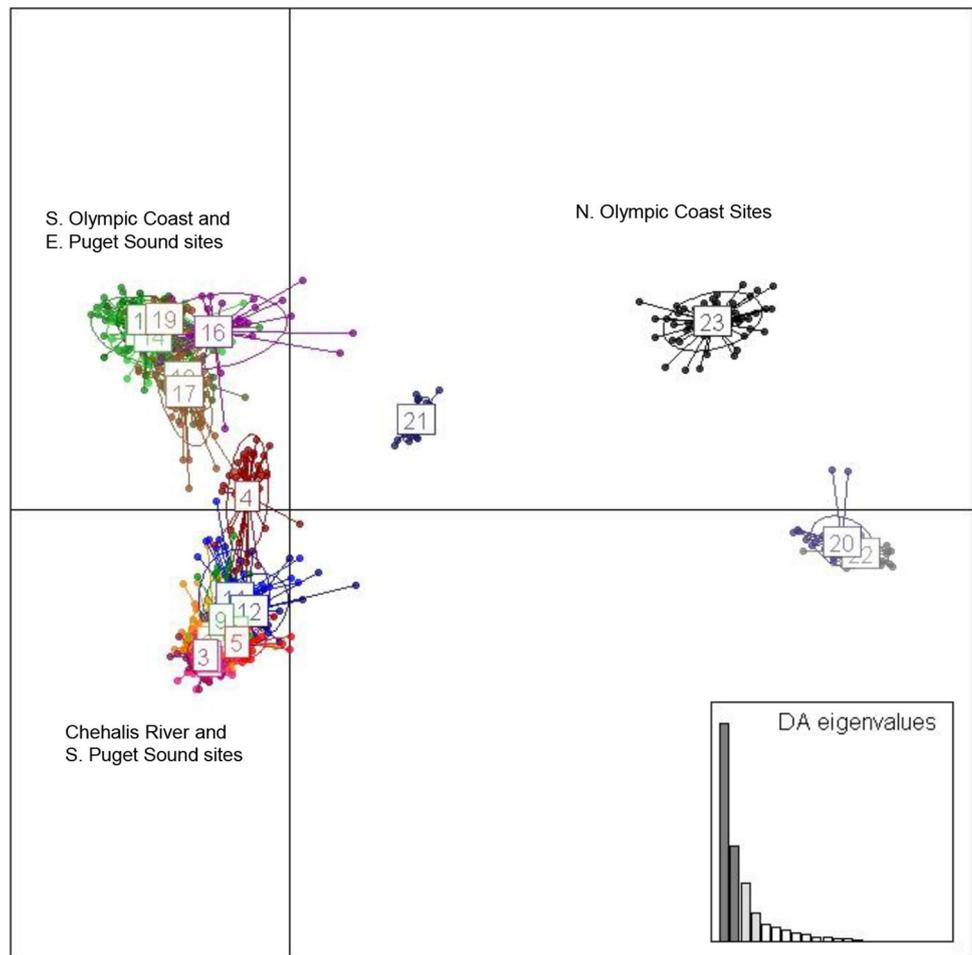
represent relatively small or declining populations and may warrant increased monitoring or additional conservation measures. Many long-term monitoring efforts utilize genetic data as an additional metric to evaluate population status (Schwartz et al. 2007; Charlier et al. 2012; Osborne et al. 2012a). This study represents the first broad scale survey of genetic variation within Olympic mudminnow and will provide useful baseline data for future monitoring efforts.

Tests for genetic bottlenecks can also identify populations that have experienced a decline in abundance. Two collection sites, James Pond and South Hanaford Creek, showed evidence of a genetic bottleneck based on a heterozygote excess. James Pond has almost completely desiccated multiple times in recent years and during these periods mudminnow likely persisted in small pockets of habitat when the size of the pond was constricted (P. Crain, Olympic National Park, *personal communication*). Although abundance data do not exist for South Hanaford Creek, relatively few individuals were observed at this site during our sampling efforts. Several collection sites showed evidence of a genetic bottleneck based on the *M*-ratio tests. This test can detect a bottleneck that occurred

upwards of 100 generations ago and provides a more historical view than the heterozygote excess test (Williamson-Natesan 2005). This time period is consistent with the urban development of much of the area that Olympic mudminnow inhabit (Mongillo and Hallock 1999) and it seems likely that abundance would have declined in many of these sites as the amount of available habitat was reduced.

Both the Pleistocene glaciation and the species' life history appear to influence the level of genetic variation within Olympic mudminnow collections. Populations in areas of former glacial refugia typically show greater levels of within population genetic variation compared to populations from formerly glaciated areas (Bernatchez and Wilson 1998; Costello et al. 2003; Stamford and Taylor 2004). Comparisons among the four geographic areas showed that genetic variation was greatest within collections from the Chehalis River Basin; the primary refugium for Olympic mudminnow during the Pleistocene glaciation (McPhail 1967; McPhail and Lindsey 1986). Areas on the Olympic Coast also remained ice free during the Pleistocene glaciation (Tabor 1975) and mudminnow likely used several coastal rivers as refugia as well. Genetic variation

Fig. 2 Scatterplot of the first two variance components of the DAPC for 23 Olympic mudminnow collection sites. Numbers on each cluster correspond to collection site locations listed in Table 1 and Fig. 1. Each *dot* represents an individual fish in the analysis and the *ellipses* represent the inertia ellipse for each collection site. The eigenvalue for the first variance component (*x*-axis) was 4,313.99 and the eigenvalue for the second variance component (*y*-axis) was 1,898.89



within collections from the Olympic Coast was substantially lower than estimates from the Chehalis River Basin. This likely reflects historical isolation among coastal sites resulting from the species' limited capacity for dispersal in these areas. The only hydrologic connection among many of the Olympic Coast sites would have been via the Pacific Ocean. Olympic mudminnow avoid salinity (Meldrim 1968), therefore migration among these sites would not have been possible. Historically isolated populations often contain private alleles (Slatkin 1985) and nearly all of the Olympic Coast collection sites contained multiple private alleles, providing further evidence of historic isolation.

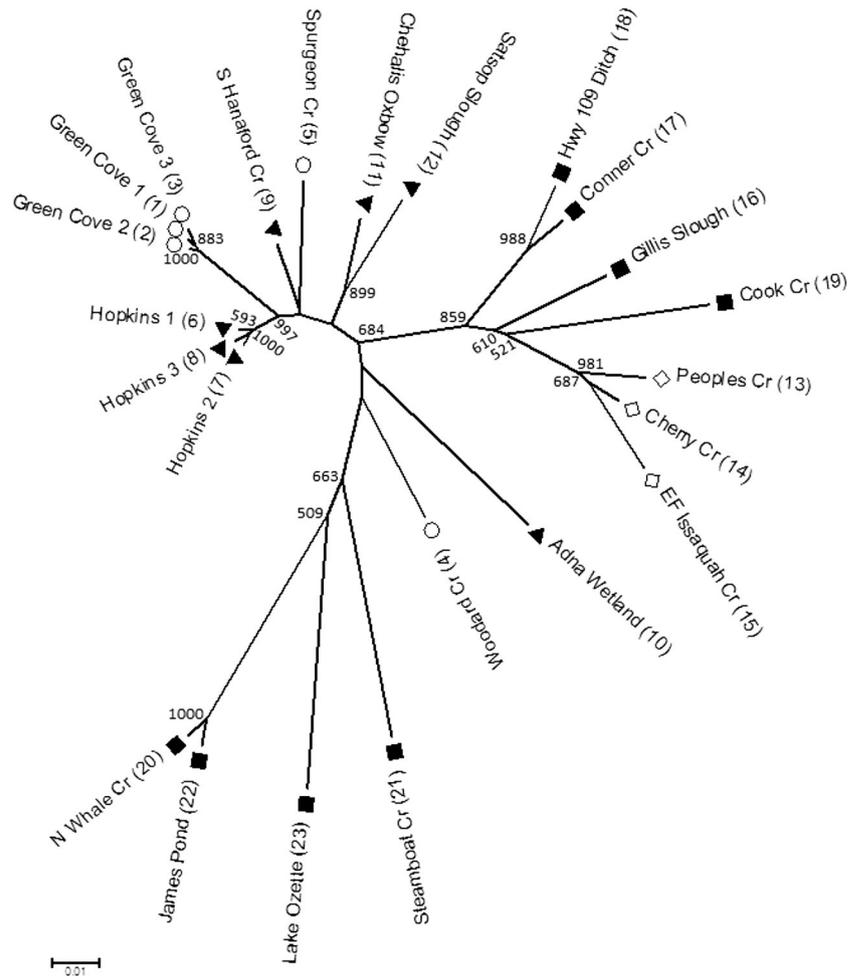
Analysis of population boundaries

Prior to establishing conservation guidelines for Olympic mudminnow, it is important that biologists have a clear understanding of what constitutes a population for the species. We employed a hierarchical sampling strategy involving collections from different geographic areas, multiple collection sites within geographic areas, and multiple collections within two sites, to determine the spatial scale that constitutes a population. The overall level

of genetic variation we observed was relatively high considering the species limited geographic distribution and suggests a strong degree of population structure exists. We observed much greater genetic variation among coastal sites; the overall F_{ST} estimate for the Olympic Coast ($F_{ST} = 0.350$) was nearly two to four times greater than the other geographic areas. Historically isolated populations typically show increased levels of genetic divergence due to limited gene flow and increased genetic drift (Costello et al. 2003; Currens et al. 2009; Ardren et al. 2011). As noted above, Olympic mudminnow in coastal habitats have been historically isolated and historically limited gene flow explains the higher level of variation among coastal collection sites relative to the other geographic areas.

Not only were estimates of genetic variation relatively high across the species' range and among geographic areas, we also observed relatively high genetic variation among many collection sites within the same geographic area, and we observed significant allele frequency differences among nearly all collection sites. The exceptions were the multiple collections from Green Cove and Hopkins Ditch where we observed pairwise F_{ST} estimates near 0.0 and no differences in allele frequencies among collections. Collectively

Fig. 3 Consensus neighbor-joining tree based on Cavalli-Sforza and Edwards' chord distances for 23 Olympic mudminnow collection sites. Numbers at the nodes represent the number of bootstrap replicates out of 1,000 that showed the displayed topology and only bootstrap values greater than 500 are displayed. Numbers in parentheses after collection site names correspond to collection site numbers in Fig. 1 and Table 1. Shapes after each collection site name correspond to the geographic areas samples were collected from. Closed triangles correspond to sites located in the Chehalis River Basin, open circles correspond to sites located in southern Puget Sound, open diamonds represent sites located in eastern Puget Sound, and closed squares represent sites located on the Olympic Coast



these data suggest that each collection site represents a genetically distinct population and that Green Cove and Hopkins Ditch each contain a single population. To date no information exists on the level of migration and gene flow among Olympic mudminnow populations and genetic data provide an important surrogate. Our data suggest that dispersal and gene flow among most populations is limited. This has important implications for Olympic mudminnow conservation; if populations are periodically lost due to habitat alterations, changes in climatic conditions, or introductions of non-native predators, it's unlikely that habitat will be recolonized by migrants from a nearby population in most cases. Maintaining large, interconnected areas such as Green Cove and Hopkins Ditch may be important to allow Olympic mudminnow to persist in a changing environment and recolonize habitat where populations have been extirpated.

Spatial structure of populations

The DAPC and NJ-tree analyses both highlight the effects of the Pleistocene glaciation on the genetic relationship among

Olympic mudminnow populations. Following deglaciation, Olympic mudminnow colonized habitat in south Puget Sound from the Chehalis River refugium (McPhail 1967; McPhail and Lindsey 1986). This shared evolutionary history is reflected in both the DAPC plot and the NJ-tree analysis where populations from these two major drainages clustered together. Coastal populations were highly diverged from the Chehalis Basin and south Puget Sound in these analyses. Many areas along the Olympic Coast remained ice free during the Pleistocene glaciation (Tabor 1975) and our data suggest that Olympic mudminnow persisted in these habitats independently of fish from the Chehalis River Basin during this period. Our results were similar to those of Rosenfeld (1983) who observed significant differences in morphometric and meristic characteristics between Olympic mudminnow from coastal populations and Chehalis Basin populations. Rosenfeld (1983) attributed these differences to a historic barrier to gene flow in the area of the Montesano Hills (between the Wynoochee and Humpulips rivers) during the Pleistocene glaciation.

Within the coastal populations there appears to be further genetic divergence. Populations from the southern

Table 5 Analysis of molecular variance (AMOVA) results

Number of groups	Population groupings	Percent of variation		
		Among groups	Among collection sites within groups	Among individuals within collection sites
2	Sites 1-15; sites 16-23	7.08 %	23.08 %	0.35 %
3	Sites 1-12; sites 13-19; sites 20-23	15.76 %	15.74 %	0.34 %
4	Sites 1-5; sites 13-15; sites 6-12; sites 16-23	9.97 %	19.02 %	0.36 %
5	Sites 1-12; sites 13-19; sites 20&22, site 21, site 23	19.74 %	12.59 %	0.39 %

The level of variation among groups and among collection sites within groups was significantly different from 0.0 for all analyses. The level of variation among individuals within collection sites was not significantly different from 0.0 for any of the analyses. Site numbers correspond to collection site numbers in Table 1 and Fig. 1

Olympic Coast (sites 16–19) grouped separately from populations from the north Olympic Coast (sites 20–23). Furthermore, the DAPC plot and pairwise estimates of F_{ST} showed that populations from the south Olympic Coast were more similar to one another compared to the populations from the north Olympic Coast, which were much more divergent from one another. Olympic mudminnow have a broader distribution on the southern Olympic Coast (Mongillo and Hallock 1999) and some populations may be periodically connected via off-channel habitats and during flood events in low elevation areas. Alternatively, our collections from the north Olympic Coast represent many of the sites Olympic mudminnow have been documented north of the Quinault River (Mongillo and Hallock 1999), and the only hydrologic connection among sample sites is via the Pacific Ocean. The difference in clustering patterns between the north and south Olympic Coast reflects the difference in landscapes and the capacity for dispersal between mudminnow from these two areas.

It's often useful to organize populations into conservation or management units to address conservation needs and to set recovery goals. Biologists have utilized several types of information to designate recovery units including behavioral, morphological, ecological, and genetic information (Nielsen 1995; Waples 1995; Fraser and Bernatchez 2001). We conducted multiple AMOVAs to determine how much genetic variation there was among different population groupings. Simply grouping populations according to

Table 6 Population pairs that showed no significant difference between pairwise estimates of F_{ST} (the level of variation among populations based on allele identity) and R_{ST} (the level of variation among populations based on allele size) based on 1,000 permutations

Collection site 1	Collection site 2	F_{ST}	R_{ST}	P value
Peoples Creek	Cherry Creek	0.061	0.159	0.106
Peoples Creek	Conner Creek	0.200	0.243	0.050
Peoples Creek	Cook Creek	0.301	0.245	0.145
Peoples Creek	E. F. Issaquah Creek	0.126	0.112	0.571
Peoples Creek	Gillis Slough	0.153	0.155	0.122
Cherry Creek	Cook Creek	0.252	0.238	0.134
Cherry Creek	E. F. Issaquah Creek	0.080	0.028	0.848
Cherry Creek	Gillis Slough	0.096	0.084	0.253
E. F. Issaquah Creek	Cook Creek	0.325	0.229	0.436
E. F. Issaquah Creek	Gillis Slough	0.198	0.113	0.619
E. F. Issaquah Creek	Hwy 109 Ditch	0.226	0.334	0.068
Green Cove 1	Green Cove 2	0.006	0.006	0.480
Green Cove 1	Green Cove 3	0.002	-0.007	0.915
Green Cove 2	Green Cove 3	0.003	0.008	0.236
Chehalis Oxbow	Satsop Slough	0.055	0.060	0.122
Chehalis Oxbow	Adna Wetland	0.212	0.302	0.096
Hopkins Ditch 1	Hopkins Ditch 2	0.003	0.004	0.291
Hopkins Ditch 1	Hopkins Ditch 3	0.004	0.014	0.148
Hopkins Ditch 1	Spurgeon Creek	0.088	0.086	0.215
Hopkins Ditch 1	Adna Wetland	0.248	0.291	0.114
Hopkins Ditch 2	Hopkins Ditch 3	0.002	-0.003	0.803
Hopkins Ditch 2	Spurgeon Creek	0.078	0.089	0.168
Hopkins Ditch 2	Adna Wetland	0.236	0.284	0.137
Hopkins Ditch 3	Spurgeon Creek	0.092	0.108	0.175
Hopkins Ditch 3	Adna Wetland	0.246	0.320	0.097
Adna Wetland	Green Cove 2	0.302	0.353	0.056
Adna Wetland	Spurgeon Creek	0.279	0.272	0.308
Adna Wetland	Woodard Creek	0.315	0.175	0.699
Gillis Slough	Cook Creek	0.235	0.142	0.405
James Pond	N.F. Whale Creek	0.030	0.013	0.588

geographic area accounted for only 9.97 % of the total genetic variation we observed, and there was nearly twice as much genetic variation among populations within groups when populations were grouped this way. AMOVA based on the relationships observed from the DAPC and NJ-tree analyses accounted for a much higher degree of genetic variation among groups. We observed the greatest amount of variation among groups when we designated five separate groups. This analysis separated geographically proximate populations from the north Olympic Coast into three

groups, which may not be reasonable from a management perspective. The best alternative may be to designate a total of three groups: (1) Chehalis River and south Puget Sound; (2) south Olympic Coast; and (3) north Olympic Coast. These three groups were apparent from both the DAPC and NJ-tree analyses and partitioning collections this way yielded the second highest proportion of variation among groups. The question of where to place the east Puget Sound populations is complicated given the origins of these populations (see below). Ultimately, it is important that additional types of data including behavioral, ecological, morphological, etc. are also considered if biologists designate multiple conservation or management units for the species.

Origins of populations in east Puget Sound

Population history can have important implications for conservation and management. Historically isolated populations may contain unique genetic material not found in other populations (Small et al. 2006, 2011; Osborne et al. 2012b) and these populations are often recognized as high priorities for conservation given their evolutionary potential (Lesica and Allendorf 1995). However, it is important to be able to distinguish historically isolated populations from undocumented introductions. Competing theories exist regarding the origins of Olympic mudminnow in east Puget Sound and data from our study provide important insight into the origins of these populations. Historically isolated populations often contain private alleles not observed in other populations (Slatkin 1985). Coastal populations of Olympic mudminnow have been historically isolated and we observed several private alleles in these populations; however, no private alleles were observed in the east Puget Sound populations as we might expect if these were relict populations that persisted near the glacial margins. If Olympic mudminnow from the Chehalis refugium colonized habitat in east Puget Sound, we would expect some degree of genetic similarity between populations in east Puget Sound and the Chehalis River Basin and south Puget Sound. However, populations in east Puget Sound were most similar to populations from the southern Olympic Coast and were quite differentiated from populations in the Chehalis River Basin and south Puget Sound. Comparisons between F_{ST} (based on allele identity) and R_{ST} (based on allele size) can be useful for inferring the history of divergence between populations (Hardy et al. 2003; Peterson and Ardren 2009). When populations have recently diverged, differences should be due primarily to genetic drift and the two estimates should be similar. When populations historically diverged, differences should also be due to step-wise mutations and estimates of R_{ST} should be significantly greater. Comparisons between F_{ST} and R_{ST}

suggest that populations in east Puget Sound were historically diverged from most of the populations we surveyed but were more recently diverged from populations from the southern Olympic Coast (i.e. no significant difference between F_{ST} and R_{ST}). Collectively these data suggest that the east Puget Sound populations were not historically isolated and that they are likely recent introductions from the southern Olympic Coast.

Introduced populations often show reduced levels of genetic variation and/or the signature of a genetic bottleneck when compared to natural populations, particularly when small numbers of founding individuals are used (Mock et al. 2004; Stephen et al. 2005). Interestingly, levels of genetic variation within the east Puget Sound populations were similar to those observed in many natural populations. Although east Puget Sound populations did not show evidence of a recent genetic bottleneck (heterozygote-excess test), all three populations showed evidence of a genetic bottleneck based on the M -ratio test. The two tests can detect a bottleneck that occurred at different time periods (Williamson-Natesan 2005) and it appears that although sufficient time has elapsed to allow the populations to recover from any genetic bottleneck associated with a recent founding event, the historic signature of a genetic bottleneck remains. The fact that genetic variation in these populations was equivalent to natural populations suggests either a large number of founding individuals, or that multiple introductions occurred. Recent anecdotal evidence suggests that translocations of Olympic mudminnow occurred from areas on the southern Olympic Coast to private ponds in the Cherry Creek drainage and additional translocations may have occurred into the Issaquah Creek drainage (M. Hallock, WDFW, *personal communication*).

Conclusions

When genetic data are incorporated into conservation planning, it's important that biologists have a clear understanding of how different forces affect genetic variation. For Olympic mudminnow, the influence of historical and contemporary forces on genetic variation has important implications for conservation. There are relatively few populations of Olympic mudminnow on the north Olympic Coast (Mongillo and Hallock 1999) and most of these populations have been historically isolated and contain unique genetic material. If these populations are extirpated, this unique genetic material would be lost and it's unlikely that Olympic mudminnow would recolonize these habitats due to the limited capacity for dispersal in these areas. As a result, populations on the north Olympic Coast represent high priorities for conservation. Alternatively, peripheral

populations in east Puget Sound are the result of undocumented introductions and likely represent a lower priority for conservation. Introduced populations may have value as a genetic reserve in the case of stochastic events or they may have experimental value (George et al. 2009). Ultimately our data help to fill information gaps and will allow biologists to develop effective conservation plans for this unique species.

Acknowledgments Funding for this study was provided by the U.S. Fish and Wildlife Service, Washington Fish and Wildlife Office. We would like to thank the following individuals for assisting with genetic sample collections: Molly Hallock (WDFW), Teal Waterstrat (USFWS), Kira Mazzi (USFWS), Dan Lantz (USFWS), Larry Gilbertson (Quinault Tribe), Pat Trotter (retired), Dan Spencer (USFWS), Hans Berge (King County), Pat Crain (NPS), and John Trobaugh (WDNR). We would also like to thank Teal Waterstrat for producing the study map, Pat Crain for sharing information regarding the history of the James Pond population, Jeanelle Miller and Molly Hallock for sharing information regarding the origins of Olympic mudminnow in east Puget Sound, and Patty Crandell, Christian Smith, and two anonymous reviewers for providing helpful comments on earlier versions of this manuscript. The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

References

- Adams B, DeHaan PW, Tabor R, Thompson B, Hawkins DK (2013) Characterization of tetranucleotide microsatellite loci for Olympic mudminnow (*Novumbra hubbsi*). *Conserv Genet Res* 5(2):573–575
- Allendorf FW, Luikart GK (2007) Conservation and the genetics of populations, 1st edn. Blackwell Publishing, Oxford
- Arden WR, DeHaan PW, Smith CT, Taylor EB, Leary R, Kozfkay CC, Godfrey L, Diggs M, Fredenberg W, Chan J, Kilpatrick CW, Small MP, Hawkins DK (2011) Genetic structure, evolutionary history, and conservation units of bull trout in the coterminous United States. *Trans Am Fish Soc* 140(2):506–525
- Bernatchez L, Wilson CC (1998) Comparative phylogeography of nearctic and palearctic fishes. *Mol Ecol* 7(4):431–452
- Brinkman TJ, Person DK, Chapin FS III, Smith W, Hundertmark KJ (2011) Estimating abundance of Sitka black-tailed deer using DNA from fecal pellets. *J Wildl Manag* 75(1):232–242
- Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution* 21:550–570
- Charlier J, Laikre L, Ryman N (2012) Genetic monitoring reveals temporal stability over 30 years in a small, lake-resident brown trout population. *Heredity* 109(4):246–253
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144(4):2001–2014
- Costello AB, Down TE, Pollard SM, Pacas CJ, Taylor EB (2003) The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). *Evolution* 57(2):328–344
- Currens KP, Schreck CB, Li HW (2009) Evolutionary ecology of redband trout. *Trans Am Fish Soc* 138(4):797–817
- Development Core Team R (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- 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(2):479–491
- Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol Bioinform* 1:47–50
- Felsenstein J (1993) PHYLIP: phylogeny inference package. (version 3.5c). <http://evolution.genetics.washington.edu/phylip/getme.html>. Accessed June 2009
- Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Mol Ecol* 10(12):2741–2752
- Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Mol Ecol* 10(2):305–318
- George AL, Kuhajda BR, Williams JD, Cantrell MA, Rakes PL, Shute JR (2009) Guidelines for propagation and translocation for freshwater fish conservation. *Fisheries* 34(11):529–545
- Glaubitz JC (2004) CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Mol Ecol Notes* 4(2):309–310
- Gomez-Uchida D, Knight TW, Ruzzante DE (2009) Interaction of landscape and life history attributes on genetic diversity, neutral divergence and gene flow in a pristine community of salmonids. *Mol Ecol* 18(23):4854–4869
- Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). <http://www.unil.ch/izea/software/fstat.html> Accessed June 2009
- Hardy OJ, Vekemans X (2002) SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol Ecol Notes* 2(4):618–620
- Hardy OJ, Charbonnel N, Freville H, Heuertz M (2003) Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. *Genetics* 163(4):1467–1482
- Harris CK (1974) The geographical distribution and habitat of the Olympic mudminnow, *Novumbra hubbsi*. Thesis, University of Washington, Schultz
- Homola JJ, Scribner KT, Elliott RF, Donofrio MC, Kanefsky J, Smith KM, McNair JN (2012) Genetically derived estimates of contemporary natural straying rates and historical gene flow among Lake Michigan lake sturgeon populations. *Trans Am Fish Soc* 141(5):1374–1388
- Jarne P, Lagoda PJJ (1996) Microsatellites, from molecules to populations and back. *Trends Ecol Evol* 11(10):424–429
- Jombart T (2008) *Adegenet*: a R package for the multivariate analysis of genetic markers. *Bioinform* 24(11):1403–1405
- Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11
- Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol Ecol Notes* 5(1):187–189
- Kendall KC, Stetz JB, Roon DA, Waits LP, Boulanger JB, Paetkau D (2008) Grizzly bear density in Glacier National Park, Montana. *J Wildl Manag* 72(8):1693–1705
- Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? *Conserv Biol* 9(4):753–760
- Lewis PO, Zaykin D (2001) Genetic Data Analysis: Computer program for the analysis of allelic data (version 1.0). <http://lewis.eeb.uconn.edu/lewishome/software.html> (accessed June 2009)
- McPhail JD (1967) Distribution of freshwater fishes in western Washington. *Northwest Sci* 41(1):1–11

- McPhail JD, Lindsey CC (1986) Zoogeography of the freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). In: Hocutt CH, Wiley EO (eds) The zoogeography of North American freshwater fishes. Wiley, New York, pp 615–638
- Meldgaard T, Nielsen EE, Loeschcke V (2003) Fragmentation by weirs in a riverine system: a study of genetic variation in time and space among populations of European grayling (*Thymallus thymallus*) in a Danish river system. *Conserv Genet* 4(6):735–747
- Meldrim JW (1968) The ecological zoogeography of the Olympic mudminnow (*Novumbra hubbsi*, Schultz 1929). Dissertation, University of Washington
- Metcalf JL, Love Stowell S, Kennedy CM, Rogers KB, McDonald D, Epp J, Keepers K, Cooper A, Austin JJ, Martin AP (2012) Historical stocking data and 19th century DNA reveal human-induced changes to native diversity and distribution of cutthroat trout. *Mol Ecol* 21(21):5194–5207
- Mock KE, Latch EK, Rhodes OE (2004) Assessing losses of genetic diversity due to translocation: long-term case histories in Merriam's turkey (*Meleagris gallopavo merriami*). *Conserv Genet* 5(5):631–645
- Mongillo P, Hallock M (1999) Washington state status report for the Olympic mudminnow. Washington Department of Fish and Wildlife, Olympia
- Nielsen JL (ed) (1995) Evolution and the aquatic ecosystem: defining unique units in population conservation. American Fisheries Society Symposium 17, Bethesda, Maryland
- Osborne MJ, Davenport SR, Hoagstrom CW, Turner TF (2010) Genetic effective size, N_e , tracks density in a small freshwater cyprinid, Pecos bluntnose shiner (*Notropis simus pecosensis*). *Mol Ecol* 19(14):2832–2844
- Osborne MJ, Carson EW, Turner TF (2012a) Genetic monitoring and complex population dynamics: insights from a 12-year study of the Rio Grande silvery minnow. *Evol Appl* 5(6):553–574
- Osborne M, Sharp A, Monzingo J, Propst DL, Turner TF (2012b) Genetic analysis suggests high conservation value of peripheral populations of Chihuahua chub (*Gila nigrescens*). *Conserv Genet* 13(5):1317–1328
- Peterson DP, Ardren WR (2009) Ancestry, population structure, and conservation genetics of Arctic grayling (*Thymallus arcticus*) in the upper Missouri River, USA. *Can J Fish Aquat Sci* 66(10):1758–1774
- Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J Hered* 90(4):502–503
- Quattro JM, Vrijenhoek RC (1989) Fitness differences among remnant populations of the endangered Sonoran topminnow. *Science* 245(4921):976–978
- Raymond M, Rousset F (1995) GENEPOP (Version-1.2)—population-genetics software for exact tests and ecumenicism. *J Hered* 86(3):248–249
- Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Cons Biol* 17(1):230–237
- Rice WR (1989) Analyzing tables of statistical tests. *Evol* 43(1):223–225
- Rosenfeld MJ (1983) Geographic variation in *Novumbra hubbsi* Schultz 1929 (Pisces: Umbridae): external meristic characters, chromosomal state and nuclear DNA content. University of British Columbia, Thesis
- Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends Ecol Evol* 22:25–33
- Slatkin M (1985) Rare alleles as indicators of gene flow. *Evol* 39(1):53–65
- Small MP, Frye AE, Von Bargen JF, Young SF (2006) Genetic structure of chum salmon (*Oncorhynchus keta*) populations in the lower Columbia River: are chum salmon in Cascade tributaries remnant populations? *Conserv Genet* 7(1):65–78
- Small MP, Burgess D, Dean C, Warheit KI (2011) Does lower crab creek in the eastern Washington desert have a native population of Chinook salmon? *Trans Am Fish Soc* 140(3):808–821
- Stamford MD, Taylor EB (2004) Phylogeographical lineages of Arctic grayling (*Thymallus arcticus*) in North America: divergence, origins and affinities with Eurasian *Thymallus*. *Mol Ecol* 13(6):1533–1549
- Stephen CL, Whittaker DG, Gillis D, Cox LL, Rhodes OE (2005) Genetic consequences of reintroductions: an example from Oregon prong horn antelope (*Antilocapra americana*). *J Wildl Manag* 69(4):1463–1474
- Strugnell JM, Watts PC, Smith PJ, Allcock AL (2012) Persistent genetic signatures of historic climatic events in an Antarctic octopus. *Mol Ecol* 21(11):2775–2787
- Tabor RW (1975) Guide to the geology of Olympic National Park. University of Washington Press, Seattle
- Taylor EB, Gow JL, Witt J, Zemplak R (2011) Connectivity among populations of pygmy whitefish (*Prosopium coulterii*) in northwestern North America inferred from microsatellite DNA analyses. *Can J Zool* 89(4):255–266
- Tilston Smith B, Escalante P, Hernandez Banos B, Navarro-Siguenza A, Rohwer S, Klicka J (2011) The role of historical and contemporary processes on phylogeographic structure and genetic diversity in the Northern Cardinal, *Cardinalis cardinalis*. *BMC Evol Biol* 11(1):136
- Trotter PC, McMillan B, Kappes D (2000) Occurrence of the Olympic mudminnow on the east side of the Puget Trough. *Northwestern Nat* 81(2):59–63
- Waples RS (1995) Evolutionary significant units and the conservation of biological diversity under the endangered species act. In: Nielsen JL (ed) Evolution and the aquatic ecosystem: defining unique units in population conservation. American Fisheries Society Symposium 17, Bethesda, Maryland, pp 8–27
- Whiteley AR, Spruell P, Allendorf FW (2004) Ecological and life history characteristics predict population genetic divergence of two salmonids in the same landscape. *Mol Ecol* 13(12):3675–3688
- Williamson-Natesan EG (2005) Comparison of methods for detecting bottlenecks from microsatellite loci. *Conserv Genet* 6(4):551–562