Herpetological Conservation and Biology 17(3):521–538.
Submitted: 6 January 2021; Accepted 21 October 2022; Published: 16 December 2022.

Do Newts at Crater Lake Represent a Distinctly Evolving Population of Rough-skinned Newts?

Andrew M. Ray[1,2,8], Stephen F. Spear[3,4], Scott F. Girdner[5], Mark W. Buktenica[5], David K. Hering[5], Jack D. Torresdal[3,6], Michael S. Parker[7], and Lisette P. Waits[3]

[1]*Crater Lake Science and Learning Center, Crater Lake National Park, Crater Lake, Oregon 97604, USA*
[2]*Greater Yellowstone Network, National Park Service, 2327 University Way, Suite 2, Bozeman, Montana 59715, USA*
[3]*Department of Fish and Wildlife Sciences, University of Idaho, 975 West 6th Street, Moscow, Idaho 83844, USA*
[4]*The Wilds, 14000 International Road, Cumberland, Ohio 43732, USA*
[5]*Crater Lake National Park, Crater Lake, Oregon 97604, USA*
[6]*Center for Ecosystem Science and Society, Northern Arizona University, Post Office Box 5620, Flagstaff, Arizona 86011, USA*
[7]*Department of Biology, Southern Oregon University, 1250 Siskiyou Boulevard, Ashland, Oregon 97520, USA*
[8]*Corresponding author, e-mail: Andrew_Ray@nps.gov*

Abstract.—**There is abundant evidence that genetic and morphological diversity is present among widespread species. This variation results from multiple processes, such as interruptions in gene flow or local adaptation. In Crater Lake National Park, a volcanic lake formed 7,700 y ago. Rough-skinned Newts (*Taricha granulosa*) subsequently colonized Crater Lake, and the lake population was described as a subspecies, the Mazama Newt (*T. g. mazamae*), characterized by dark ventral pigmentation. The sub-specific designation is not recognized by all authorities. In this study, we used a combination of genetic, morphological, and coloration data to test whether newts within the Crater Lake caldera form a distinct population segment (DPS). We found that the ventral surfaces of Crater Lake newts were darker and that head shapes and body condition were distinguishable from Rough-skinned Newts sampled throughout Oregon. Using microsatellite loci and mitochondrial DNA sequence data, we also found genetic differentiation between the caldera population and newts sampled from locations outside the caldera. Clustering analyses of microsatellite loci segregated Crater Lake newts from newts collected outside the caldera. Moreover, newts just outside the caldera were more similar to newts hundreds of kilometers distant than to the more proximal newt population within the caldera. Genetic diversity was also lower in newts found in the caldera compared to populations outside. Combined, our integrative approach provides evidence that Crater Lake newts are a distinct population that meets criteria as a DPS. This isolated population is threatened by introduced crayfish, and therefore conservation measures designed to protect this population are warranted.**

Key Words.—amphibians; distinct population; National Park Service; *Taricha granulosa*

Introduction

In this era of rapid biodiversity decline, threatened or endangered species are the most common motivator for ecological studies or conservation actions (Goble et al. 2012; Evans et al. 2016). Yet, conservation biologists today recognize the importance of protecting both rare and common taxa to preserve functioning ecosystems (Jiguet and Julliard 2006). Common species have a disproportionate impact on ecosystem processes and are often recognized for their role in creating and maintaining habitat (Frimpong 2018); their conservation provides indirect protections to many other species and microhabitats. Moreover, there is abundant evidence that genetic and morphological diversity is present among common, geographically widespread species (Gustafson et al. 2019). This diversity may be advantageous for exploiting a variety of habitat types across the range of a species and persisting through periods of environmental stochasticity. When natural and artificial barriers interrupt gene flow or migration is limited, however, isolation of otherwise widespread species can lead to inbreeding depression and loss of genetic variation (Madsen et al. 1996; Rowe and Beebee 2003). Fragmentation from barriers also can result in small, isolated populations with increased vulnerability to stochastic events (e.g., Freake et al. 2018). In such instances, distinct populations of otherwise common species can become locally threatened (Jamieson et al. 2008). Depending on the ecological and evolutionary context, isolated populations created by fragmentation may represent valuable reservoirs of distinct genetic diversity, or conversely, they may function as population sinks. In these scenarios, it is important to assess both ecological and evolutionary distinctiveness of isolated populations (e.g., Garner et al. 2005). Although the

loss of distinct populations may not threaten the overall persistence of common species, the adaptive diversity present in those populations is vulnerable to extinction, as is the speciation potential of reproductively isolated populations within newly formed habitats (see Barluenga et al. 2006).

Regulatory protections for many common or geographically widespread species are minimal, even within areas formally designated for protection. In the U.S., National Park Service (NPS) units and U.S. Fish and Wildlife Service refuges represent the core of the federal conservation network (Lawrence et al. 2011; Leslie 2014), yet federal policies lack national scale efforts emphasizing the importance of protecting common species for their biodiversity benefits on NPS lands (Doremus 2002; Leslie 2014). This is true even though protected areas can contain large portions of the biodiversity of a region. For example, waters within NPS units provide critical habitat for more than 60% of the recognized freshwater fish species in the USA. The status of these species within the NPS network and the status of distinct populations in some instances remains unclear (Williams et al. 2011). Less is known about the benefits of NPS lands to amphibian diversity; however, a survey of amphibian occurrence across the USA indicated that lands managed by the NPS had some of the highest rates of population decline (Adams et al. 2013). Furthermore, the patchiness and unusual natural features that characterize many protected areas (Doremus 2002) likely contribute to the presence of distinct and isolated populations. Despite an overarching goal of biodiversity conservation, many protected areas still face the increasing and interactive threats of climate change, invasive species, and novel pathogens (Halstead et al. 2022).

One example of a conservation concern of a common species within a protected area is the case of the Rough-skinned Newt (*Taricha granulosa*) within Crater Lake National Park. The Rough-skinned Newt is listed as a species of Least Concern on the Red List of the International Union for Conservation of Nature (IUCN 2015) and globally Secure through NatureServe (http://www.natureserve.org/explorer). The species ranges from southeast Alaska to central coastal California in western North America. Within the unusual environment of the Crater Lake caldera in southern Oregon, however, a geographically isolated population of *T. granulosa* occurs. The Mazama Newt (*T. granulosa mazamae*), as it is locally known, was formally described in the 1940s (Myers 1942) and proposed as a subspecies because of the unusually dark ventral pigmentation present on individuals within Crater Lake. The unusual color patterns of newts occupying Crater Lake have been noted by several naturalists (Myers 1942; Farner and Kezer 1953; Buktenica et al. 2015), but *T. granulosa mazamae*, the designation assigned to Crater Lake newts, is not a recognized subspecies in all authoritative sources including Crother (2017), for example. Mazama Newts, however, are present in the Integrated Taxonomic Information System (http://www.itis.gov), NatureServe (http://www.natureserve.org/explorer), and the Oregon Biodiversity Information Center (2019). Uncertainty and even disagreement on the taxonomic assignment exists among biologists and managers interpreting available taxonomic information on Crater Lake newts. For example, the subspecies is listed by NatureServe (http://www.natureserve.org/explorer) as Critically Imperiled but is not listed as a Species of Greatest Conservation Need by the Oregon Conservation Strategy (Oregon Department of Fish and Wildlife 2016). This uncertainty may hamper protections for the population, which is under current threat of extirpation due to displacement by introduced crayfish (Girdner et al. 2018).

The scenario of the Mazama Newt and other taxa with inconsistently recognized specific or subspecific status (see Mace 2004) presents a challenge for land managers regarding the prioritization of conservation actions (Game et al. 2013). In particular, the designation of subspecies has long been a controversial topic, and debates continue about how to define subspecies or incompletely separated species and their evolutionary relevance (Hillis 2019; de Quieroz 2020; Hillis 2021; Padial and De la Riva 2021). The concept of integrative taxonomy largely abandons debates on how best to classify sublineages (as populations, subspecies, or species; see de Quieroz 2020 and Hillis 2021) and instead considers as species any independently evolving lineages (whether completely or incompletely separated) and whose distinction can be corroborated through multiple lines of evidence (Padial et al. 2010). This view is not universally accepted, and criticisms include the taxonomic confusion that may result from this species concept (Hillis 2021). Although concepts of subspecies and sublineages are important for emphasizing biodiversity within common species that exhibit geographic variation (see de Quieroz 2020; Hillis 2021), they do not yet provide concrete criteria that land managers can use to define and protect potentially distinct and threatened lineages of otherwise common species.

In the U.S., protections of imperiled taxa occur under the Endangered Species Act (ESA; 1973). The definition of a taxonomic unit receiving protection under the ESA was expanded in the 1990s when the species definition was broadened to include species, subspecies, and for vertebrates, distinct population segments (DPS; U.S. Fish and Wildlife Service 1996). To be protected under the Endangered Species Act, a DPS must meet three criteria: (1) the population must be

discrete or clearly separated from other populations of the same taxonomic group as a consequence of physical barriers or other factors; (2) it must possess ecological or biological significance stemming from its occurrence in an unusual setting or because of its status as the only surviving representative of a unusual taxon that is otherwise common or abundant elsewhere; and (3) the conservation status of the population segment, when treated as if it were a species, must meet the definitions of threatened or endangered of the Act (U.S. Fish and Wildlife Service 1996). Land managers must evaluate these criteria to determine appropriateness of federal protection for imperiled taxa at the sub-specific level (May et al. 2011).

The Mazama Newt occurs within the Crater Lake caldera, a closed lake basin formed from the eruption of Mount Mazama 7,700 y ago and because of the geologic history of this basin, newts within Crater Lake have been separated from other newt populations for a maximum of only about 7,000 y. As a result, if the Mazama Newt is currently an independently evolving lineage, it almost certainly would appear as an incompletely separated lineage, as there has not been sufficient time for patterns such as reciprocal monophyly to occur across mitochondrial loci. Additionally, previous work using mitochondrial loci demonstrated relatively low variation in Rough-skinned Newts in their range north of California (Kuchta and Tan 2005). Therefore, a reasonable hypothesis is that newts that initially colonized the caldera would have been genetically similar to other Oregon populations and any distinctiveness from Oregon populations would have occurred due to evolution within the caldera.

The aim of this study was to evaluate intraspecific variation of Rough-skinned Newts in Oregon including the population occupying Crater Lake that has inconsistently been recognized as a subspecies. We sought to resolve uncertainty about the taxonomy of newts within Crater Lake, evaluate distinctiveness of newts in Crater Lake following definitions within the ESA, and provide information to support policy and management plans of Crater Lake National Park. Accordingly, the goal of the study was not to evaluate diversity across the entire range of the species, but rather to consider intraspecific variation at a regional scale in the vicinity of Crater Lake. Previous work had identified color differences between newts inside and outside the Crater Lake caldera (Myers 1942; Farner and Kezer 1953; Buktenica et al. 2015). We sought to more thoroughly characterize the discreteness of the Mazama Newt using quantitative measures of morphological and genetic discontinuity, specifically ventral coloration, morphology (head shape), and variation in both the nuclear and mitochondrial genome. We believe that measurable distinctness in these characters would imply that the Mazama Newt is an independently evolving lineage and thus would support the definition of the population as a DPS, potentially making the taxon eligible for protection under the ESA.

Materials and Methods

Study sites.—From 2010 to 2012 we sampled Rough-skinned Newts from Crater Lake and 12 other locations (wetlands, stream backwaters, ponds, and lakes) in western Oregon, USA. Two thirds of the newts sampled were putative Mazama Newts collected within Crater Lake (Table 1). Crater Lake is a large (53 km^2) ultraoligotrophic sub-alpine lake (1,883 m above sea level) that occupies the caldera of the former Mt. Mazama (Bacon 1983). Known for its depth (594 m maximum depth) and extremely clear water (mean Secchi depth = 31 m), the lake contains approximately 35 km of shoreline surrounded by a steep (> 30°) caldera wall. Cobble and boulder predominate the shoreline substrate, but bedrock, gravel, and sand-dominated shorelines are also present in isolated locations. The littoral zone is narrow and steep (> 30°) and drops quickly to deep water. Wave-cut platforms exist above steeper slopes in some areas of the lake (e.g., south and southwest side) where talus material allows platform formation. Phantom Ship and Wizard Island are the only islands in the lake. Newts were collected from six shoreline locations within Crater Lake (Fig. 1; Supplemental Information Table S1). Skell Channel (SC), Devil's Backbone (DB), and Merriam Point (MP) are located on the west shoreline. Phantom Ship (PS) and Eagle Point (EP) are located on the south shore, and Lady of the Lake (LL) is located on the eastern shore.

We also sampled five locations outside of Crater Lake but within or bordering Crater Lake National Park. These sites included Spruce Lake (SL; Farner and Kezer

Table 1. Sample sizes and general geographic localities for all morphological and genetic analyses of Rough-skinned Newts (*Taricha granulosa*) included in this study. Within caldera represents any newt sampled within Crater Lake, Oregon, USA; Outside caldera represents newts sampled at one of the sites in the vicinity of Crater Lake but outside the lake; and northwest Oregon represents samples from five sites sampled in northwest (NW) Oregon and distant from Crater Lake National Park. Refer to the text for specific information on each variable.

Response Variable	# within caldera	# outside caldera	# NW Oregon
Body condition index	222	69	0
Ventral surface color	173	73	0
Head shape morphometrics	11	5	15
Microsatellite genotypes	229	74	89
Mitochondrial: cytochrome *b*	156	43	0
Mitochondrial: concatenated	45	15	0

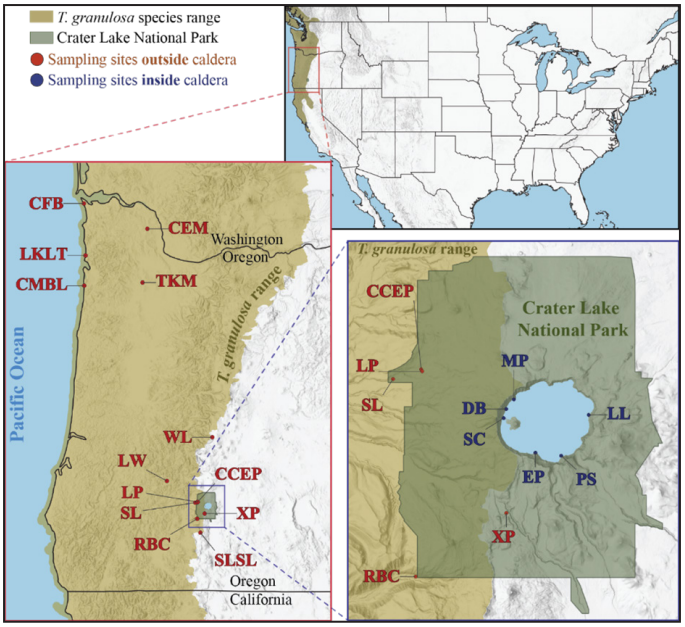

Figure 1. Study area map showing sampling locations inside and outside of Crater Lake National Park. Inset map shows the location of Crater Lake National Park in western Oregon, USA. Sampling site abbreviations are as follows: Devil's Backbone (DB), Eagle Point (EP), Lady of the Lake (LL), Merriam Point (MP), Phantom Ship (PS), and Skell Channel (SC) within Crater Lake. Outside the caldera, the locations include Crater Creek Eastern Pond (CCEP), Camp Emerald Forest (CEF), Coffenbury Lake (CFB), Chamberlain Lake (CMBL), Lake Lytle (LKLT), Lily Pond (LP), Lake in the Woods (LW), Red Blanket Creek (RBC), Spruce Lake (SL), South Lake (SLSL), Lake Tilikum (TKM), Waldo Lake (WL), and Xanterra Ponds (XP). Although not shown, the range of Rough-skinned Newts (*Taricha granulosa*) extends north to Alaska, USA.

1953), Lily Pond (LP), and Crater Creek Eastern Pond (CCEP) within the Crater Creek drainage; the Xanterra Ponds (XP), a lined artificial complex of sewage settling ponds and an earthen overflow pond located near park headquarters (Bury and Wegner 2005); and an off-channel pond in the Red Blanket Creek (RBC) drainage, near the southwest corner of the park (Farner and Kezer 1953). These sites were all within approximately 15 km of the Crater Lake caldera.

Also, in the southern or central Oregon Cascades mountains but farther from Crater Lake, we sampled newts from Lake in the Woods (LW; Stokes et al. 2015), Waldo Lake (WL), and South Lake (SLSL; Marangio 1978). Lake in the Woods and South Lake are small, high elevation lakes, and Waldo Lake is a large (25 km^2) natural lake of moderate depth (average 38 m; Swanson et al. 2000). In northwest Oregon, we collected samples from within or immediately surrounding five lakes: Coffenbury Lake (CFB), Lake Lytle (LKLT), Chamberlain Lake (CMBL), Camp Emerald Forest (CEF), and Lake Tilikum (TKM; Fig. 1, Supplemental Information Table S1).

Sampling.—We captured juvenile and adult newts from July through September by dip-netting in small, shallow habitats or with minnow nets or by hand when snorkeling in deep lakes. At a few sites (e.g., CFB), we also captured newts in terrestrial habitats immediately surrounding the lake margin. Once captured, we transferred newts to a 3.8 L bucket with native water. We used a standard flatbed scanner (CanoSCan LiDE 200; Canon U.S.A., Inc., Melville, New York, USA) without color controls to digitally scan (Costa et al. 2009) the ventral surfaces of post-metamorphic individuals from sites within Crater Lake National Park. We handled individuals with nitrile gloves and lightly held them with a dampened cloth atop the scanning surface. We rinsed scanner surfaces with lake water and patted them dry with a separate cloth before scanning a new individual. At all sites, we collected tissue samples by clipping a segment of tail 2–5 mm in length from each newt (Jones et al. 2001). We dipped scissors in 95% ethanol and flamed them between samples. After they were sampled, we returned animals to the point of capture, usually within 30 min. We euthanized newts collected for morphological analysis by cutaneous administration of 20% Benzocaine in the field (Gamble 2014) and we deposited the specimens in the University of Idaho (Moscow, Idaho, USA) Herpetology Collection.

Color analysis.—We analyzed all scanned newt images using Image J image processing and analysis freeware (National Institutes of Health; http://rsbweb.nih.gov/ij/). We completed on-screen assessments of newt coloration using the ventral surface from head to vent and excluding legs and tail; we defined this area

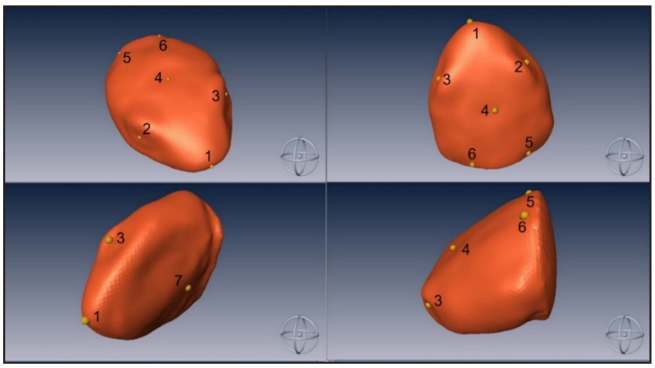

Figure 2. Representation of morphometric landmarks used to conduct three-dimensional head shape analysis of Rough-skinned Newts (*Taricha granulosa*). Landmark numbers are as follows: 1 is tip of snout; 2 is center of right eye; 3 is center of left eye; 4 is dorsal centered on the parietal; 5 is right dorsal on the prootic-exoccipital bone; 6 is left dorsal on the prootic-exoccipital bone; and 7 is ventral centered on throat between quadrate bones.

as the region of interest (ROI; *sensu* Beukema 2011). In brief, we defined the perimeter of the ROI using 45–55 visually selected vertices. When feet and toes overlapped the ROI on the scanned image, we excluded them from the selection. When possible, we excluded areas within the ROI that were reflective in the original scan (because of water or other foreign objects present on the scanner glass) to prevent those lightly colored pixels from misrepresenting the true color of the newt. We then converted the cropped ROI to grayscale image with 8 bits per sampled pixel yielding 256 different possible intensities. The grayscale intensity range spanned from 0, representing true black, to 255 representing complete white. The number of pixels available per individual varied due to differences in size, condition, or because of water or other interruptions removed from the ROI (see above). Regardless of these differences, we summarized grayscale intensity levels for all pixels within the ROI of each individual. To remove the effect of pixel number and standardize presentation of grayscale intensity across individuals, we used cumulative frequency distributions of grayscale intensities and standardized each grayscale summary by the total number of pixels evaluated. Using this approach, we were able to present a summary of all distinct grayscale intensities (ranging from 0 to 255) and their representative number of occurrences for each scanned individual. We then summarized the cumulative frequency distribution of grayscale intensities for newts sampled inside and outside of the caldera. From these distributions we identified the grayscale maximum (grayscale intensity with the greatest number of occurrences) for each individual and then compared the medians of these grayscale maxima from newts sampled inside and outside the caldera, using a non-parametric Mann Whitney *U* rank sum test.

Morphometric analysis.—We calculated body condition index by dividing the mass of each newt, measured to the nearest 0.1 g (Ohaus Scout Pro SP401; Ohaus Corporation, Parsippany, New Jersey, USA), by its snout-vent-length (SVL; Karraker and Welsh 2006). Snout-vent-length was measured to the nearest millimeter using a plastic ruler. Sexual dimorphism occurs in Rough-skinned Newts, with males typically larger and having a more prominent (larger and longer) vent (Myers 1942; Bishop 1943). In addition, males have longer and more flattened tails during the breeding season (Janzen and Brodie 1989). We could not determine the sex of all individuals easily, so we pooled all individuals for our analysis of body condition. We used the non-parametric Mann Whitney *U* Rank Sum test to evaluate whether median body condition index differed between newts from Crater Lake and those collected elsewhere.

We constructed three-dimensional models of newt heads for 11 individuals sampled from Skell Channel within Crater Lake, five individuals from Spruce Lake, and five individuals each from three of the northwest Oregon sites (CFB, CMBL, LKLT). We used a NextEngine 3D Scanner (NextEngine, Inc., Santa Monica, California, USA) to create a 3D mesh surface with up to 11,350 individual data points per individual. We exported each model into AmiraTM software (ThermoFisher Scientific, Waltham, Massachusetts, USA) and simplified each model to 5,002 data points. We set landmarks at the tip of the snout (the farthest point forward), in the middle of each eye, on the parietal bone approximately centered and anterior between the right and left squamosal on the dorsal surface, on the dorsal surfaces of each of the right and left prootic-exoccipital bones, and lastly on the ventral surface of the throat centered between the quadrates (Fig. 2). These landmarks were used to align each model into a common reference system for comparison. We then analyzed each model and its corresponding landmark file using spherical harmonics (SPHARM; Shen et al. 2009). To do this, we first aligned the specimen models using the landmark files and then combined and

resized each model. We standardized centroid size, the square root of the sum of squared distances of a set of landmarks from their centroid, to account for variation in head size. We used SPHARM to generate a small set of spherical harmonic axes that described variation in head shape. After this procedure, each specimen had a set of coordinates corresponding to changes in three-dimensional head shape that we used for statistical analyses. We also carried out a Discriminant Analysis to identify the axes that best differentiated among the populations using the predict function in package MASS (Venables and Ripley 2002) in R version 3.6.2 (R core team 2019). To summarize the predictive value of this function for each population, we calculated the proportion of times that individuals were reclassified into the correct population based on their morphology. We used Linear Discriminant axes one and two against each other to graphically show patterns of head shape variation across populations.

Microsatellite DNA analysis.—We extracted genomic DNA from 297 newt tail samples using the Qiagen DNEasy Blood and Tissue Kit (Qiagen Sciences, Inc., Germantown, Maryland, USA), including negative controls. We included 229 individuals sampled from within Crater Lake and 74 individuals sampled from locations outside of the caldera. Individuals sampled within Crater Lake represented six regions of the lake and individuals from outside the caldera were from three separate ponds (LW, LP, and SL). Additionally, we included 89 individuals from the northwest Oregon sites (CFB, LKLT, CMBL, CEF, and TKM; Fig. 1). We used five microsatellite loci previously developed for *T. granulosa* (Tgr01, Tgr02, Tgr06, Tgr10, Tgr14; Jones et al. 2001). We ran PCRs for two multiplexed microsatellite panels using the Qiagen Multiplex PCR kit (Qiagen, Inc.). We ran 7 µL reactions for each PCR reaction regardless of multiplex panel. For Multiplex 1, we used 3.5 µL Multiplex PCR Master Mix, 0.24 µm of Tgr01 forward and reverse primer, 0.32 µm of Tgr02 forward and reverse primer, 0.57 µm of Tgr06 forward and reverse primer, and 1 µL of template DNA. For Multiplex 2, we used 3.5 µL Multiplex PCR Master Mix, 0.7 µL Multiplex PCR Q solution, 0.43 µm of Tgr10 forward and reverse primer, 0.29 µm of Tgr14 forward and reverse primer, and 1 µL of template DNA. Multiplex 1 PCR conditions consisted of an initial denaturation step of 95° C for 15 min, followed by 32 cycles of 95º C for 30 s, 56º C for 90 s, and 72º C for 60 s, and a single final extension at 60º C for 30 min. For Multiplex 2, we used a touchdown protocol with an initial denaturation step of 95º C for 15 min, 10 cycles of 95º C for 30 s, an initial annealing temperature of 58º C (decreasing by 1º each cycle) for 90 s, and 72º C for 60 s, 26 cycles of 95º C for 30 s, 48º C for 90 s, and 72º C for 60 s, with a single final extension of 60º C for 30 min. All PCR products were run on an Applied Biosystems 3130xl Genetic Analyzer at the University of Idaho Laboratory for Ecological, Evolutionary and Conservation Genetics using Applied Biosystems LIZ 500 as a size standard and genotyped using Applied Biosystems Genemapper software (Foster City, California, USA).

We used Microsatellite Analyzer (MSA) v4.05 to convert microsatellite genotypes to the various needed file formats. We used Genepop v3.4 (http://genepop.curtin.edu.au/) to test for Hardy-Weinberg equilibrium and any instances of linkage disequilibrium, using the default parameters. We estimated average number of alleles per locus and expected heterozygosity across sites using GDA v1.0 (http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php). As presence of full siblings can bias estimates of population structure based on clustering algorithms, we used Colony 2.0.6.7 (Jones and Wang 2010) to identify likely full sibling relationships within each site. We combined all individuals within the caldera because there are no barriers between caldera sampling sites; all isolated ponds were analyzed separately. We assumed both male and female polygamy and inbreeding and did not estimate any genotyping error. If full siblings were identified, we retained only one individual within the group for further clustering analysis. We identified the most likely number of clusters across all individuals using STRUCTURE v2.3.4 (Pritchard et al. 2000) and estimated the range of K based on the mean and median membership proportion method proposed by Puechmaille (2016), with supporting evidence based on the log likelihood of each value of K. We evaluated both the median K value across replicates for each threshold and the maximum K value across replicates. We examined clustering at a threshold of 0.8 site membership coefficient (based on Q-values) to a particular cluster. We ran 20 replicates at each K = 1–15 for 50,000 iterations after a 100,000-replicate burn-in period. We calculated the value of each metric using StructureSelector (Li and Liu 2018). Proportion membership to each cluster was integrated over all replicate runs and visualized using CLUMPAK (Kopelman et al. 2015). If results indicated multiple values of K, we ran STRUCTURE separately on each resulting cluster until no more subdivision was detected using a K equal to the number of remaining sites in the hierarchical partition and using the same metrics as above. If a site had individuals assign to at least two different clusters at a membership threshold greater than 0.8, we included all individuals at that site in each hierarchical run that included that cluster. Because we had high individual sampling in three sites (Phantom Ship, Skell Channel, and Spruce Lake) and fewer than 20 individuals in all other sites, we also conducted

the same STRUCTURE analytical workflow with a random subset of 20 samples each from Phantom Ship, Skell Channel, and Spruce Lake along with each of the other sites. We conducted five replicate workflows for the subsampled dataset, and all individuals within the subsampled sites had an equal chance of being selected for each replicate run. Each subsampled dataset was analyzed in STRUCTURE as described for the full dataset.

Mitochondrial DNA analysis.—We sequenced three regions of the mitochondrial genome in a subset of individuals to gain further insight into patterns of genetic variation of newts both inside and outside the caldera. The regions were cytochrome *b* and two sequences of the 16s-tRNAMet-ND2 region. We initially focused on the cytochrome *b* locus because this region was sequenced in a previous study on Rough-skinned Newt genetic structure across the Pacific Northwest (Kuchta and Tan 2005). That study did not sample newts within the immediate vicinity of Crater Lake, but did sample populations within Oregon, and thus allowed us to identify if any haplotypes were shared with other populations in Oregon. We amplified 487 bp of cytochrome *b* using the primer pair MVZ15 and MVZ16 (Moritz et al. 1992) for 156 individuals within Crater Lake and 43 individuals outside the caldera. Because there appeared to be relatively low diversity across cytochrome *b* within Oregon, we also sequenced a subsample for two other primer pairs that have been used in newt studies: 1,174 bp of the 16s-tRNAMet region using the primer pair L3002 (Macey et al. 1997) and H4419 (Macey et al. 1998), and 548 bp of the NADH dehydrogenase subunit 2 (ND2) region using the primer pair L4437 and H5934 (Macey et al. 1997). We generated a concatenated sequence across the three sequenced regions representing 45 individuals from the Crater Lake caldera and 15 individuals from outside the caldera but within the region (14 from Spruce Lake and one from Red Blanket Creek). We chose to analyze both cytochrome *b* alone along with the full concatenated set because of the previous data regarding haplotypic diversity in cytochrome *b*, whereas the other two regions had limited sequence data for Rough-skinned Newts and so there was limited value in analyzing them separately. For each of the two mitochondrial datasets, we identified the number of haplotypes, estimated nucleotide diversity within each geographical region and F_{ST} between Crater Lake and other populations outside of Crater Lake and tested for significant genetic differentiation between the two populations using a Chi-square test. All analyses of sequence data were done using DnaSP v5 (Librado and Rozas 2009). We calculated haplotype networks for each set using the pegas package (Paradis et al. 2021) in R v3.6.3 (R core team 2021) using custom code (Toparslan et al. 2020).

Results

Color.—Ventral surfaces of Rough-skinned Newts within Crater Lake exhibited varying patterns of dark pigmentation that were not detected on individuals sampled outside the caldera (Fig. 3). Ventral surfaces of individuals sampled from within and outside the

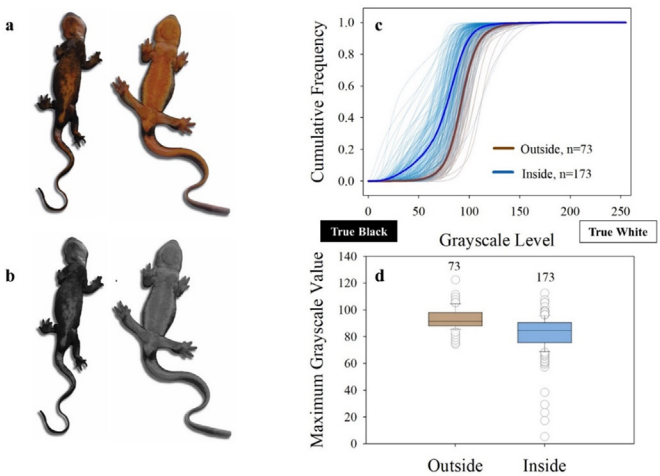

Figure 3. Ventral surface coloration summary for Rough-skinned Newts (*Taricha granulosa*) included in this study. (a) Examples of ventral true coloration of (left) a Mazama Newt from Crater Lake, Oregon, USA, and (right) a Rough-skinned Newt from nearby Spruce Lake, Oregon. Color scans (a) were converted to grayscale images (b) with 8 bits per sampled pixel yielding 256 different intensities. The intensity range spans from 0 (true black) to 255 (complete white) and grayscale intensity summaries are shown for individuals sampled inside (n = 173) and outside (n = 73) the Crater Lake caldera using cumulative frequency distributions. (c) Average cumulative frequency distribution for each group. (d) Grayscale maxima or the grayscale intensity value with the most number of occurrences summarized for Rough-skinned Newts included in this study.

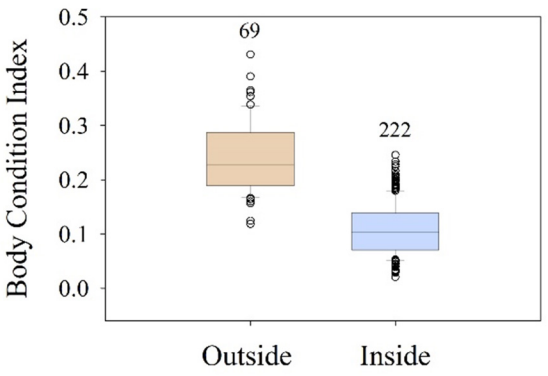

Figure 4. Body Condition Index (BCI) summary for Rough-skinned Newts (*Taricha granulosa*) included in this study in Oregon, USA. The BCI was calculated by dividing the mass of each individual by its snout-vent-length. Sample sizes used to summarize BCI inside and outside the Crater Lake caldera are shown above each boxplot.

caldera had overlapping grayscale intensity frequency distributions (Fig. 3), but the darkest newts, those with cumulative frequency distributions that were closer to the true black end of the grayscale intensity continuum, were newts sampled from sites within Crater Lake (Fig. 3). Using maximum grayscale intensities values from the grayscale intensity summary of each individual, we also show that newts in Crater Lake were darker (U = 2785.5, $P < 0.001$) and had a lower (i.e., darker) median maximum gray level (median = 85; lower quartile [Q1] = 76, upper quartile [Q3] = 91; n = 173) compared with newts outside the caldera (median = 92; Q1 = 88.5, Q3 = 98.5; n = 73; Fig. 3). Despite these differences, there was much greater variation in maximum grayscale intensity levels for newts inside Crater Lake (coefficient of variation [CV] = 17.3) versus outside (CV = 8.8).

Morphometrics.—The body condition index for *T. granulosa* in habitats outside of Crater Lake (median = 0.228, Q1 = 0.189, Q3 = 0.288) was more than twice that of individuals sampled within Crater Lake (median 0.104, Q1 = 0.072, Q3 = 0.139) and the medians of these two groups differed significantly ($U = 675.5$, outside n = 69, inside n = 222, $P < 0.001$; Fig. 4). We also found variation in the body condition indices among sampling locations within Crater Lake ($F_{5,216} = 2.26$, $P < 0.001$; Table 2). Within Crater Lake, individuals from Skell Channel, Lady of the Lake, Devil's Backbone, and Eagle Point had a higher condition factor than individuals present on the island known as Phantom Ship (Table 2).

Each of the five sites analyzed for head shape morphometrics could be differentiated based on the first two linear discriminant axes (Fig. 5). There was distinct separation of CFB newts along axis 1 (LD1) and Crater Lake newts along axis 2 (LD2). The main distinction between SL newts, sampled just kilometers from Crater Lake, and Crater Lake newts was larger centroid sizes (representative of larger overall volume of head size) in SL populations, consistent with reduced body condition in newts within the caldera. Predictive assignment tests did assign one Crater Lake individual to SL, and one

Table 2. Sample numbers (n), mean snout to vent length (SVL; in mm), total length (TL; in mm), mass (g), and Body Condition Index (BCI) for newts sampled within Crater Lake, Oregon, USA, and in ponds and lakes outside the caldera. Means and standard deviations (in parentheses) for all samples are summarized by location.

Location	n	SVL (mm)	TL (mm)	Total Mass (g)	BCI
Within Crater Lake					
Skell Channel	96	66.88 (16.13)	141.31 (39.52)	9.68 (5.12)	0.13 (0.05)
Crater Lake near Phantom Ship	64	47.72 (13.30)	99.16 (32.15)	3.88 (2.87)	0.07 (0.03)
Lady of the Lake	20	60.35 (12.67)	129.90 (30.41)	7.01 (3.42)	0.11 (0.03)
Devils Backbone	10	65.70 (12.45)	138.70 (25.59)	8.55 (3.73)	0.13 (0.04)
Eagle Point	20	59.55 (10.68)	127.55 (21.73)	6.37 (2.54)	0.10 (0.03)
Meriam Point	10	57.70 (8.00)	111.30 (20.23)	5.27 (2.05)	0.09 (0.02)
Outside Caldera					
Spruce Lake	54	71.74 (0.88)	164.57 (2.40)	17.75 (0.58)	0.25 (0.01)
Lily Pond	7	71.86 (1.26)	154.86 (5.95)	17.74 (1.21)	0.25 (0.01)
Crater Creek East Pond	2	71.00 (0.00)	148.50 (1.50)	12.70 (0.60)	0.18 (0.01)
Lake in the Woods	2	72.50 (2.50)	181.00 (9.00)	17.90 (5.30)	0.24 (0.06)
Red Blanket Creek	1	76.00	166.00	17.40	0.23
South Lake	1	68.00	111.00	11.40	0.17
Xanterra Pond	1	62.00	140.00	9.70	0.16
Waldo Lake	1	52.00	113.00	8.30	0.16

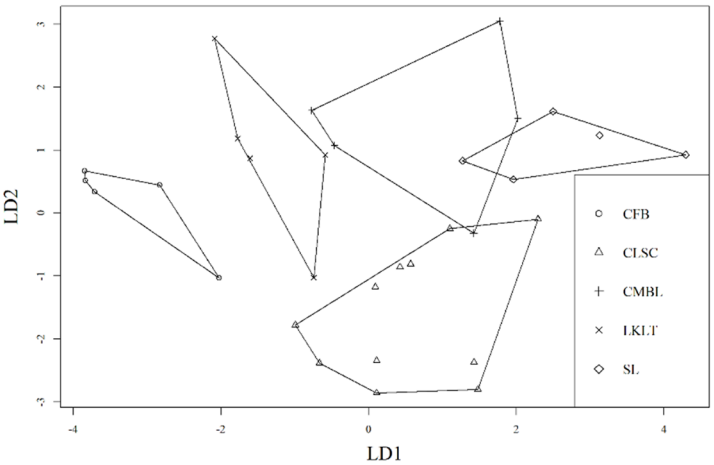

Figure 5. Summary of three-dimensional head shape models for Rough-skinned Newts (*Taricha granulosa*) collected as part of this study in Oregon, USA. Eleven individuals were included from Skell Channel in Crater Lake (CLSC) and five individuals were from Spruce Lake (SL) located outside the caldera but just 12 km from Crater Lake. In addition, five individuals were included from three of the northwestern Oregon sites Coffenbury Lake (CFB), Lake Chamberlain (CMBL), and Lake Lytle (LKLT). Summaries were generated using six landmarks at fixed positions for each model. Models and landmark files were analyzed using spherical harmonics and summarized along two axes.

CMBL sample was assigned to LKLT, but otherwise individuals were assigned to the site from which they were collected (Table 3). When a so-called ambiguous category was added to the predict pop function, misassigned sites were categorized as ambiguous rather than to incorrect localities.

Microsatellite DNA.—We found no violations of Hardy-Weinberg expectations or evidence of linkage disequilibrium among loci. Metrics of genetic diversity were consistently higher outside than inside the caldera. Averaged across the different sampling sites, average number of alleles per locus by site was 6.1 outside the caldera compared to 4.4 inside. Expected heterozygosity averaged 0.83 outside the caldera compared to only 0.68 inside.

Table 3. Population assignments based on three-dimensional head shape models for Rough-skinned Newts (*Taricha granulosa*) collected as part of this study. Eleven individuals were included from Skell Channel in Crater Lake, Oregon, USA (CLSC) and five individuals were from Spruce Lake (SL) located outside the caldera but just 12 km from Crater Lake. In addition, five individuals were included from three of the northwestern Oregon sites: Coffenbury Lake (CFB), Lake Chamberlain (CMBL), and Lake Lytle (LKLT). Column headings represent true site of origin, with row headings represent site of assignment.

Location	Crater Lake	Spruce Lake	Northwest Oregon Lakes		
			CFB	CMBL	LKLT
Crater Lake	10	0	0	0	0
Spruce Lake	1	5	0	0	0
CFB	0	0	5	0	0
CMBL	0	0	0	4	0
LKLT	0	0	0	1	5

We did detect evidence of some full sibling pairs within the caldera and in a subset of the other pond sites, although the proportion of full siblings was small overall. We estimated 212 unique family groups out of the 229 individuals genotyped within the caldera. Other sampled sites with full siblings included SL (42 unique groups out of 49 samples), LP (six unique groups out of seven samples), CMBL (19 unique groups out of 20 samples), LKLT (18 unique groups out of 20 samples), and TKM (15 unique groups out of 16 samples).

Population clustering based on STRUCTURE using only one sample per sibling group demonstrated a divergence based on whether a sample was collected from inside or outside of the caldera. Across all samples, the best supported number of clusters was 2–3 depending on specific metric, with $K = 3$ estimated by 3 out of the 4 metrics (Supplemental Information Fig. S1). The only clear subdivision based on membership bar plots (Fig. 6 and Supplemental Information S2), however, was one cluster represented by the six sites within the Crater Lake caldera and the second cluster represented individuals outside the caldera, regardless of whether they were collected in southern or northwest Oregon. Furthermore, when summarized across all replicates, no individual was assigned to the third cluster with a Q-value > 0.8. Admixture of newts in the caldera with outside populations was low, with one individual from LP assigning to the caldera cluster > 0.8, and likewise one individual from SC in the caldera assigning to the outside cluster > 0.8. Overall, 95% of individuals from sites outside the caldera assigned to the outside cluster > 0.8 and similarly 94% of individuals from within the caldera assigned to the caldera cluster > 0.8. The pattern of $K = 3$ produced two possible clustering

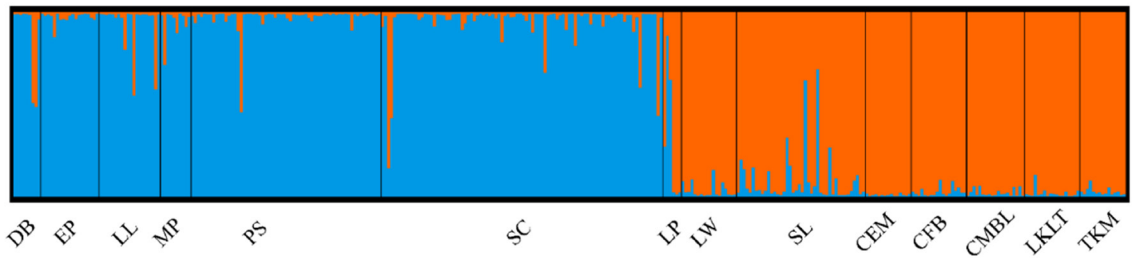

Figure 6. Genetic clustering results of Rough-skinned Newts (*Taricha granulosa*) based on a STRUCTURE analysis assuming K = 2. Orange represents the Crater Lake cluster and blue represents the cluster outside the caldera, including northwest Oregon, USA, populations.

modes: a major mode with a third cluster largely represented by SL (but with a high degree of admixture among all sites outside the caldera) and a minor mode of a third cluster was almost completely admixed within the caldera cluster, such that each individual had some ancestry assigned to the two clusters suggesting over-splitting at K = 3 (Supplemental Information Fig. S2). For hierarchical clustering analyses, we included LP with the caldera cluster (due to one individual > 0.8) and for the outside cluster, included SC due to the one individual there assigning to that cluster. For the caldera subdivision, no further substructure was indicated, as all metrics identified K = 1. The outside cluster (with SC) represented either two clusters (based on MedMean K and MaxMean K) or three (based on MedMed K and MaxMed K), however, K = 3 had membership > 0.8 to all three clusters, so we considered this subdivision for further analysis (Supplemental Information Fig. S3-S5). The first cluster was primarily the caldera site SC, with one individual from LP assigning > 0.8 (Supplemental Information Fig. S5). The second cluster was primarily represented by SL, with some individuals from LW, and one individual from the northern Oregon site TKM clustering > 0.8 (Supplemental Information Fig. S5). Finally, the third cluster consisted of all northern Oregon sites as well as LW (Supplemental Information Fig. S5). When each of the three clusters were run individually, all metrics estimated a K = 1, with no further substructure.

Across the five subsampled replicates to create a more even sampling, all five replicates resulted in an estimation of two clusters across all metrics, following the general pattern of a caldera cluster and a cluster representing all outside sites (Supplemental Information Figs. S6-S23). No individuals within the caldera assigned to the outside cluster at a percentage > 0.8. Lily Pond (LP) was admixed in all five scenarios, with different individuals assigned to both clusters. Spruce Lake (SL) had different individuals assigned to both clusters in three of the five replicates. In the hierarchical analysis, there was no further substructure within the outside cluster for any of the five replicates. Within the caldera cluster (including LP and sometimes SL), there was substructure in each of the five replicates, although there were slightly different patterns among replicates. In the three instances in which both LP and SL had evidence of admixture, subsequent clustering separated caldera sites from both LP and SL. In two of these cases, however, caldera site DB had evidence of admixture with the LP and SL cluster, and in one instance, caldera site SC had evidence of admixture with the LP and SL cluster. Subsequent hierarchical testing separated out SC, but in both cases, DB remained clustered with LP and SL. In the two replicates in which only LP had evidence of admixture with the caldera sites, no further substructure was detected.

Mitochondrial DNA.—There were 14 haplotypes within the cytochrome *b* gene that diverged by only 1–2 base pairs. Most individuals (n = 164) assigned to a single haplotype that not only included individuals from both within and outside the caldera, but also matched a widespread haplotype seen in Oregon and southern Washington (Kuchta and Tan 2005). The other haplotype found in Oregon by Kuchta and Tan (2005) also matched another haplotype found in seven individuals, (six from Spruce Lake and one from Crater Lake Skell Channel). The third haplotype found in both populations was not identified by Kuchta and Tan (2005) but represents an intermediate sequence between the two previously discussed haplotypes, and thus may be a haplotype widespread throughout Oregon. There was a fourth haplotype found in one individual in each population that was not found by Kuchta and Tan (2005) nor is it an intermediate sequence to their haplotypes. The remaining 10 haplotypes were only found in a single population; eight found only in Crater Lake and two found only outside the caldera. Despite a number of shared haplotypes and a lack of haplotype clustering by region (Fig. 7A), there were clear differences between the regions in mitochondrial genetic diversity and F_{ST}. Nucleotide diversity outside the caldera was 0.00225, nearly an order of magnitude greater than the diversity of 0.00034 within the caldera. Similarly, the average number of nucleotide differences was 1.094 outside

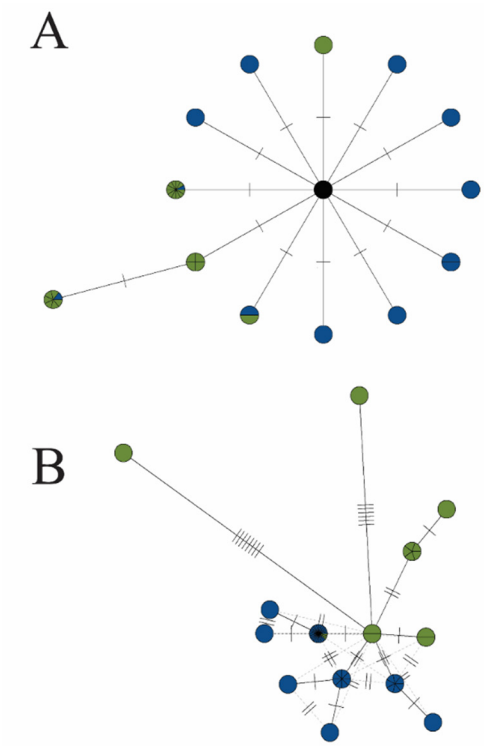

Figure 7. Haplotype network for (A) the cytochrome *b* mitochondrial region and (B) the cytochrome *b*, 16s-tRNAMet, and ND2 concatenated sequences for Rough-skinned Newts (*Taricha granulosa*) both within the Crater Lake caldera and outside the caldera in southern Oregon, USA. Circle divisions represent individuals with that haplotype, color-coded by region. Blue represents within the caldera and green represents outside the caldera. The central haplotype A is found in 144 caldera individuals and 20 outside individuals. Perpendicular marks represent the number of base pair differences between connected haplotypes. Solid lines among haplotypes represent the most parsimonious network and dashed lines represent alternative links.

compared to 0.167 within the caldera. The level of genetic divergence (F_{ST}) was 0.16.

Across the 60 individuals for which we had concatenated sequence data across all three loci, we detected 14 haplotypes. There was only one shared haplotype, which represented the most common haplotype and was found in 47% of individuals. Six haplotypes were only found outside the caldera and seven haplotypes within the caldera only. In contrast to cytochrome *b* alone, the concatenated network was generally structured by region and outside haplotypes were more clearly differentiated than haplotypes within the caldera (Table 4; Fig. 7B). This was also reflected by the average number of nucleotide differences, which was higher outside the caldera (3.581) compared to inside (1.366). Furthermore, the nucleotide diversity was 0.00162 outside compared to 0.00062 within the lake. The estimate of F_{ST} between the two areas was 0.18.

Table 4. Mitochondrial DNA summary values by gene and location for Rough-skinned Newts (*Taricha granulosa*) sampled inside and outside the Crater Lake caldera, Oregon, USA. See Table 1 for sample sizes for each mitochondrial locus. Abbreviations are WCC*b* = within caldera cytochrome *b*, WCC = within caldera concatenated, OCC*b* = outside caldera cytochrome *b*, OCC = outside caldera concatenated.

	WCC*b*	WCC	OCC*b*	OCC
# unique haplotypes	8	7	2	6
# shared haplotypes	4	1	4	1
# nucleotide differences	0.167	1.366	1.094	3.581
Nucleotide diversity	0.00034	0.00062	0.00225	0.00162

Discussion

Our study revealed differences in coloration, morphology, and genetic structure that support the hypothesis that newts within the Crater Lake caldera are a distinct, although incompletely separated, independently evolving population. Specifically, we confirmed that the ventral surfaces of Mazama Newts were variable but, on average, darker and that head size and condition factors were distinguishable from other Rough-skinned Newts sampled throughout Oregon. Using microsatellite loci and mtDNA sequences, we demonstrated differentiation between the caldera population of newts and newts sampled from Oregon locations outside the caldera. Clustering analyses based on microsatellite DNA generally segregated the data into two clusters; one cluster included all sites sampled within Crater Lake and the second cluster included individuals from all sites outside the caldera that ranged from northwest Oregon to locations only 12 km from the Crater Lake caldera. Finally, the concatenated mitochondrial loci had multiple haplotypes that were unique to Crater Lake and haplotypes were primarily grouped between caldera and outside, although without evidence of reciprocal monophyly. Together, these multiple lines of evidence demonstrate that Mazama Newts are distinguishable from populations located outside the caldera and meet the discreteness criterion of a DPS.

The original designation of the Mazama Newt as a subspecies of Rough-skinned Newt was based on qualitative descriptions of dark skin pigmentation on newts within the Crater Lake caldera (Myers 1942; Farner and Kezer 1953). Our analysis quantified that the ventral surfaces of Crater Lake newts are significantly darker, usually characterized by dark blotches, compared with individuals sampled from other habitats. There was significant variation in the extent of blotchiness among individuals, but no newts sampled outside the caldera exhibited this ventral color pattern. Because of extremely low dissolved organic carbon concentration, ultraviolet radiation penetrates to great depths in Crater

Lake (Hargreaves et al. 2007). Many aquatic and semi-aquatic organisms have physiological strategies that assist in repairing or preventing UV-B- induced damage (Bancroft et al. 2007; Häkkinen et al. 2002; Wiegand et al. 2004) or minimize exposure to elevated UV-B (Garcia et al. 2009). Screening pigments like melanin can help reduce the effects of UV-B radiation in aquatic organisms (Häkkinen et al. 2002; Garcia et al. 2009). In salamanders, including Rough-skinned Newts, a darkening response to UV-B exposure was apparent after just 5 d of exposure (Belden and Blaustein 2002). Given the high UV-B levels at this elevation (1,883 m) and high transparency of water in Crater Lake, the darkened dorsal and ventral pigmentation quantified here is possibly an adaptive physiological response to UV-B conditions present in Crater Lake. Future research would be beneficial to determine the extent that the pigmentation represents local genetic adaptation or a phenotypically plastic response to this unique habitat.

Our morphometric data show that Mazama Newts have lower body condition and have head volumes that differ from newts sampled from ponds and lakes outside the caldera. The low body condition of newts within Crater Lake is likely associated with the unusually low productivity and ultraoligotrophic conditions of the lake. Photographs of newts published in Myers (1942) show similarly slender newts suggesting lower body condition has been typical for this population for several decades. Head size and shape analysis suggested distinctive morphologies across each tested site, although Mazama Newts were divergent along a different axis than the remaining sites, particularly with respect to overall head volume. In fishes, variations in head and jaw shape have been described for sympatric morphotypes and represent adaptations to divergent foraging strategies (Markevich et al. 2018). While variations in head geometry among conspecifics are not unexpected and can occur as a result of competitor density, presence of predators, and differing habitat conditions (Ivanović et al. 2009; Vega-Trejo et al. 2013), consistent variation among populations can be helpful to distinguish divergent morphotypes of amphibian populations (Vieira et al. 2008). Rough-skinned Newts are known to have morphological variation in a variety of characters that are related to locality, season, and habitat type (Riemer 1958; Pimentel 1960; Livezey and Wyllie 1961). As a result, it is likely that there is considerable plasticity in this trait that is environmentally influenced, given the high assignment of sampling site based on head shape. Further study is needed to determine which of the described characters are genetically controlled, whether they have adaptive ecological significance, and to identify other traits that may further distinguish this population from populations throughout the range of the species.

Stokes et al. (2015) demonstrated that newts occurring at high-elevation breeding sites, including Crater Lake, exhibit significantly lower or non-measurable levels of tetrodotoxin (TTX), a potent neurotoxin produced by newts as an anti-predation defense (Brodie 1968; Hanifin et al. 1999), when compared to low-elevation populations. The explanation for this variation is unclear but may be related to a lack of reptilian predators at higher elevations (McCain 2010) or that energy demands for producing toxin manifest in reduced toxicity in high elevation habitats where productivity is low (Stokes et al. 2015). Further work is needed to determine whether the TTX levels in the Crater Lake population provides a meaningful character for distinguishing this population.

Both nuclear DNA microsatellite and mtDNA results also support genetic divergence between newts found in Crater Lake and those found in habitats outside the caldera. Newts outside the caldera but within Crater Lake National Park clustered with geographically distant sites in northwest Oregon, rather than with the much closer Mazama Newt population. Our results, along with previous work (Kuchta and Tan 2005; Ridenhour et al. 2007; Bakkegard 2008), suggest that Rough-skinned Newts typically have high levels of gene flow and connectivity across Oregon, but the Crater Lake caldera was a notable exception, with only a small number of individuals (≤ 5) showing admixture between the caldera and LP/SL. In two of the subsampled hierarchical clustering replicates, LP clustered with both caldera and outside sites when those two groups were tested separately, indicating that the site is either well admixed, or perhaps more likely, cannot be definitively assigned due to low sample size for LP (six individuals) and the low number of microsatellite loci we had available. Increasing sample size and loci would likely clarify these relationships. There is a greater degree of differentiation among newt populations in northern California analyzed by Kuchta and Tan (2005) and it is possible that unique lineages exist in that portion of the range as well, although further sampling is needed.

Although cytochrome *b* haplotypes displayed shallow divergence and little structuring by region, the concatenated haplotype network demonstrated divergent structure between the caldera and SL, albeit without reciprocal monophyly. The one shared haplotype among the two regions (the most common caldera haplotype) and a central haplotype found only in SL is consistent with at least a historic relationship between the two regions. This suggests that the initial colonizing newts into the Crater Lake caldera were part of the same population as the ancestors of the current Spruce Lake population outside the caldera. As expected, haplotypes within the caldera all had shallow divergences, with 1–2 bp separating most haplotypes. In contrast, haplotypes found in SL were typically separated by several base

pairs consistent with either a longer evolutionary history or greater admixture with other populations. Furthermore, mitochondrial loci had values of F_{ST} that indicate high differentiation between the two regions.

Levels of genetic diversity are low in Crater Lake for both types of genetic markers, which is consistent with genetic drift associated with a founder effect from a newly colonized population subsequently maintained by geographic isolation. Given the relatively sudden appearance of Crater Lake and the steep, exposed walls surrounding the lake, there was almost certainly an initial founder effect for the Crater Lake newt population that likely led to an immediate difference in allele frequencies that has been maintained through isolation. Although there exist models such as Isolation with Migration (IM; Hey 2010) that allow investigators to test these specific hypotheses, the recent formation of Crater Lake (and therefore a recent divergence time) combined with our low number of loci are likely to be problematic for IM and other similar models (Hey et al. 2015). In the Mount St. Helens blast zone in Washington, USA, newts were able to readily colonize distant ponds and maintained high gene flow (Bakkegard 2008). Additionally, the recolonized populations at Mount St. Helens showed no evidence of reduced genetic diversity only two decades after the eruption, in stark contrast to the pattern observed at Crater Lake many generations following its formation. This comparison indicates that an initial colonization bottleneck is unlikely to be solely responsible for the current pattern of genetic isolation and reduced diversity in Crater Lake. We expect high landscape resistance to connectivity given that the walls of the caldera serve as a prominent barrier to frequent movement of newts. A secondary, although untested, hypothesis is that the modern road that encircles the lake and seasonal vehicular traffic further restricts even infrequent immigration. While there has been no local support for the secondary hypothesis, evidence from elsewhere shows that road crossing patterns of Rough-skinned Newts are unusual among species in the Pacific Northwest. Unlike other species that crossed roads at night, peak road crossing of newts occurred during daylight hours corresponding with periods of higher vehicular traffic (Schuett-Hames et al. 2019).

Federal protections afforded by a DPS classification can assist with the management of populations of conservation concern by enabling targeted management of distinct populations of otherwise common species where management actions are merited (Oyler-McCance et al. 2014). Recognition of a sub-specific taxon as a DPS requires meeting the established legal criteria of discreteness, significance, and conservation status. A key piece of this definition is the discreteness criterion where population units express distinct ecological, phenotypic, and genetic characters (Waples 1991; Moritz 1994; Crandall et al. 2000). Although exact criteria vary among alternative definitions, the population of newts in Crater Lake are consistent with DPS criteria of genetic differentiation and phenotypic distinctiveness.

The significance aspect of the DPS designation requires the population to possess ecological or biological significance based on its occurrence in an unusual setting, its status as the only surviving population of an unusual taxon that is otherwise common or abundant elsewhere or documented genetic differences from other populations of same species (U.S. Fish and Wildlife Service 1996). May et al. (2011) refer to this as the ecological exchangeability of the population. The newts in Crater Lake occupy an unusual setting, an ultra-oligotrophic lake that is recognized as one of the clearest lakes in the world (Girdner et al. 2020) and possesses optical characteristics comparable to regions of the open ocean (Boss et al. 2007). These unusual properties of Crater Lake and the unusual setting in the caldera of the former Mt. Mazama have likely contributed to the distinct coloration, morphology, and genetic structure of newts of Crater Lake. Further, newts likely played a key ecological role as top aquatic predators in this ecosystem prior to the introduction of fish and crayfish (Umek 2016).

Finally, previous work has described threats to newts of Crater Lake posed by introduced fish and crayfish and demonstrated that Mazama Newts have been displaced by nonnative crayfish along the Crater Lake shoreline (Buktenica et al. 2015; Girdner et al. 2018). Ongoing monitoring demonstrates a declining conservation status of newts over multiple years. As of summer 2021, crayfish occupied 85% of the rocky shoreline of Crater Lake, an increase from 44% in 2008. During that same period, newts declined from 33% occupancy of the shoreline in 2008 to 26% in 2021 (Buktenica et al. 2015; unpubl. data). Additional declines in abundance and distribution are anticipated and could lead to extinction of newts in Crater Lake (Girdner et al. 2018). If this population segment were evaluated in a manner consistent with ESA standards (i.e., treating the DPS as a species), we believe the changing distributions of newts and crayfish and decreasing occurrence of newts are trends that indicate listing of this taxon as Endangered may be warranted.

While this population belongs to a species that is secure in its overall status and has widespread distribution, we argue that the population meets DPS criteria, and its presence inside a national park alone warrants strong consideration for protection (Berger 2003). Populations with unusual phenotypes or important ecosystem roles are likely to have considerable biodiversity value even with high genetic drift, and conservation plans should clearly define this important component of biodiversity within protected areas (Jenkins et al. 2015; Cooper et

al. 2019). Such clarification would serve as the basis for conservation priorities and management action. In the case of newts in Crater Lake, a DPS designation will help boost support for lake-wide control efforts to remove or reduce primary threats (i.e., introduced crayfish), supplementation strategies including a captive breeding program, and an enhanced (i.e., demographic) monitoring program to track population trends and future responses to management actions. Protecting this and other imperiled amphibian populations will remain a vexing conservation problem, but reversing declines is possible when resource managers have access to local, population-specific information.

Acknowledgments.—Many people have contributed to newt ecology and crayfish invasion studies at Crater Lake. In particular, we would like to recognize Sudeep Chandra, John Umek, Rene Henry, Kristin Beem, Andrew Dinlinger, Monisha Sugla and Jeremy Mack. Rebecca Johnson, Brita Olson, Isaiah Hoyer and Brannon Durant assisted by helping with the DNA extraction and sequencing for this study. Caitlyn Brendal assisted with digital image processing and Ben LaFrance provided assistance with study area map. All unique mitochondrial haplotypes were submitted to Genbank (accession numbers OP314905-OP314918 for cytochrome *b*, OP314919-OP314925 for 16s-tRNAMet, and OP314930-OP314937 for ND2). All work was approved by Crater Lake National Park under Research Permit # CRLA-2011-SCI-011; 2011 – 2013, the Institutional Animal Care and Use Committee of Montana State University (authorization number: MSU 2011-40), the Institutional Animal Care and Use Committee of the University of Idaho (Protocol number 2008-44), and animals were handled under the Oregon Scientific Taking Permit: 108-12.

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Supplemental Information: http://www.herpconbio.org/Volume_17/Issue_3/Ray_etal_2022_Suppl

Andrew M. Ray is an Ecologist with the Greater Yellowstone Network of the U.S. National Park Service in Bozeman, Montana, USA. Andrew received an M.S. from Northern Michigan University, Marquette, Michigan, USA, and a Ph.D. from Idaho State University, Pocatello, Idaho, USA. He coordinates long-term monitoring of amphibians for the National Park Service in Yellowstone and Grand Teton national parks. Previously, Andrew worked as the Science Coordinator with the Crater Lake Science and Learning Center where he worked on a distinct population of Rough-skinned Newts in Crater Lake. (Photographed by David Thoma).

Stephen F. Spear is currently a Research Biologist with the Upper Midwest Environmental Sciences Center of the U.S. Geological Survey in La Crosse, Wisconsin, USA. Previously he worked as the Director of Wildlife Ecology at The Wilds and as a Conservation Scientist with the Orianne Society. His interests include conservation genetics, environmental DNA, endangered species head-starting and reintroduction, and restoration of wildlife habitat. He earned his Ph.D. from Washington State University (Pullman, Washington USA) and a M.S. from Idaho State University in Pocatello, Idaho USA. Throughout his career, a major focus of his research has been to use molecular tools to inform questions rooted in ecology, with an emphasis on relevance for applied management. (Photographed by Chloe Moore).

Scott F. Girdner is an Aquatic Biologist at Crater Lake National Park, Oregon, USA, where he manages a long-term limnological monitoring program on Crater Lake. He began studies of inland waters at the Institute of Limnology in Uppsala, Sweden, while getting a B.S. in Biology from Chico State University, California, USA. He received his M.S. from Oregon State University in Corvallis, USA, studying the ecology of high mountain ponds and lakes in Mount Rainier National Park, Washington, USA. His research interests include mountain lakes and using long-term monitoring data to address scientific questions. (National Park Service Photo).

Mark W. Buktenica began studying Crater Lake in 1985 and retired from Crater Lake National Park in 2018. As Aquatic Branch Chief and Ecologist, he exercised his passion for long-term limnological monitoring and stream restoration, and still participates with these programs today. (Photographed by Maggie Buktenica).

David K. Hering is an Aquatic Ecologist at Crater Lake National Park, Oregon, USA. He received a B.S. in Biology from the University of Oregon, Eugene, USA, and an M.S. in Fisheries from Oregon State University, Corvallis, USA. His interests include life-history diversity and behavior of freshwater fish, effects of invasive species, and conservation of native fish and amphibians. For 15 y, David has worked to protect imperiled trout in the Upper Klamath Basin of Oregon, and he recently assisted a project to restore an alpine lake ecosystem in Slovenia. (Photograph courtesy of the U.S. National Park Service).

Jack D. Torresdal is a Science Teacher at Northland Preparatory Academy in Flagstaff, Arizona, USA. He earned his MS in Biology from Northern Arizona University, Flagstaff (2020), where he worked with state and federal agencies to research native fish reintroduction ecology. He worked previously in Trinidad, West Indies, as a Project and Field Station Manager for 6 y, studying evolution in wild populations of Trinidadian guppies (Poeciliidae), the effects of mining on river ecosystems, and sky-island biogeography of the endemic Golden Treefrog (*Phytotriades auratus*). (Photographed by Diana Gerardo).

Michael S. Parker is Professor Emeritus in the Biology Department at Southern Oregon University, Ashland, USA, where he has taught courses in Herpetology, Vertebrate Natural History, Fish Biology, Aquatic Ecology, and Environmental Ethics for 29 y. He has a B.S. in Biology from Southern Oregon State College, Ashland, USA, an M.S. in Aquatic Ecology from the University of Nevada, Las Vegas, USA, and Ph.D. in Ecology from the University of California, Davis, USA. His research focuses on stream ecology and the conservation of amphibians and reptiles in the Klamath Mountains ecological province. (Photographed by Emmery Parker).

Lisette P. Waits is a Distinguished Professor in the Department of Fish and Wildlife Sciences at the University of Idaho, Moscow, Idaho, USA. She has a B.S. in Genetics from the University of Georgia, Athens, USA, and a Ph.D. in Genetics from the University of Utah, Salt Lake City, USA. Her research is focused in the fields of conservation genetics and molecular ecology with particular focus on developing methods for noninvasive genetic monitoring of wildlife and landscape genetics. She is an elected fellow of The Wildlife Society and the American Association for the Advancement of Science. (Photographed by John Koprowski).