Type N Experimental Buffer Treatment Study: Post-harvest comparison of genetic diversity and demographic findings for three stream-associated amphibians

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Washington State Forest Practices Adaptive Management Program

The Washington State Forest Practices Board (FPB) has established an Adaptive Management Program (AMP) by rule in accordance with the Forests & Fish Report (FFR) and subsequent legislation. The purpose of this program is to:

> *Provide science-based recommendations and technical information to assist the FPB in determining if and when it is necessary or advisable to adjust rules and guidance for aquatic resources to achieve resource goals and objectives. The board may also use this program to adjust other rules and guidance.* (Forest Practices Rules, WAC 222-12-045(1)).

To provide the science needed to support adaptive management, the FPB established the Cooperative Monitoring, Evaluation and Research (CMER) committee as a participant in the program. The FPB empowered CMER to conduct research, effectiveness monitoring, and validation monitoring in accordance with WAC 222-12-045 and Board Manual Section 22.

Report Type and Disclaimer

This technical report contains scientific information from research or monitoring studies that are designed to evaluate the effectiveness of the forest practices rules in achieving one or more of the Forest and Fish performance goals, resource objectives, and/or performance targets. The document was prepared for the Cooperative Monitoring, Evaluation and Research Committee (CMER) and was intended to inform and support the Forest Practices Adaptive Management program. The project is part of the Type N Experimental Buffer Treatment Study, and was conducted under the oversight of the Landscape and Wildlife Scientific Advisory Group (LWAG).

This document was reviewed by CMER and was assessed through the Adaptive Management Program's independent scientific peer review process. CMER has approved this document for distribution as an official CMER document. As a CMER document, CMER is in consensus on the scientific merit of the document. However, any conclusions, interpretations, or recommendations contained within this document are those of the authors and may not reflect the views of all CMER members.

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Full Reference

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1-1. ABSTRACT

Genetic monitoring provides a complementary approach to demographic monitoring, and can provide additional information on a population's response to disturbance. For instance, genetic monitoring can identify rapid declines in population size and non-random mating that may lead to future inbreeding depression. As part of the Type N Experimental Buffer Treatment Study, we assessed the genetic response of three stream-associated amphibian species (Coastal Tailed Frog, Cope's Giant Salamander, and Coastal Giant Salamander) before and after clearcut timber harvest of small headwater basins. We used a Before-After Control-Impact (BACI) study design to evaluate amphibian genetic response to four experimental treatments, including an unharvested reference (i.e., in the harvest rotation but withheld from harvest) and three riparian buffer treatments involving clearcut harvest of the entire basin. Riparian buffer treatments included a two-sided 50-ft (15.2-m) riparian leave-tree buffer along the entire riparian management zone (RMZ; 100% treatment), a two-sided 50-ft (15.2-m) riparian buffer along at least 50% of the RMZ, consistent with the Washington State Forest Practices buffer prescription for non-fish-bearing streams (FP treatment), and clearcut harvest throughout the entire RMZ (0% treatment). We used several metrics to characterize levels of genetic diversity before and after harvest. These included number of full siblings, allelic diversity, heterozygosity, inbreeding coefficient, and heterozygosity excess. We compared changes in genetic diversity 7-8 years post-harvest to results from the analysis of demographic data collected at the same study sites in the two years post-harvest. We also identified genetic clusters across study sites to determine the role of gene flow in the observed genetic response. Overall, we found little evidence for a change in genetic diversity as a result of riparian buffer treatments. No significant treatment effects were observed between the pre- and post-harvest periods for Coastal Tailed Frogs. Cope's Giant Salamander generally lacked evidence of significant treatment effects for most metrics, although we saw a significant pairwise decrease in F_{IS} for the 100% treatment relative to the FP treatment (-0.10, $p = 0.02$). We observed a significant ($p = 0.03$) treatment effect for the change in number of unique full sibling families of Coastal Giant Salamanders, which was driven by the observed decrease (-24.0) in unique full sibling families within the 100% treatment. There were no treatment effects on the change in proportion of full sibling families, and therefore the significance of the decline is due to a decrease in sampling numbers. We observed a significant change in F_{IS} for Coastal Giant Salamanders ($p = 0.05$) that resulted from a decrease in both the 100% treatment (-0.17; $p = 0.02$) and the 0% (-0.16; $p = 0.03$) relative to the reference. Decreases in F_{IS} typically indicate mating between more distantly related individuals and is consistent with a shift in immigration/emigration dynamics. Several sites showed a signature of recent genetic bottlenecks based on an increase in heterozygosity excess for both the Coastal Tailed Frog and the Cope's Giant Salamander. However, we detected significant heterozygosity excess in both the reference and treatment sites, suggesting no clear pattern due to treatment. We detected no evidence of heterozygosity excess for Coastal Giant Salamanders. We did not see clear associations of genetic patterns with the demographic analyses conducted on data collected immediately post-harvest. We note that the demographic and genetic analyses do not represent concurrent sampling. Both Coastal Tailed Frogs and Coastal Giant Salamanders had high levels of gene flow among sites in both pre- and post-harvest periods. Cope's Giant Salamander has much more restricted levels of gene flow overall, although there was genetic connectivity among nearby sites. There was a slight decrease in genetic differentiation post-harvest in Cope's Giant Salamander, which could be consistent with the decrease in F_{IS}. However, for all three species,

genetic structure is likely influenced by surrounding basins in addition to site-level treatment effects. Although we do not see evidence of a change in genetic diversity due to clearcut timber harvest and alternative riparian buffer treatments, we caution that increased sample size and additional sampling across future generations may be necessary to detect a trend, as simulations have demonstrated that steady declines are often not detected until several generations post-decline.

1-2. **INTRODUCTION**

Genetic monitoring (Schwartz *et al.* 2007) has emerged as a complementary approach to typical demographic monitoring approaches due to the development of genetic markers that respond at a relatively fine temporal and spatial scale. At a broad scale, genetic diversity is required to provide the raw material for populations to evolve in response to environmental changes and therefore persist over the long term. Techniques to estimate demographic trends in population size (e.g., mark-recapture or detection probability) require multiple sampling occasions and intense sampling effort. In contrast, accurately characterizing genetic structure generally requires sampling a smaller proportion of the population and as few as one site visit within a period of interest (Pierson *et al.* 2015). It can become difficult to determine decreases in reproductive success in long-lived species over short periods. In cases in which larval or juvenile stages are sampled, the young individuals may only be offspring of a small number of parents from the previous generation. Genetic markers can help estimate the number of unrelated individuals at a site, infer the predominant mating system, and provide opportunities for mark-recapture through genetic identification of the same individual.

The simplest indicator of genetic diversity is the number of alleles present in a population. When strong population declines occur, alleles are typically lost from the population (Luikart *et al.* 1998; Allendorf and Luikart 2007) suggesting allelic diversity may be one of the better measures for genetic monitoring. In fact, Hoban *et al.* (2014) demonstrated through simulations that a change in the average number of alleles per locus had the highest power to detect evidence of a population decline. Additional common measures of genetic diversity include heterozygosity and Wright's inbreeding coefficient ($F_{IS)}$. Although heterozygosity and F_{IS} had lower power in the simulations conducted by Hoban *et al.* (2014), the authors assumed random mating. Non-random mating has a strong effect on both observed heterozygosity and F_{IS}, and both metrics are often used as an assessment of whether mating is random (Allendorf and Luikart 2007). Relatively low heterozygosity and increased F_{IS} are signs of mating among close relatives, whereas the opposite patterns are often indicative of increased immigration into the focal site.

Genetic data can provide information on individual family relationships (parentage, siblings) and estimate effective population size (N_e), both of which complement demographic estimates such as census population size of adults and larvae. While genetics have long been used to describe pedigrees in well-sampled systems, only recently have researchers had the statistical tools to estimate family relationships for populations in which pedigree information is lacking (Wang 2004). With relatively few genetic loci, we can now estimate full siblings and putative parents with confidence given sufficient sampling effort. While estimating family groups is a useful rationale for genetic monitoring, estimating effective population size is often the focus of genetic studies (Waples 2005; Luikart *et al.* 2010). The reason for this emphasis is that effective

population size provides the best indication of the evolutionary potential of the population (Waples 2005). There are several estimators for effective population size; however, high variability frequently exists among estimators and the best estimator is often situation-specific (Gilbert and Whitlock 2015). Therefore, effective population size may not be a consistent metric to include in genetic monitoring studies.

Most genetic monitoring (herein defined as the use of genetic markers to estimate genetic diversity and connectivity) has focused on single samples to assess the current genetic diversity of populations, or has used genetic information to detect individuals or populations of secretive taxa (i.e., non-invasive environmental sampling). Evaluations of temporal data have typically used historical samples to compare with recent samples, and often span many generations (Kekkonen *et al.* 2011). Examples of genetic monitoring that follow or even resemble a Before-After Control-Impact (BACI) design (Eberhardt 1976; Green 1979), such as the one we report on here, are rare. Relevant examples include experimental removal of bush rats to assess changes in genetic structure (Peakall and Lindenmayer 2006), change in lizard genetic structure before and after a major drought (Vandergast *et al.* 2016), and the genetic response of sailfin mollies to a major hurricane in Florida (Apodaca *et al.* 2013). These studies all detected differences in genetic differentiation among populations but relatively little change in genetic diversity. Furthermore, none of these studies examined changes in parameters such as increased presence of full siblings or higher relatedness.

Amphibians are sensitive to changes in environmental conditions (Stuart *et al.* 2004) and thus are used as an indicator to monitor changes in forested environments due to natural and anthropogenic disturbance (e.g., Welsh and Ollivier 1998; Lawler *et al.* 2010). Management practices that reduce structural complexity of aquatic and terrestrial habitats can negatively impact stream-associated amphibian species in forested environments (Corn and Bury 1989; Kroll 2009). While some have concluded that some stream-associated amphibians are sensitive to forest management (Bury and Corn 1988; Welsh *et al.* 2005; Hawkes and Gregory 2012; Maigret *et al.* 2014), many researchers have documented stream-associated amphibians in abundance in streams located in forest stands with a history of timber harvest (Russell *et al.* 2004; Hayes *et al.* 2006; Olson *et al.* 2014).

During the Forests & Fish (USFWS 1999) negotiations leading to the development of Washington State's current Forest Practices rules, several stream-associated amphibians were selected for protection in perennial non-fish-bearing streams (Type N Waters) including: Coastal Tailed Frog (*Ascaphus truei*); and Olympic, Columbia and Cascade Torrent Salamanders (*Rhyacotriton olympicus*, *R. kezeri*, and *R. cascadae*). At the time of negotiations almost no published studies addressed the efficacy of riparian buffers for maintaining these amphibian species in Type N Waters or provided clear or compelling guidance addressing conservation needs of stream-associated amphibians.

Most studies of amphibian response to timber management in headwater basins have been based on monitoring changes in occupancy or abundance (e.g., Diller and Wallace 1999; Wilkins and Peterson 2000; Jackson *et al.* 2007; Kroll *et al.* 2010; Pollett *et al.* 2010; Olson *et al.* 2014). Over short periods, amphibian demography can be highly variable (Pechmann *et al.* 1991). Genetic structure would not be expected to change as rapidly and can give a better indication of long-term response to disturbance. In this study, we compare amphibian genetic structure before and

eight years after timber harvest with variable retention riparian buffer treatments. Although we likely do not have power to detect smaller genetic changes eight years since disturbance (i.e., timber harvest; Hoban *et al.* 2014), genetic monitoring may be more sensitive to severe bottlenecks or an increase in inbreeding than demographic measures alone.

1-3. BACKGROUND AND STUDY DESIGN

In the Type N Experimental Buffer Treatment Study (hereafter, Type N Study) we evaluated the effectiveness of current riparian management prescriptions for non-fish-bearing streams (Type N waters) for western Washington. We compared the effectiveness of the current riparian buffer prescription in maintaining riparian structures, functions and processes important to the riparian forest and associated aquatic system to buffer alternatives. A primary focus was stream-associated amphibians (specifically Coastal Tailed Frog [*A. truei*], and torrent [*Rhyacotriton*] and giant [*Dicamptodon*] salamanders). We also evaluated the response of riparian tree mortality and tree fall, in-channel wood recruitment and loading, stream temperature and shade, discharge, nutrient export, suspended sediment export, channel characteristics, litterfall input and detritus export, biofilm and periphyton, and macroinvertebrate export.

An important component of our study was the characterization of genetic diversity for stream-associated amphibians to refine information for making predictions about both short- and long-term responses of amphibians to timber management. An evaluation of amphibian populations at multiple levels of biological organization (e.g., demographic and genetic) and in conjunction with measures of ecosystem conditions (e.g., stream temperature, stream channel characteristics) is imperative for understanding the potential consequences of human-induced disturbances (Surasinghe 2013; Clipp and Anderson 2014). Baseline, pre-harvest measures of genetic structure were reported for Coastal Tailed Frog, Coastal Giant Salamanders (*D. tenebrosus*) and Cope's Giant Salamanders (*D. copei*) in The Type N Experimental Buffer Treatment Study: Baseline Measures of Genetic Diversity and Gene Flow of Three Stream-associated Amphibians (Spear *et al.* 2011), hereafter Baseline Genetic Report. Simulation data based on the baseline genetic measures indicated that we would have power to detect severe reductions in population size (i.e. effective population sizes reduced to 6-34 individuals). Torrent salamanders were not included in the genetic analysis since three species of the genus (*R. cascadae*, *R. kezeri* and *R. olympicus*) occur throughout the study area, but are distributed geographically in a non-overlapping manner that would confound treatment comparisons.

The pre-harvest genetic sampling for Coastal Tailed Frogs, Cope's Giant Salamander, and Coastal Giant Salamander not only provided the baseline and comparison for the present study, but data were combined with additional sampling to evaluate genetic connectivity across the forest landscapes of western Washington. Coastal Tailed Frogs exhibited extensive genetic connectivity across both the Olympic Peninsula and the South Cascades, including a rapid genetic recovery of the Mt. St. Helens blast zone (Spear and Storfer 2008; Spear *et al.* 2012). While overall gene flow was high in all landscapes, there was some evidence of a negative effect of timber harvest on genetic connectivity in Olympic National Forest (Spear and Storfer 2008) and a greater negative impact of climatic variables in the salvage logged areas surrounding Mt. St. Helens (Spear *et al.* 2012). Furthermore, there were evidence of population bottlenecks in the harvested forest in the Olympics (Spear and Storfer 2008). That study was unable to identify the

exact mechanism leading to the observed genetic effects, which the present study could help address. A landscape genetic study with Cope's Giant Salamander did not directly address forest management practices, but did examine genetic connectivity within each of the three main regions sampled in this study (Trumbo *et al.* 2013).The study found different landscape factors that were significant by region, with stream connectivity being most important in the Olympics and Willapa Hills, whereas heat load index had the greatest effect on connectivity in the South Cascades. Finally, Dudianec *et al.* (2012) examined Coastal Giant Salamander genetic structure in response to several environmental variables, finding a strong response of genetic connectivity to frost-free period in the South Cascades, but no significant effects on connectivity in the Willapa Hills. As with the Cope's Giant Salamander, this study was not specifically designed to evaluate the impact of forest management.

1-3.1. STUDY SITES AND TREATMENTS

Study sites included 17 Type N basins of second-growth forested stands located on hard rock (i.e., competent) lithologies. Each watershed was a perennially flowing, non-fish-bearing, first-, second- or third-order stream basin (*sensu*, Strahler 1952) located along the Clearwater, Humptulips and Wishkah rivers in the Olympic Mountains; the North, Willapa, Nemah, Grays and Skamokawa rivers and Smith Creek in the Willapa Hills; and the Washougal River and Hamilton and Trout creeks in the southern Cascades (N45°48.42' – N47°38.87', W122°15.88' – W124°12.07', elevation 22 – 730 m; **Figure 1**). Watersheds occurred in managed Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) dominated second-growth forests on private, state and federal timberlands. Watersheds ranged from 12.5 to 76.1 ha (measured utilizing a Geographic Information System (GIS), specifically ArcMap (ESRI 2004) and were dominated (≥84% of total watershed area) by forest stands with ages ranging from 32 to 80 years.

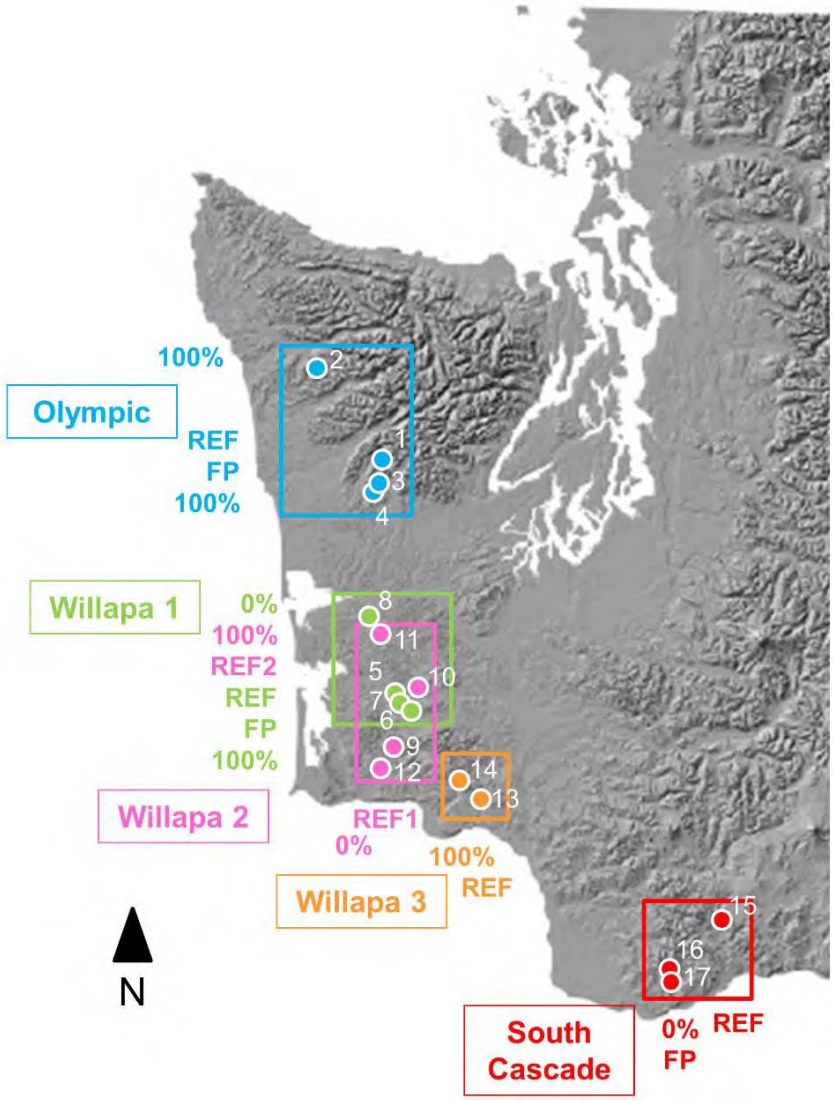

Figure 1. Distribution of study sites and treatment allocation for the Type N Study, 2006 – 2010. Study sites are blocked (grouped) based on geography. The five blocks are color-coded such that sites in a block are the same color. Treatments include REF (reference), and 100%, FP (Forest Practices) and 0% buffer treatments. Site IDs used for genetic clustering figures are shown in white.

We established four treatments: three different riparian buffer configurations in basins that were otherwise fully clearcut to maximize the potential impact of treatments, and a reference (i.e., control) with no timber removal (see McIntyre *et al.* 2018c, Chapter 3 - Management Prescriptions). The intent of this design was to harvest riparian management zones (RMZs) at intensities both greater and less than current Forest practice rules such that we could evaluate the relative effectiveness of alternative treatments in meeting the four key goals established by the Washington Forest Practices Board (WFPB; see McIntyre *et al.* 2018a, Chapter 1 - Introduction and Background). The four experimental treatments included (**Figure 2**):

1) **Reference (REF):** unharvested reference with no timber harvest activities within the study site during the study period;

2) **100% treatment (100%):** clearcut harvest with a riparian leave-tree buffer (i.e., two-sided 50-ft [15-m]) throughout the RMZ;

3) **Forest Practices treatment (FP):** clearcut harvest with current Forest Practices riparian leave-tree buffer (i.e., clearcut harvest with a two-sided 50-ft [15-m] riparian buffer along ≥ 50% of the RMZ, including buffers prescribed for sensitive sites [side-slope and headwall seeps, headwater springs, Type Np intersections and alluvial fans]). In practice, the stream length buffered ranged from 55% to 73% of the Type N stream length.; and

4) **0% treatment (0%):** clearcut harvest with no riparian buffer retained within the RMZ.

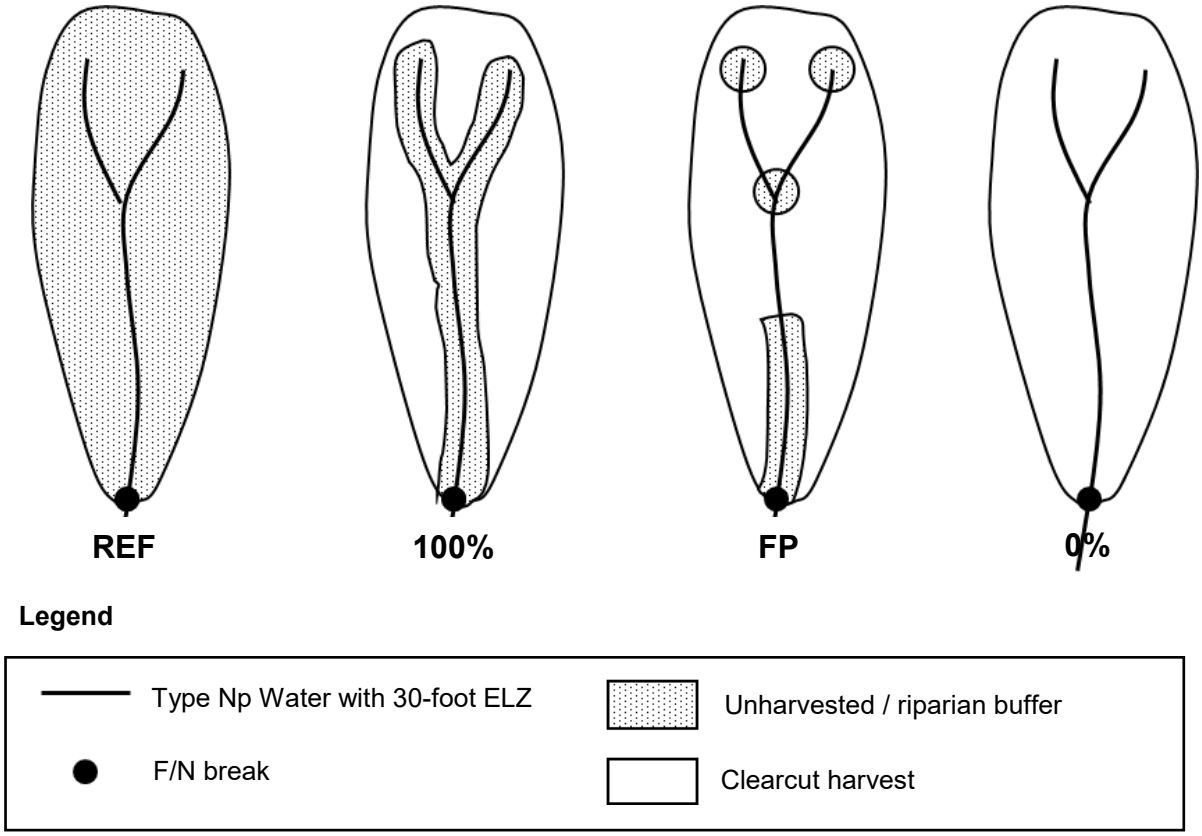

Figure 2. Schematic of the four experimental treatments included in the Type N Study. Treatments include unharvested reference sites (REF) and sites receiving a clearcut harvest with one of three riparian buffer treatments along the Type Np Water RMZ: two-sided 50-ft (15-m) riparian buffers of 100%, Forest Practice (FP), and 0%. FP and 100% treatments include 56-ft (17-m) radius buffers around Type Np intersections and headwater springs. All streams are protected by a two-sided 30-ft (9-m) equipment limitation zone (ELZ).

To maximize the influence of the riparian buffer treatments and reduce confounding effects, harvest units were applied to the entire Type N basin, where possible. In five study sites <1% – 15% of a basin could not be harvested due to regulatory or logistic constraints (see McIntyre *et al.* 2018c, Chapter 3 - Management Prescriptions). Harvest unit size was operationally meaningful, ranging from 12 ha (30 ac), the unit size identified by landowners as the minimum typically harvested, to 54 ha (133 ac). The maximum harvest unit size allowable under Forest Practices is 49 ha (120 ac) without an exception based on review by an interdisciplinary science team (WFPB 2001).

Besides application of the treatment, harvest practices followed Forest Practices rules (e.g., a 30-ft [9-m] equipment limitation zone (ELZ) was maintained along all Type Np and Ns[1] Waters, regardless of treatment). We blocked (grouped) study sites based on geography to minimize variability and randomly assigned sites within each block to one of the four treatments, when possible (see McIntyre *et al.* 2009). Sites within a block were located within the same physiographic region (Olympic, Willapa Hills, and South Cascade). We had one block of four sites in the Olympic region, two blocks of four sites each and one block of two sites in the Willapa Hills region, and one block of three sites in the South Cascade region (

Table 1; **Figure 1**). We established an acronym for each study site, based on the combination of the block to which it was assigned and the treatment applied. We use these acronyms in tables and figures throughout this report (**Table 2**). Identification numbers, referenced in **Table 2**, were used in the Baseline Genetic Report.

Table 1. Distribution and numbers of sites among geographic blocks and treatments for the Type N Study.

Block	Number of Sites	Treatment			
		REF	100%	FP	0%
Olympic (OLYM)	4	1	1	1	1
Willapa 1 (WIL1)	4	1	1	1	1
Willapa 2 (WIL2)	4	2	1	0	1
Willapa 3 (WIL3)	2	1	1	0	0
South Cascade (CASC)	3	1	0	1	1
Total	17	6	4	3	4

[1] A Type Ns Water, or non-perennial, seasonally intermittent stream includes all segments of natural waters within the bankfull width of the defined channels that are not Type S, F, or Np Waters. These are seasonal, non-fish habitat streams in which surface flow is not present for at least some portion of a year of normal rainfall and are not located downstream from any stream reach that is a Type Np Water. Ns Waters must be physically connected by an above-ground channel system to Type S, F, or Np Waters (WAC 222-16-030).

Table 2. Study site acronyms for each block and treatment combination. For cross reference purposes, the identification number used in the Baseline Genetic Report (Report ID) is included. Site ID is the identification number used in all genetic clustering figures.

Block	Treatment	Site Acronym	Report ID	Site ID
Olympic	Reference	OLYM-REF	1099	1
	100% treatment	OLYM-100%	363	2
	FP treatment	OLYM-FP	1197	3
	0% treatment	OLYM-0%	1236	4
Willapa 1	Reference	WIL1-REF	3098	5
	100% treatment	WIL1-100%	3111	6
	FP treatment	WIL1-FP	3110	7
	0% treatment	WIL1-0%	2260	8
Willapa 2	Reference 1	WIL2-REF1	3437	9
	Reference 2	WIL2-REF2	3074	10
	100% treatment	WIL2-100%	2468	11
	0% treatment	WIL2-0%	3576	12
Willapa 3	Reference	WIL3-REF	5785	13
	100% treatment	WIL3-100%	3914	14
South Cascade	Reference	CASC-REF	5378	15
	FP treatment	CASC-FP	5595S	16
	0% treatment	CASC-0%	5595N	17

1-3.2. STUDY TIMELINE

We collected the baseline data for all response metrics presented in the Baseline Genetic Report during the pre-harvest period (2006 – 2008). Harvest occurred in buffer treatment from July 2008 through August 2009. We collected two years of post-harvest data in the extended study period, seven and eight years after harvest (2015 and 2016). Few studies have provided size and age information for tailed frogs and giant salamanders. The best data available for tailed frogs are for the Rocky Mountain Tailed Frog (*A. montanus*), a species that is closely related to the Coastal Tailed Frog and was considered the same species until recently (Neilson *et al.* 2001). Daugherty and Sheldon (1982) estimated the size of 8+ year old Rocky Mountain Tailed Frogs in Montana to be ≥44 mm snout-vent length (SVL) for females and ≥40 mm SVL for males. Sagar *et al.* (2007) found that second- and third-year Coastal Giant Salamanders in coastal Oregon ranged from 54 to 99 mm SVL and that aquatic adults four years of age and older had SVLs ≥100 m. Similar size and age information for Cope's giant salamander were not available, so we will assume that the same size and age classes apply to both giant salamander species. All larval individuals of all taxa encountered during our post-harvest sampling would have been born after treatment implementation. We estimate that all post-metamorphic tailed frogs less than 42 mm SVL and giant salamanders less than 100 mm SVL would be younger than eight years, and thus also born after treatment implementation.

1-3.3. BASELINE AMPHIBIAN GENETICS

As part of the Baseline Genetic Report, we measured stream-associated amphibian genetic diversity and genetic differentiation within and among populations to provide insight into trends in population size and to identify the level of migration among sites. We also used genetic identification to differentiate between larval giant salamanders. The added benefits of including amphibian genetics, in addition to demographic information, included (see Spear *et al.* 2011):

1) Genetic markers are the best means of ensuring unambiguous identifications for the two giant salamander species.

2) Stream-associated amphibian population estimates are usually larval-biased. Multiple amphibian larvae can be the offspring of one parent, and larvae typically suffer high mortality rates prior to metamorphosis. Therefore, larval abundance estimates are unlikely to fairly represent adult population size. Genetic markers can estimate the number of unique families that are represented and identify sibling groups.

3) Genetic diversity statistics can be used to infer changes in population size, and populations with decreased genetic diversity and increased levels of inbreeding may not be sustainable at the scale of tens to hundreds of generations.

4) Genetic data can be used to estimate the degree of gene flow across a study area.

1-4. **OBJECTIVES**

The population genetics portion of the Type N Study had five main objectives:

1) Correctly identify giant salamander individuals to species level (Cope's or Coastal) and identify hybrids in sites where the two species co-occur. Evaluate changes in the proportion of each species and the extent of hybridization post-harvest.

2) Generate post-harvest measures of genetic diversity (i.e., allelic richness, heterozygosity and inbreeding coefficient) for each species and study site and test whether these measures differ from those obtained during the pre-harvest period.

3) Test for evidence of recent population bottlenecks.

4) Estimate spatial extent of gene flow (through genetic clustering) for both the pre-harvest and post-harvest periods. Results will indicate the potential effect of gene flow on metrics of within-basin genetic diversity, as well as identify any changes in number of genetic clusters between periods.

5) Provide a framework for future genetic monitoring of amphibian populations, both in our study sites and in Type N basins across the landscape.

1-5. MATERIALS AND METHODS

1-5.1. AMPHIBIAN SAMPLING AND GENETIC TISSUE COLLECTION

We collected tissue samples for genetic analysis from 23 June through 2 November in 2006 – 2008 (pre-harvest period), and 2015 and 2016 (post-harvest period). We used two amphibian sampling methodologies, each designed to detect and capture both tailed frogs and giant salamanders: light-touch (Lowe and Bolger 2002) and rubble-rouse sampling (Bury and Corn 1991). Both are commonly used for surveys of headwater amphibians in the Pacific Northwest (e.g., Welsh and Lind 1996; Wilkins and Peterson 2000; Steele *et al.* 2003; Russell *et al.* 2004; Quinn *et al.* 2007). Sampling efforts were intensive, covering much of the stream length in every study site. Study stream length ranged from 325 m (1,066 ft) to 2,737 m (8,980 ft). We conducted light-touch sampling along every tributary, in 10-m sample reaches systematically distributed between a point located up to 100 m below the fish-end-point and upstream to each tributary headwall, with a minimum of 25% of the total stream length in each site sampled each year. In addition to this site-wide sampling, we divided the 200 meters above the fish-end-point into 10 meter segments and sampled 1-m rubble-rouse plots randomly located within each of these 20 intervals. Additional rubble-rouse sampling was conducted in post-harvest years in study sites where wood obstructions prevented light-touch sampling. In this instance, plots were 3-m long and the number of plots per site depended on the proportion of the stream length in which light-touch sampling could not be effectively implemented (seeMcIntyre *et al.* 2018b, Chapter 15 - Stream-associated Amphibians for details on the distribution and number of plots per site). We made an effort to collect tissue from individuals distributed throughout the entire stream network to better characterize the genetic population structure.

We used sterilized dissecting scissors to obtain tissue samples. We collected tail tissue from larval tailed frogs and larval and post-metamorphic giant salamanders. The target tissue size was 0.5 cm^2 collected with a single snip to the tip of the tail. We collected a single toe from metamorphosed tailed frogs. Tissue samples were stored in 96% ethanol. We had a minimum goal of sampling 30 individuals per taxa and study site. Individuals with evidence of previous tissue removal were not sampled. For sites where the minimum of 30 samples per taxa were not obtained with standard light-touch and rubble-rouse sampling, we conducted additional nocturnal light-touch surveys, and diurnal light-touch, rubble-rouse and kick sampling (e.g., Arkle and Pilliod 2010) to increase sample sizes.

1-5.2. LABORATORY METHODS

We extracted Coastal Tailed Frog (N = 360) and giant salamander (N = 1,106) tissues collected in the post-harvest period with the Qiagen DNeasy Tissue kit, as per manufacturer's protocol (Qiagen Inc., Germantown, MD). Tailed frog and giant salamander samples were amplified at 13 (Spear *et al.* 2008) and 11 microsatellite loci (Steele *et al.* 2008) respectively. PCR amplifications were carried out with Qiagen Master Mix (Qiagen Inc., Germantown, MD). Samples that did not amplify at >70% of the loci or had identical genotypes were not included in the analyses. Full descriptions of the PCR conditions and thermal profiles for each species multiplex reaction can be found in **Appendix A** and **Appendix B**. Primer sequences are listed in Spear *et al.* (2008) and Steele *et al.* (2008). PCR products were visualized on an ABI 3730

sequencer (Applied Biosystems, Foster City, CA) concurrent with LIZ500 size standard. Alleles were scored using the GENEMAPPER 5 software (Applied Biosystems, Foster City, CA).

1-5.3. SALAMANDER SPECIES IDENTIFICATION

We used the program NewHybrids (Anderson and Thompson 2002) to identify pure or hybrid individuals of Coastal or Cope's Giant Salamander. NewHybrids uses a Bayesian framework to calculate posterior probabilities of sampled individuals belonging to six categories of hybrid status: pure Coastal, pure Cope's, F1 hybrid, F2 hybrid, backcross on Coastal, and backcross on Cope's. We selected Jeffrey's prior (a non-informative objective prior) for θ and π. NewHybrids was run with a burn-in of 10,000 followed by 100,000 iterations. We classified individuals into three categories based on the posterior probabilities: 1) Pure Coastal or Cope's, 2) Hybrid, and 3) No call (data were insufficient or ambiguous). A posterior probability of 0.90 or greater determined the classification of an individual to either a Coastal or Cope's Giant Salamander. An individual was classified as a hybrid if the sum of all hybrid classes was 0.90 or higher. Individuals that had insufficient or ambiguous data (posterior probability for pure or hybrid class ranging between 0.48 – 0.75) were classified as no call. However, we recorded the category with the highest posterior probability in the final table.

1-5.4. GENETIC ANALYSES

We first prepared genotypes in the software CONVERT (Glaubitz 2004) for further downstream conversion of genetic data files for the various population genetic analysis software packages we used. We tested each locus and site for violations of Hardy-Weinberg equilibrium and presence of linkage disequilibrium using Genepop on the web (Raymond and Rousset 1995; Rousset 2008). We also tested for proportion of null alleles (a common cause of Hardy-Weinberg violations) using the software FreeNA (Chapuis and Estoup 2007).

We tested for the presence of full siblings using Colony v2 0.6.3 (Wang 2004). This is especially important because a high proportion of our samples were larval individuals that may have a high representation of family groups. In the Baseline Genetic Report (Spear *et al.* 2011) we removed siblings from the dataset for genetic analyses because of concerns that the presence of full siblings can bias results (Goldberg and Waits 2010). However, recent research has indicated that automatically removing siblings may not give a better representation of actual population genetic structure, especially for estimates of genetic diversity (Waples and Anderson 2017). Therefore, we elected to retain all unique individuals while using the proportion and number of full siblings as a potential indicator of treatment effects. We note that this means that some of the pre-harvest measures presented herein may not exactly match tables from the Baseline Genetic Report (Spear *et al.* 2011). For the Colony analyses we assumed polygamy for both sexes, did not include a Bayesian prior (i.e. did not assume a particular distribution in advance), and included the null allele rates for each locus as potential error affecting estimation. We used Cervus v3.0.7 (Kalinowski *et al.* 2007) to estimate the probability that two individuals would have the same genotype by chance (P_{ID}). We estimated P_{ID} for both unrelated individuals and for full siblings.

We estimated several measures of genetic diversity using the software GDA 1.1 (Lewis and Zaykin 2002). Variables included average number of alleles per locus, observed heterozygosity,

and Wright's inbreeding coefficient (F_{IS}). Number of alleles per locus is the clearest measure of genetic diversity at the site level as it simply records the number of alleles present. The average number of alleles can be misleading if variation in sample size exists; more individuals sampled increases the chance for additional alleles. Therefore, we also calculated allelic richness. Allelic richness estimates the number of alleles that would be present if sample sizes were equal across all sites and is based on the lowest sample size available. As our comparison of interest was pre- versus post-harvest, we calculated allelic richness separately for each site incorporating sample size of both periods. Allelic richness was calculated using Fstat (Goudet 2001). Observed heterozygosity is the measure of the proportion of individuals that have two different alleles at a locus. It can be seen as a measure of genetic diversity within individuals at a site. F_{IS} is typically estimated by comparing the difference between the observed heterozygosity and the theoretical levels of heterozygosity expected based on the number of alleles. If mating is random, then we would expect observed heterozygosity to match expected heterozygosity and F_{IS} would be zero. If mating occurs between individuals that are more genetically similar than random, the F_{IS} increases (hence the name of the metric). F_{IS} can also be negative if mating occurs among individuals that are more distantly related than random. F_{IS} ranges from -1 to 1, but values are typically much lower than one. For instance, a F_{IS} of 0.05 can represent the equivalence of mating between first cousins. We also attempted to include effective population size as a metric of genetic diversity. However, the method used to estimate effective population size (Onesamp; Tallmon *et al.* 2008) in the Baseline Genetic Report (Spear *et al.* 2011) is only available as a web interface and was no longer available for the post-harvest analysis. We used another common estimator based on linkage disequilibrium (LDNe; Do *et al.* 2014) and noted both large confidence intervals and little consistency with the pre-harvest Onesamp estimates for the same individuals. Based on this we did not include effective population size in our current analysis.

We tested for significant reductions in effective population size (bottlenecks) using two methods: the heterozygosity excess test (Cornuet and Luikart 1996) and shifted allele distributions (Luikart *et al.* 1998). Spear *et al.* (2011) included a third test in the Baseline Genetic Report based on a metric known as M-ratio (Garza and Williamson 2001). We did not retain the M-ratio for this analysis as Peery *et al.* (2012) demonstrated through simulations that the M-ratio test was susceptible to a high Type I error rate. The first method, heterozygosity excess test, detects bottlenecks through an increase in expected heterozygosity relative to theoretical expectations. This increase in expected heterozygosity is an ephemeral signature after a bottleneck because allelic diversity is lost before heterozygosity begins to decrease. We tested for heterozygosity excess in the software Bottleneck (Piry *et al.* 1999). We assumed a two-phase mutation model with 10% multistep mutations and 12% variance in size of multi-step mutations. This was the recommended value by Piry *et al.* (1999). Peery *et al.* (2012) note that though the exact justification for these values is not clear, heterozygosity excess is relatively robust to the exact parameter values. We assessed significance using a Wilcoxon sign-rank test. The second test, a shifted allele distribution, measures the loss of rare alleles. Microsatellites are typically characterized by a high proportion of rare alleles. Rare alleles are lost first in a bottleneck and therefore we expect that the distribution of allele frequencies should shift toward a greater frequency of more common alleles. The test for shifted allele distribution was also implemented in the Bottleneck software.

Finally, we estimated the amount of genetic connectivity and scale of genetic neighborhoods using the Bayesian population clustering algorithm STRUCTURE (Pritchard *et al.* 2000). While

population clustering is determined by factors at a much broader scale than our treatment sites, understanding the scale of genetic connectivity is useful to understand the degree to which migration might influence site-level genetic results. One of the difficulties of using STRUCTURE and other clustering algorithms is determining the best supported number of clusters (*K*). In the Baseline Genetic Report (Spear *et al.* 2011) we used a hierarchical partitioning of sites using the second order rate of change in model likelihood, a statistic known as Δ*K* (Evanno *et al.* 2005). However, recent research has highlighted problems with the Δ*K* metric, specifically that it tends to select a *K* of 2 and underestimates substructure (Puechmaille 2016; Janes *et al.* 2017). The hierarchical approach of running STRUCTURE at multiple levels helps to address the weakness in the Δ*K* approach, but can complicate interpretation of clustering plots. Puechmaille *et al.* (2016) developed four related metrics that they found to be more informative across a wider range of population scenarios. These metrics calculate the proportion membership of each individual to each of the *K* clusters. The mean or median (depending on exact metric) of cluster membership is calculated across each sampling site, and the number of clusters that have a mean/median membership of a threshold (0.5 or greater) in at least one population is recorded. Therefore, the method only considers clusters that can be assigned to at least one sampling site. For each method (the mean or median), the metric identifies two values of *K*: one that is the median value among all replicates, and one that is the maximum value among all replicates. The four metrics are abbreviated MedMedK (median value of *K* from the median membership), MaxMedK (maximum value of *K* from the median membership), MedMeanK (median value of *K* from the mean membership), and MaxMeanK (maximum value of *K* from the mean membership). Puechmaille *et al.* (2016) recommends calculating each of the four metrics at thresholds ranging from 0.5-0.8 to evaluate the most consistent number of clusters. We used the median value of *K* from the 16 different possibilities (four types of metrics multiplied by four different thresholds). We also ran STRUCTURE for the pre-treatment genetic data using the same metrics and method for choosing *K*. This allowed us to identify any change in genetic admixture within a site that may have become more or less differentiated post-harvest. For each of the replicate STRUCTURE runs, we used the admixture model with a burn-in of 100,000 iterations and 100,000 iterations after burn-in. We ran 20 replicates for each value of number of clusters and we considered *K* and up to be the total number of sites for which sampling occurred for each species. We did not include a location prior and we assumed correlated allele frequencies. We averaged runs and calculated metrics using StructureSelector (Li and Liu 2018).

1-5.5. STATISTICAL ANALYSES

We used a linear mixed effects model to assess the effect of treatment on change in measures of genetic diversity. Our fixed effects were time (pre- or post-harvest), treatment, and the treatment × time interaction. Our random effects were block and site. We evaluated whether there was a significant treatment × time effect using a Wald-type test on linear contrasts of fixed effects. Consistent with McIntyre *et al.* (2018b, Chapter 15 - Stream-associated Amphibians), we used an alpha of 0.10. We believe this approach is appropriate for applying a reasonable level of confidence to significant results, in light of the lower number of replicates due to the constraints of the field BACI design and time separating sampling periods. If a treatment × time effect less than 0.1 existed, we examined pairwise contrasts among each treatment combination. We used the nlme package (Pinheiro *et al.* 2015) in R v.3.2.3 (R Core Team 2015). We assessed models for lack-of-fit using residual diagnostic plots. In sum, we performed 18 significance tests for

mixed effect models across all species and genetic diversity metrics, 18 significance tests for pairwise contrasts, and 37 significance tests for the post-harvest heterozygosity excess tests.

1-6. **RESULTS**

Of the Coastal Tailed Frogs included in the analysis for the post-harvest period (N = 354), 31% were larvae, 21% were in the process of metamorphosing, and 48% were post-metamorphic. Of the post-metamorphic individuals, less than 1% were greater than 42 mm SVL (**Figure 3**). Of the giant salamanders included in the analysis for the post-harvest period (N =1,073), greater than 99% were ≤100 mm SVL, less than 1% were >100 mm SVL, and less than 1% were post-metamorphs (**Figure 4**).We detected greater numbers of both taxa in the pre-harvest period: Coastal Tailed Frog N = 573, giant salamanders N = 1,504 (Spear *et al.* 2011).

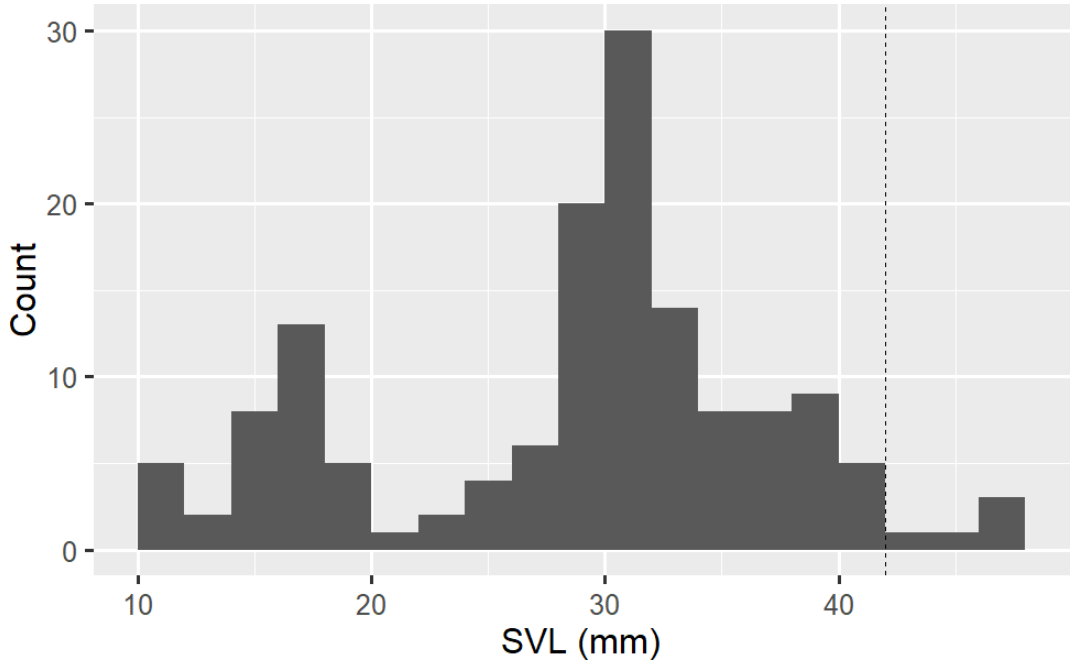

Figure 3. Size distribution of post-metamorphic Coastal Tailed Frog snout-vent lengths (SVL) for animals sampled in the post-harvest period. The dotted line at 42 mm SVL is the minimum size for which we estimated post-metamorphic tailed frogs to be younger than eight years, and thus also born after treatment implementation

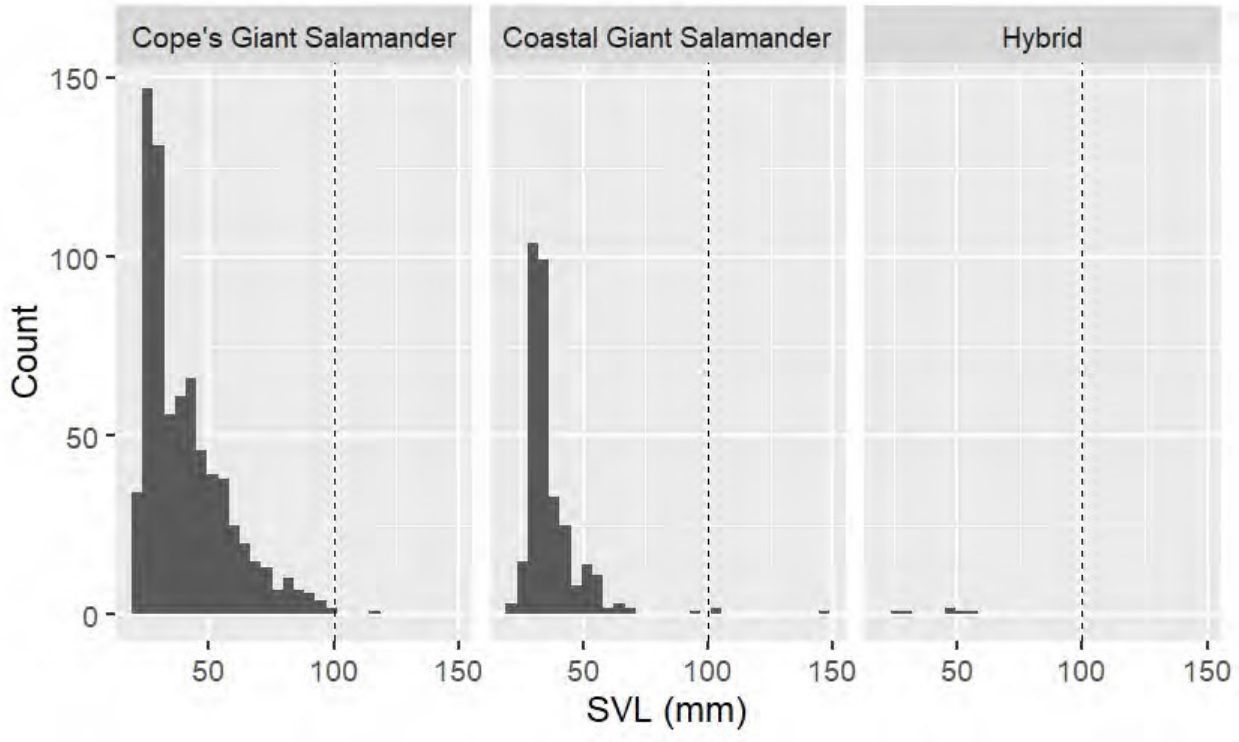

Figure 4. Size distributions of larval, neotenic and post-metamorphic giant salamander snout-vent lengths (SVL) for Cope's (N = 737), Coastal (N = 329) and hybrid (N = 7) giant salamanders sampled in the post-harvest period. The dotted line at 100 mm SVL is the minimum size for which we estimated giant salamanders to be younger than eight years, and thus also born after treatment implementation.

1-6.1. GENETIC DIVERSITY

1-6.1.1. Sample size and full sibling groups

1-6.1.1.a. Coastal Tailed Frog

A total of 353 unique tailed frog individuals successfully amplified at >70% of the loci (**Table 3**). Consistent with the Baseline Genetic Report (Spear *et al.* 2011), four loci (A14, A2, A29, A3) had evidence of null alleles and violations of Hardy-Weinberg equilibrium at multiple sites. Therefore, as with the Baseline Genetic Report (Spear *et al.* 2011), we eliminated these loci from further analysis. None of the nine remaining loci had evidence of null alleles or consistent Hardy-Weinberg violations. Sample number ranged from 3-56 individuals (mean = 24.86, SE = 4.22; **Appendix C**). Even though fewer individuals were sampled post-harvest across treatments (**Table 3**; **Figure 5**), there was no difference in the magnitude of change between treatments (F = 1.47; P = 0.28; **Table 4** and **Table 5**). The P_{ID} for unrelated tailed frog individuals was 5×10^{-19} and the full sibling P_{ID} was 2×10^{-5}. Therefore, it is highly unlikely we would infer unrelated individuals as within a family group due to chance. The average number of

unrelated individuals (i.e. unique family groups) was 22.64 (SE = 4.08) in the post-harvest period (**Appendix C**). This average is lower than that seen in the pre-harvest period (33.5), but the difference can be entirely explained by a change in sample size (**Figure 5** and **Figure 6**). We detected no significant treatment effect on the change in number of unique full-sibling family groups (F = 1.40, P = 0.30; **Table 6**; **Figure 6**). The pattern of between-treatment change in family number was also very similar to overall sample size (**Table 7**).

Table 3. Sample size for each species from post-harvest genetic sampling. Samples that did not amplify at >70% of the loci or had identical genotypes were not included in the total. Hybrid represents crosses between the two giant salamander species. Site ID is the identification number referred to throughout the genetic clustering figures.

Block	Site	Site ID	Coastal Tailed Frog	Giant Salamanders		
				Cope's	Coastal	Hybrid
OLYM	REF	1	56	90	0	0
	100%	2	5	52	0	0
	FP	3	7	26	0	0
	0%	4	33	41	0	0
Subtotal			**101**	**209**	**0**	**0**
WIL1	REF	5	27	14	30	1
	100%	6	3	15	23	0
	FP	7	13	26	37	1
	0%	8	26	58	0	0
Subtotal			**69**	**113**	**90**	**2**
WIL2	REF1	9	49	92	30	1
	REF2	10	18	39	2	1
	100%	11	28	55	0	0
	0%	12	13	74	23	0
Subtotal			**108**	**260**	**55**	**2**
WIL3	REF	13	33	28	22	1
	100%	14	7	6	34	1
Subtotal			**40**	**34**	**56**	**2**
CASC	REF	15	35	48	30	1
	FP	16	0	59	65	0
	0%	17	0	14	33	0
Subtotal			**35**	**121**	**128**	**1**
Grand total			**353**	**737**	**329**	**7**

Table 4. Within-treatment change (Estimate) and 95% CI in number of individuals sampled between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error. The p-value for this model was 0.28, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	-0.67	6.30	-16.87	15.53
100%	-24.67	13.69	-83.58	34.25
FP	-29.00	12.00	-181.52	123.52
0%	-13.67	16.15	-83.15	55.82

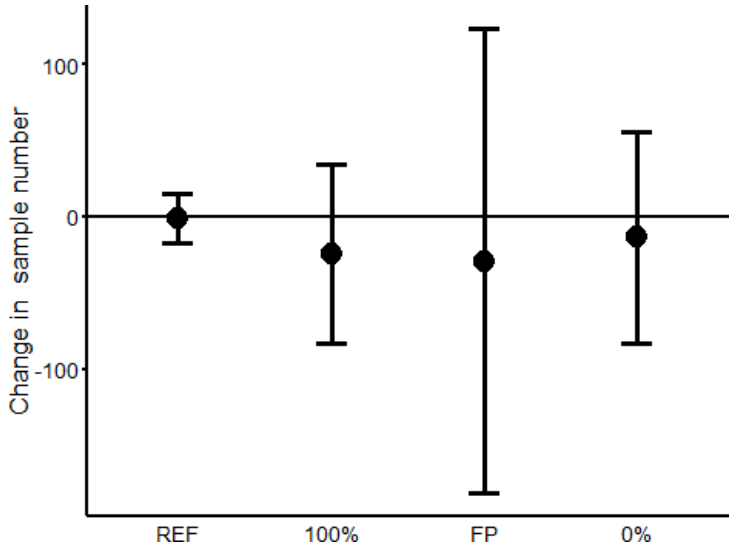

Figure 5. Within-treatment change and 95% CI in number of individuals sampled between pre- and post-harvest sampling for Coastal Tailed Frogs.

Table 5. Pairwise contrasts of the between-treatment change (Estimate) in the number of individuals sampled between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	-24.00	14.43	-52.28	4.28
FP vs. REF	-28.30	16.67	-60.97	4.37
0% vs. REF	-13.00	14.43	-41.28	15.28
100% vs FP	4.33	18.64	-32.20	40.86
100% vs. 0%	-11.00	16.67	-43.67	21.67
FP vs. 0%	-15.00	18.64	-51.53	21.53

Table 6. Within-treatment change (Estimate) and 95% CI in number of unique full-sibling families sampled between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error. The p-value for this model was 0.30, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.33	5.57	-13.99	14.66
100%	-22.33	14.31	-83.91	39.24
FP	-25.00	13.00	-190.23	140.23
0%	-12.33	14.10	-73.00	48.33

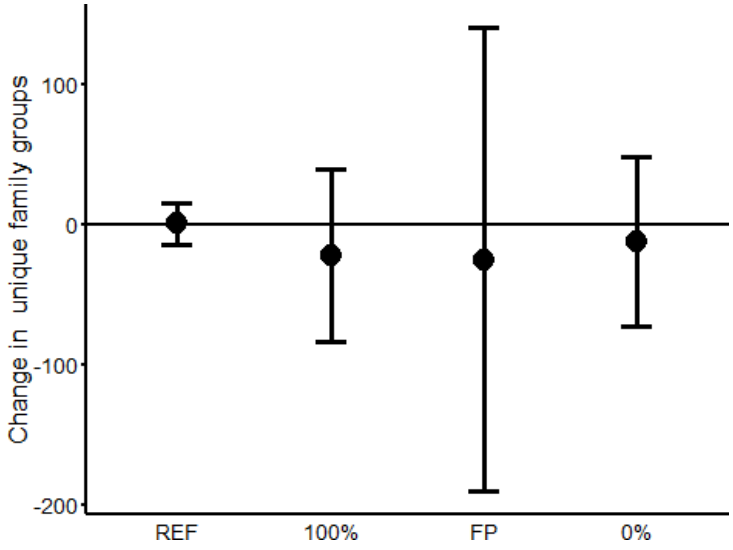

Figure 6. Within-treatment change and 95% CI in number of unique full-sibling families sampled between pre- and post-harvest sampling for Coastal Tailed Frogs.

Table 7. Pairwise contrasts of the between-treatment change (Estimate) in the number of unique full-sibling families sampled between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	-22.67	13.58	-49.29	3.95
FP vs. REF	-25.33	15.69	-56.08	5.42
0% vs. REF	-12.67	13.58	-39.29	13.95
100% vs FP	2.67	17.54	-31.71	37.05
100% vs. 0%	-10.00	15.69	-40.75	20.75
FP vs. 0%	-12.67	17.54	-47.05	21.71

1-6.1.1.b. Giant salamander species identification

We were able to obtain unique genotypes from a total of 1,073 giant salamander individuals. We removed two of the eleven markers (D05 and D24) for the purposes of species identification because they were not reliably scored. The presence/absence marker (D15) was also removed since it does not amplify in Coastal Giant Salamander. We therefore used a total of eight loci for species identification. We identified a total of 737 Cope's Giant Salamanders, 329 Coastal Giant Salamanders, and 7 hybrids (**Table 3**). Hybrids made up less than 1% of all samples. There was

no significant difference (F = 0.78; P = 0.54) in the proportion of Cope's giant salamanders per site between the pre-harvest and post-harvest period (

Table 8 and **Table 9**; **Figure 7** and **Figure 8**).

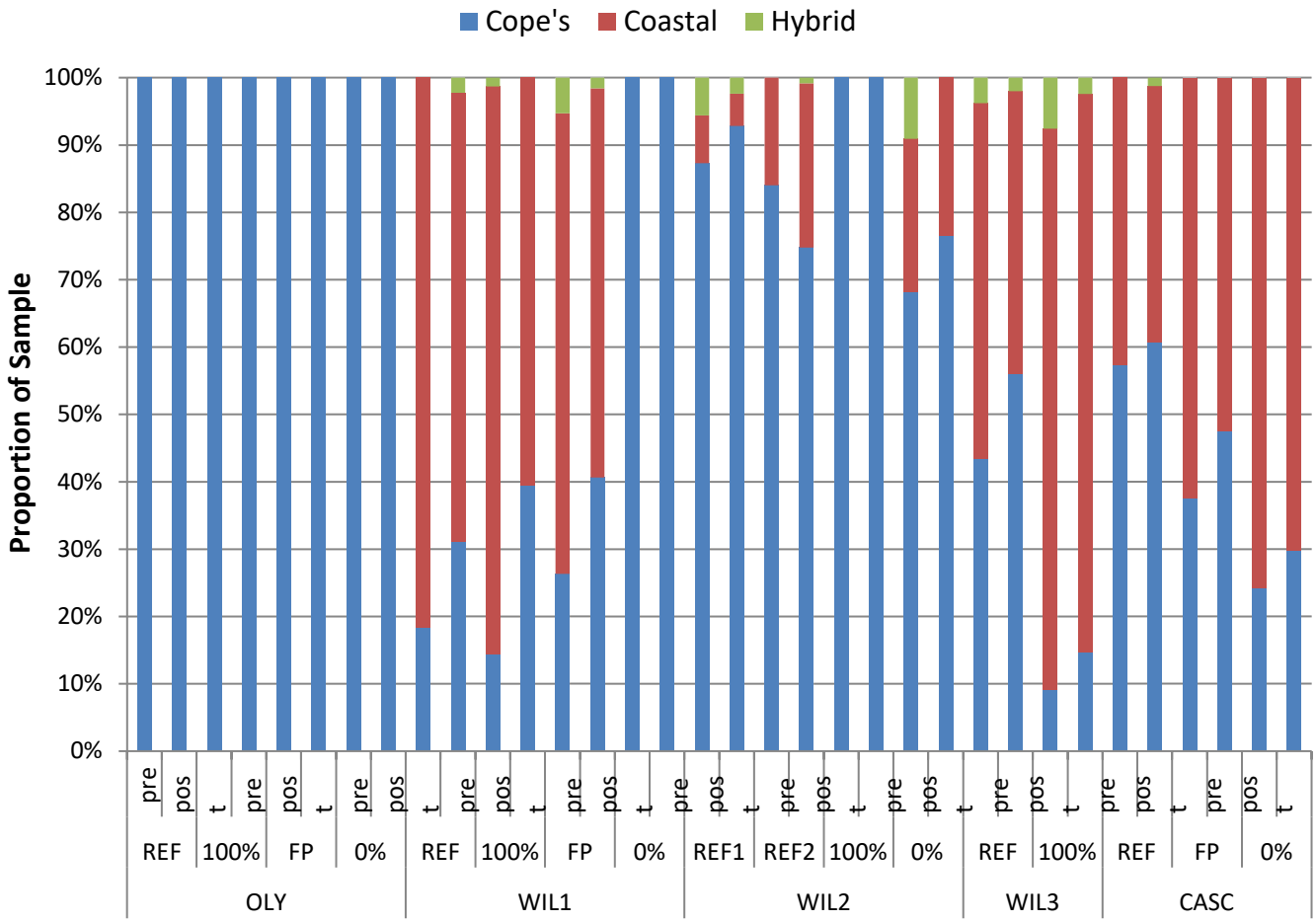

Figure 7. Proportion of Cope's, Coastal, and hybrid Giant Salamanders individuals sampled in the pre-harvest (pre) and post-harvest (post) periods. Each column represents the total sample collected in a site within each period.

Table 8. Within-treatment change (Estimate) and 95% CI in proportion of Cope's Giant Salamander sampled between pre- and post-harvest sampling for all giant salamanders. SE represents standard error. The p-value for this model was 0.54, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.03	0.06	-0.13	0.20
100%	0.16	0.09	-1.01	1.34
FP	0.13	0.06	-0.63	0.90
0%	0.07	0.00	0.01	0.13

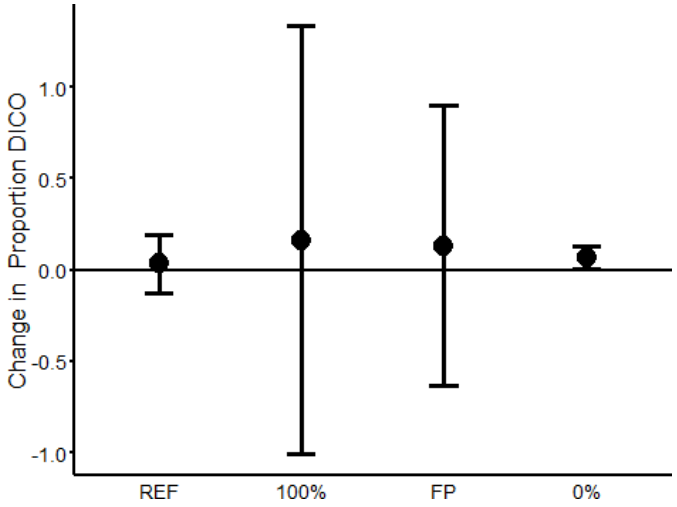

Figure 8. Within-treatment change and 95% CI in proportion of Cope's Giant Salamander (DICO) sampled between pre- and post-harvest sampling relative to total giant salamanders (sum of Cope's, Coastal, and hybrid).

Table 9. Pairwise contrasts of the between-treatment change (Estimate) in the proportion of overall giant salamanders that were Cope's Giant Salamanders between pre- and post-harvest sampling. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	0.13	0.1	-0.07	0.33
FP vs. REF	0.10	0.1	-0.10	0.30
0% vs. REF	0.04	0.1	-0.16	0.24
0% vs. FP	0.03	0.12	-0.21	0.27
0% vs. 100%	0.09	0.12	-0.15	0.33
FP vs. 100%	0.06	0.12	-0.18	0.30

1-6.1.1.b.(i). Cope's Giant Salamander

We did not see any consistent evidence of Hardy-Weinberg violations by loci or of null alleles, thus, all eleven loci were retained for Cope's Giant Salamander. Sample size per site ranged from 6-92 (mean=43.35, SE = 6.35; **Appendix D**). There was no treatment effect on change in overall sample size (F = 0.63; P = 0.61) and little change in sample size from the baseline genetic survey. Only the reference sites had a confidence interval that did not overlap zero (**Table 10** and **Table 11**; **Figure 9**). The P_{ID} for unrelated Cope's Giant Salamander individuals was 4×10^{-19} and the full sibling P_{ID} was 4×10^{-6}. The proportion of unique family groups was high with an average of 85% (**Appendix D**) While there was no difference in change in proportion by treatment (F = 0.66; P = 0.59), all treatments had an increased proportion of unique families relative to the baseline surveys (**Table 12** and **Table 13**; **Figure 10**).

Table 10. Within-treatment change (Estimate) and 95% CI in number of individuals sampled between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error. The p-value for this model was 0.61, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	11.50	5.66	-3.04	26.04
100%	2.50	6.33	-17.65	22.65
FP	-2.67	4.06	-20.12	14.78
0%	-2.25	15.35	-51.09	46.59

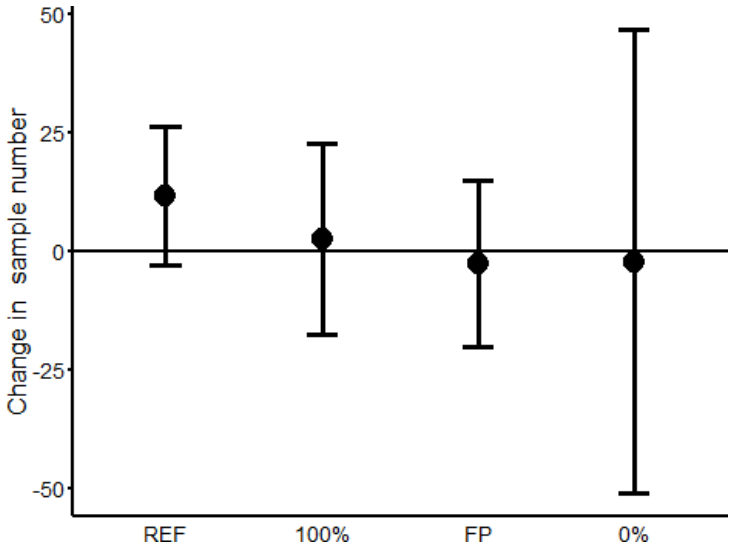

Figure 9. Within-treatment change and 95% CI in number of individuals sampled between pre- and post-harvest sampling for Cope's Giant Salamander.

Table 11. Pairwise contrasts of the between-treatment change (Estimate) in the number of individuals sampled between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	-9.00	11.83	-32.19	14.19
FP vs. REF	-14.17	12.96	-39.57	11.23
0% vs. REF	-13.75	11.83	-36.94	9.44
100% vs FP	5.17	14.00	-22.27	32.61
100% vs. 0%	4.75	12.96	-20.65	30.15
FP vs. 0%	-0.42	14.00	-27.86	27.02

Table 12. Within-treatment change (Estimate) and 95% CI for the proportion of unique full-sibling families relative to total sample size between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error. The p-value for this model was 0.59, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.02	0.06	-0.13	0.29
100%	0.11	0.05	-0.06	0.47
FP	0.11	0.08	-0.25	0.44
0%	0.14	0.10	-0.16	0.52

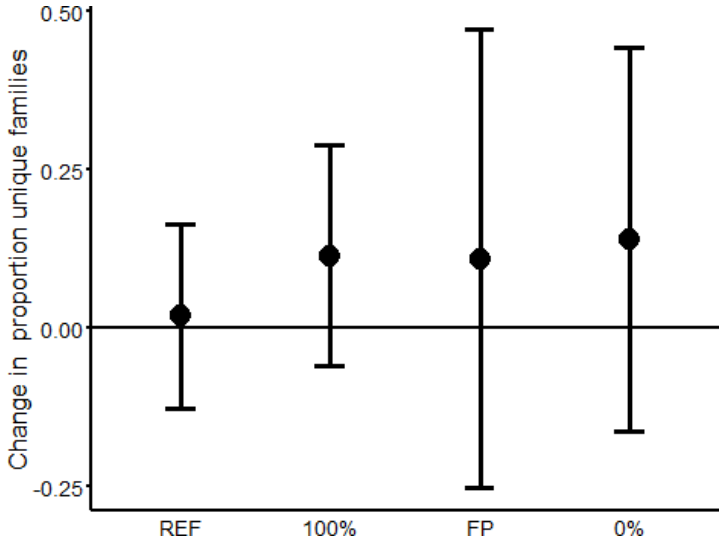

Figure 10. Within-treatment change and 95% CI in proportion of unique full-sibling families relative to total sample size between pre- and post-harvest sampling for Cope's Giant Salamander.

Table 13. Pairwise contrasts of the between-treatment change (Estimate) in proportion of unique full-sibling families relative to total sample size between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	0.09	0.10	-0.11	0.29
FP vs. REF	0.09	0.10	-0.11	0.29
0% vs. REF	0.12	0.10	-0.08	0.32
100% vs FP	0.00	0.11	-0.22	0.22
100% vs. 0%	-0.03	0.10	-0.23	0.17
FP vs. 0%	-0.03	0.11	-0.25	0.19

1-6.1.1.b.(ii). Coastal Giant Salamander

Of the 11 total giant salamander loci only five loci (D04, D13, D18, D23, D24) did not have high instances of missing data or evidence of null alleles in Coastal Giant Salamander samples. Therefore, we only retained these five loci for further analysis. Sample size ranged from 2-65 (mean of 29.91, SE = 4.52; **Appendix E**). We did not test for differences by treatment in sample number due to violations of normality (and inability of transformations to correct this) but we did model the difference in both number of unique families and proportion of unique families to gain insight into whether there were differences in overall sample size. The P_{ID} for unrelated Coastal Giant Salamander individuals was 6×10^{-5} and the full sibling P_{ID} was 0.03. Due to the fewer number of loci, there is a greater chance of identical genotypes by chance in this species compared to Coastal Tailed Frog or Cope's Giant Salamander. However, there is still only a 6 in 100,000 chance that unrelated individuals would have the same genotype, and therefore we are confident our estimated family groups are not likely to consist of unrelated individuals. The number of unique families per site averaged 23.55 (SE = 3.83; **Appendix E**). There was a significant treatment effect in the change in number of unique full sibling family groups (F = 5.15; P = 0.03). This effect was driven by a decrease in the number of unique family groups in the 100% treatment (**Table 14**; **Figure 11**) and all pairwise comparisons with the 100% treatment were significantly different (**Table 15**). There were no treatment effects with respect to change in proportion of full sibling family groups relative to total sample size (F = 1.14; P = 0.40; **Table 16** and **Table 17**; **Figure 12**). Therefore, the significant decline in full-sibling family groups in the 100% treatment is due to a decrease in overall number of individuals.

Table 14. Within-treatment change (Estimate) and 95% CI in number of unique full-sibling families sampled between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error. The p-value for this model was 0.03, which indicated a significant difference based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	5.40	3.80	-5.16	15.96
100%	-24.00	10.00	-151.10	103.10
FP	4.50	2.50	-27.28	36.28
0%	-1.00	7.00	-89.97	87.97

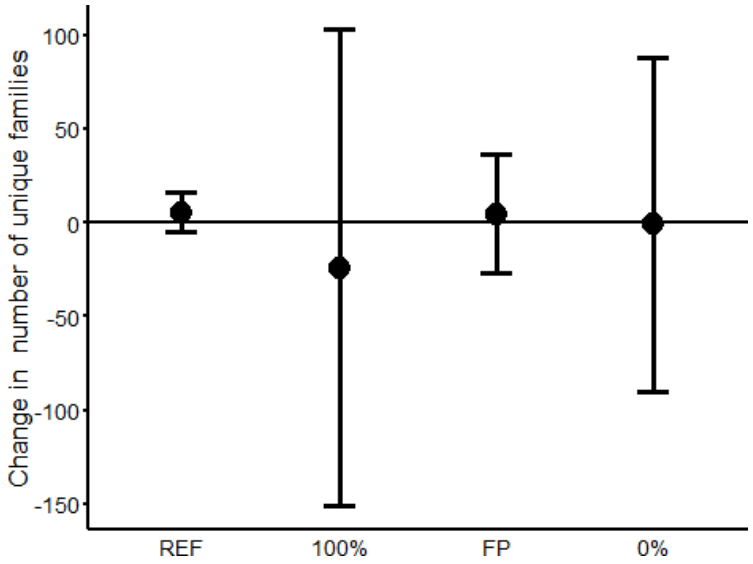

Figure 11. Within-treatment change and 95% CI in number of unique full-sibling families sampled between pre- and post-harvest sampling for Coastal Giant Salamander.

Table 15. Pairwise contrasts of the between-treatment change (Estimate) in the number of unique full-sibling families sampled between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first. We used an alpha of 0.10 to assess significance based on the p-value.

Contrast	Estimate	SE	P-value	95% CI	
				Lower	Upper
100% vs. REF	-29.4	7.74	0.01	-44.57	-14.23
FP vs. REF	-0.90	7.74	0.91	-16.07	14.27
0% vs. REF	-6.40	7.74	0.44	-21.57	8.77
100% vs FP	-28.50	9.26	0.02	-46.65	-10.35
100% vs. 0%	-23.00	9.26	0.04	-41.15	-4.85
FP vs. 0%	5.50	9.26	0.57	-12.65	23.65

Table 16. Within-treatment change (Estimate) and 95% CI for the proportion of unique full-sibling families relative to total sample size between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error. The p-value for this model was 0.40, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.17	0.09	-0.07	0.42
100%	0.34	0.10	-0.88	1.56
FP	0.40	0.07	-0.53	1.33
0%	0.18	0.11	-1.20	1.56

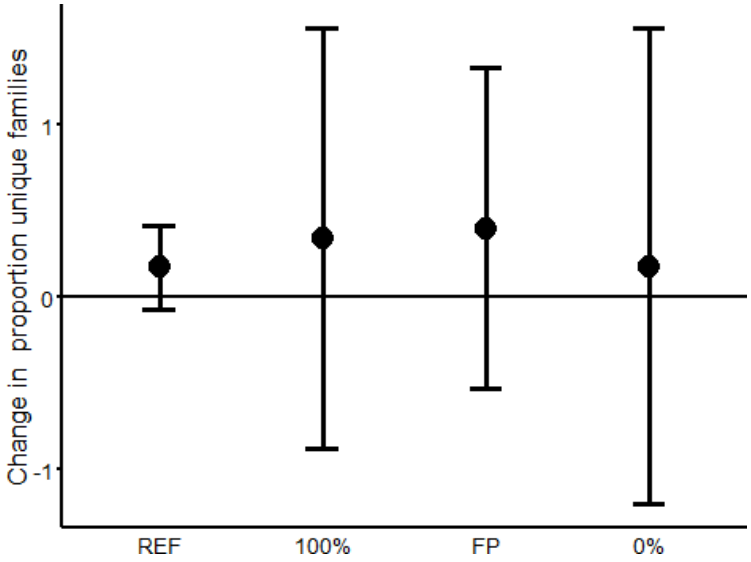

Figure 12. Within-treatment change and 95% CI in proportion of unique full-sibling families relative to total sample size between pre- and post-harvest sampling for Coastal Giant Salamander.

Table 17. Pairwise contrasts of the between-treatment change (Estimate) in proportion of unique full-sibling families relative to total sample size between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	0.17	0.14	-0.10	0.44
FP vs. REF	0.23	0.14	-0.04	0.50
0% vs. REF	0.00	0.14	-0.27	0.27
100% vs FP	-0.06	0.17	-0.39	0.27
100% vs. 0%	0.16	0.17	-0.17	0.49
FP vs. 0%	0.22	0.17	-0.11	0.55

1-6.1.2. Allelic Diversity

1-6.1.2.a. Coastal Tailed Frog

The average number of alleles per locus ranged from 4.7 to 19.0 with an average of 13.6 (SE = 1.12; **Appendix C**). We detected no treatment effect on differences in average number of alleles per locus for Coastal Tailed Frogs (F = 1.65; P = 0.24; **Table 18** and **Table 19**; **Figure 13**). The pattern in allele number was very similar to the pattern observed for sample size demonstrating that total allelic diversity largely followed sample size in this system. We did not find a

significant treatment effect on change in allelic richness (F = 1.27; P = 0.34). However, there was a consistent trend among buffer treatments with respect to mean change in allelic richness (**Table 20** and **Table 21**; **Figure 14**). There was a mean increase in richness from the reference in the 100% treatment, with corresponding lower means as the forested buffer decreased, and very little mean difference between the reference and 0% treatment.

Table 18. Within-treatment change (Estimate) and 95% CI in average number of alleles per locus between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error. The p-value for this model was 0.24, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	-0.35	0.76	-2.30	1.59
100%	-6.74	4.73	-27.09	13.60
FP	-5.83	2.39	-36.20	24.53
0%	-2.30	3.34	-16.66	12.07

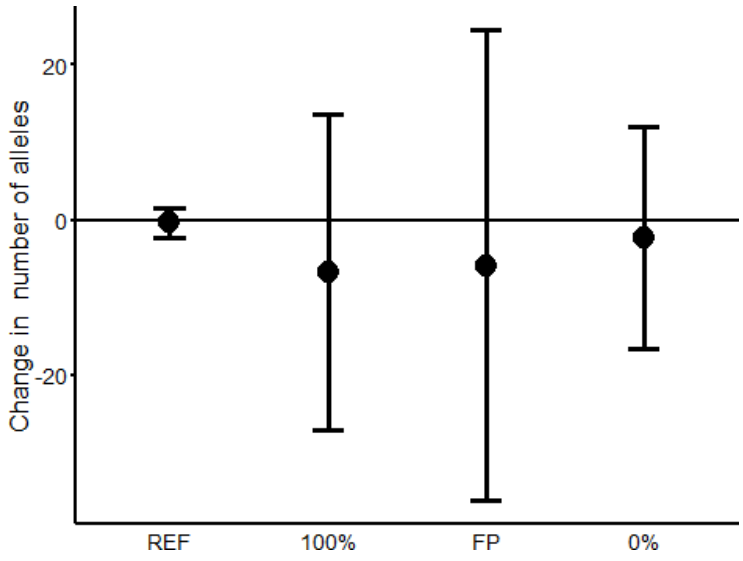

Figure 13. Within-treatment change and 95% CI in average number of alleles per locus between pre- and post-harvest sampling for Coastal Tailed Frogs.

Table 19. Pairwise contrasts of the between-treatment change (Estimate) in the average number of alleles per locus between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	-6.39	3.20	-12.66	-0.12
FP vs. REF	-5.48	3.70	-12.73	1.77
0% vs. REF	-1.94	3.20	-8.21	4.33
100% vs FP	-0.91	4.13	-9.00	7.18
100% vs. 0%	-4.44	3.70	-11.69	2.81
FP vs. 0%	-3.54	4.13	-11.63	4.55

Table 20. Within-treatment change (Estimate) and 95% CI in allelic richness between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error. The p-value for this model was 0.34, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	-0.14	0.18	-0.60	0.31
100%	0.89	0.72	-2.22	4.01
FP	0.25	0.64	-7.92	8.41
0%	-0.33	0.66	-3.15	2.49

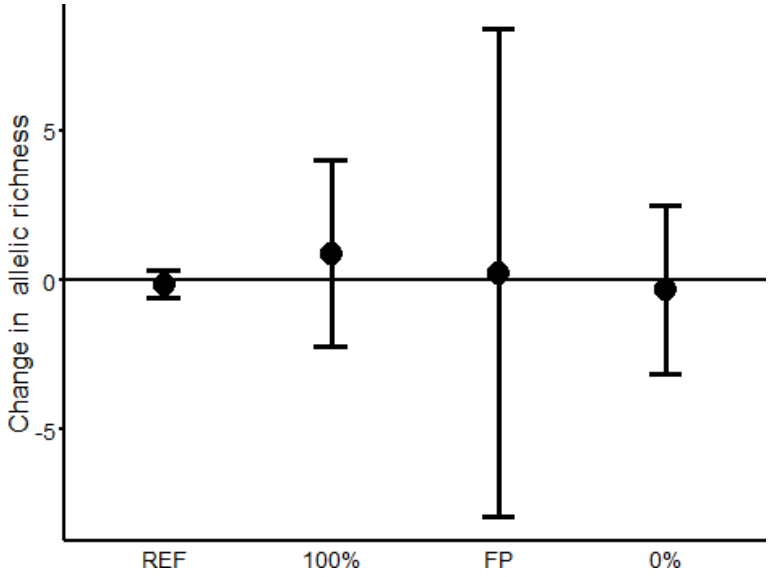

Figure 14. Within-treatment change and 95% CI in allelic richness between pre- and post-harvest sampling for Coastal Tailed Frogs.

Table 21. Pairwise contrasts of the between-treatment change (Estimate) in allelic richness between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	1.04	0.61	-0.16	2.24
FP vs. REF	0.39	0.71	-1.00	1.78
0% vs. REF	-0.19	0.61	-1.39	1.01
100% vs FP	0.65	0.79	-0.99	2.20
100% vs. 0%	1.23	0.71	-0.16	2.62
FP vs. 0%	0.58	0.79	-0.97	2.13

1-6.1.2.b. Cope's Giant Salamander

Average allelic number per loci ranged from 4.0 to 15.0 with a mean of 9.4 alleles (SE = 0.72; **Appendix D**). There was no evidence of a treatment effect on the change of either average number of alleles per locus or allelic richness for Cope's Giant Salamander (allele number: F = 0.22; P = 0.88, allelic richness: F = 0.30; P = 0.82). The allelic diversity was very stable across all treatments compared to the baseline condition with the average change being less than one (**Tables 22-25**; **Figure 15** and **Figure 16**).

Table 22. Within-treatment change (Estimate) and 95% CI in average number of alleles per locus between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error. The p-value for this model was 0.88, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.20	0.13	-0.12	0.52
100%	-0.18	0.54	-1.89	1.53
FP	-0.03	0.51	-2.22	2.16
0%	-0.07	0.44	-1.46	1.32

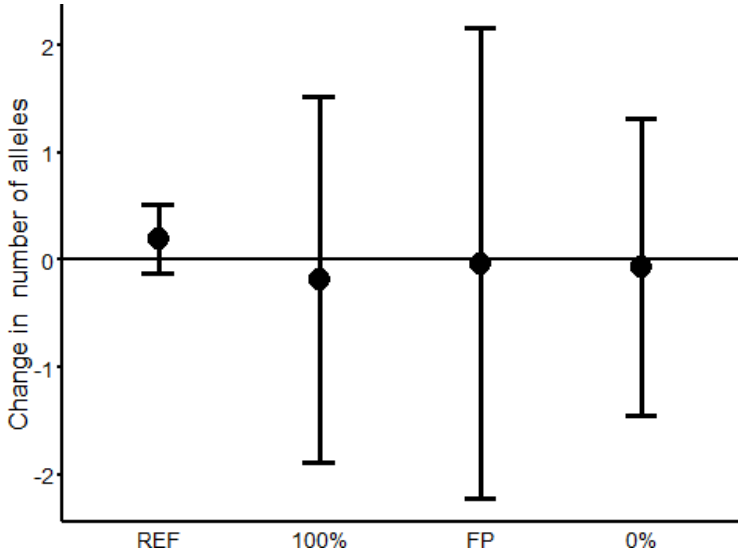

Figure 15. Within-treatment change and 95% CI in average number of alleles per locus between pre- and post-harvest sampling for Cope's Giant Salamander.

Table 23. Pairwise contrasts of the between-treatment change (Estimate) in the average number of alleles per locus between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	-0.38	0.50	-1.36	0.60
FP vs. REF	-0.23	0.55	-1.31	0.85
0% vs. REF	-0.27	0.50	-1.25	0.71
0% vs. FP	-0.15	0.59	-1.31	1.01
0% vs. 100%	-0.11	0.55	-1.19	0.97
FP vs. 100%	0.04	0.59	-1.12	1.20

Table 24. Within-treatment change (Estimate) and 95% CI in allelic richness between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error. The p-value for this model was 0.82, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	-0.18	0.15	-0.57	0.21
100%	0.06	0.23	-0.68	0.81
FP	-0.07	0.34	-1.53	1.39
0%	-0.06	0.08	-0.31	0.18

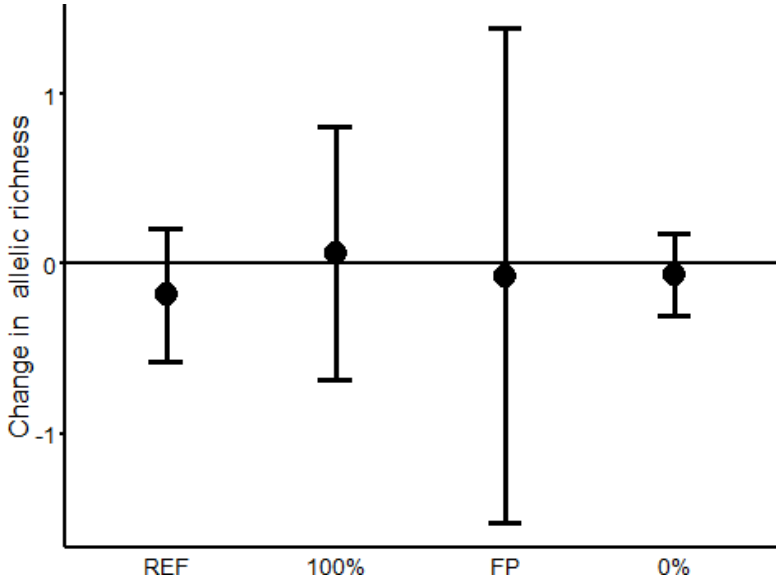

Figure 16. Within-treatment change and 95% CI in allelic richness between pre- and post-harvest sampling for Cope's Giant Salamander.

Table 25. Pairwise contrasts of the between-treatment change (Estimate) in allelic richness between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	0.25	0.26	-0.26	0.76
FP vs. REF	0.11	0.28	-0.44	0.66
0% vs. REF	0.12	0.26	-0.39	0.63
100% vs FP	0.13	0.31	-0.48	0.74
100% vs. 0%	0.13	0.28	-0.42	0.68
FP vs. 0%	-0.01	0.31	-0.62	0.60

1-6.1.2.c. Coastal Giant Salamander

Average alleles per locus in Coastal Giant Salamander sites ranged from 2.2 to 7.8 with an average of 5.6 (SE = 0.46; **Appendix E**). We did not detect any treatment effect on the number of alleles per locus (F = 2.29; P = 0.17) and differences among periods were generally small with only the 100% treatment having a mean change greater than one (**Table 26** and **Table 27**; **Figure 17**). We did not test for differences in allelic richness due to violations of normality assumptions.

Table 26. Within-treatment change (Estimate) and 95% CI in average number of alleles per locus between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error. The p-value for this model was 0.17, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.72	0.79	-1.48	2.92
100%	-3.20	1.20	-18.45	12.05
FP	-0.90	0.30	-4.71	2.91
0%	-0.70	1.90	-24.85	23.45

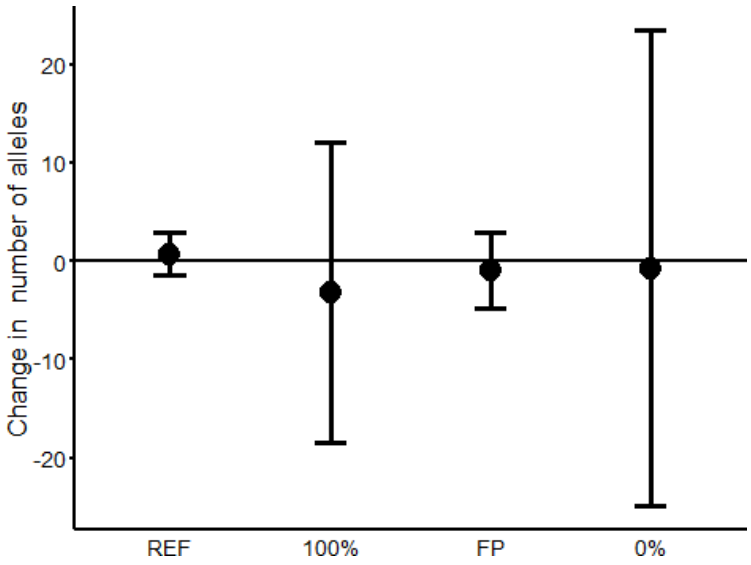

Figure 17. Within-treatment change and 95% CI in average number of alleles per locus between pre- and post-harvest sampling for Coastal Giant Salamander.

Table 27. Pairwise contrasts of the between-treatment change (Estimate) in the average number of alleles per locus between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	-3.92	1.51	-6.88	-0.96
FP vs. REF	-1.62	1.51	-4.58	1.34
0% vs. REF	-1.42	1.51	-4.38	1.54
100% vs FP	-2.30	1.81	-5.85	1.25
100% vs. 0%	-2.50	1.81	-6.05	1.05
FP vs. 0%	-0.20	1.81	-3.75	3.35

1-6.1.3. Observed Heterozygosity

1-6.1.3.a. Coastal Tailed Frog

Observed heterozygosity was high, ranging from 0.83 to 0.94 within sites with an overall average of 0.88 (SE = 0.01; **Appendix C**). There was no treatment effect on heterozygosity (F = 2.12; P = 0.16), and the overall magnitude of change was small relative to overall heterozygosity (**Table 28** and **Table 29**; **Figure 18**).

Table 28. Within-treatment change (Estimate) and 95% CI in observed heterozygosity between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error. The p-value for this model was 0.16, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.02	0.01	-0.01	0.06
100%	-0.03	0.02	-0.10	0.04
FP	-0.01	0.04	-0.50	0.48
0%	0.02	0.01	-0.04	0.08

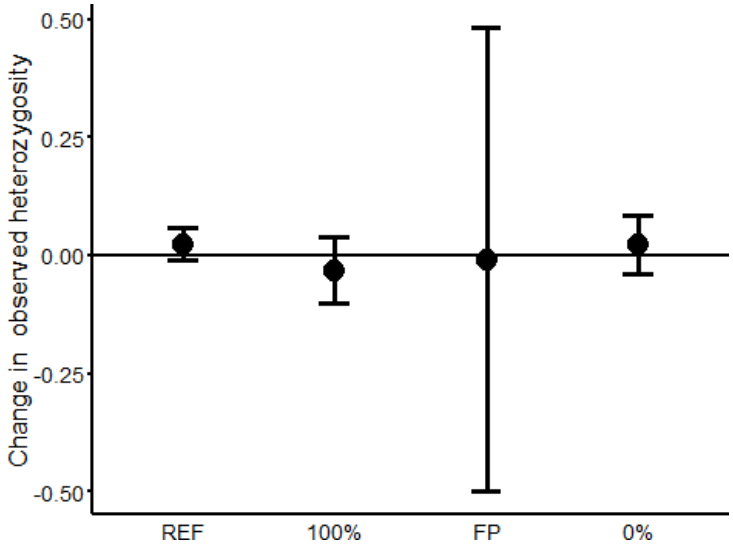

Figure 18. Within-treatment change and 95% CI in observed heterozygosity between pre- and post-harvest sampling for Coastal Tailed Frogs.

Table 29. Pairwise contrasts of the between-treatment change (Estimate) in observed heterozygosity between pre- and post-harvest sampling for Coastal Tailed Frogs. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	-0.05	0.02	-0.09	-0.01
FP vs. REF	-0.03	0.03	-0.09	0.03
0% vs. REF	0.00	0.02	-0.04	0.04
100% vs FP	-0.02	0.03	-0.08	0.04
100% vs. 0%	-0.05	0.03	-0.11	0.01
FP vs. 0%	-0.03	0.03	-0.09	0.03

1-6.1.3.b. Cope's Giant Salamander

Observed heterozygosity in Cope's Giant Salamander sites ranged from 0.45 to 0.84 with an average of 0.72 (SE = 0.03; **Appendix D**). We logit-transformed observed heterozygosity values for analysis due to violations of normality assumptions. There was no treatment effect on the change in heterozygosity (F = 1.31; P = 0.31). The mean observed heterozygosity in all treatments was higher than baseline averages (**Table 30** and **Table 31**; **Figure 19**).

Table 30. Within-treatment change (Estimate) and 95% CI in logit-transformed observed heterozygosity between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error. The p-value for this model was 0.31, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.12	0.05	-0.02	0.25
100%	0.16	0.04	0.04	0.27
FP	0.02	0.04	-0.16	0.20
0%	0.06	0.05	-0.09	0.21

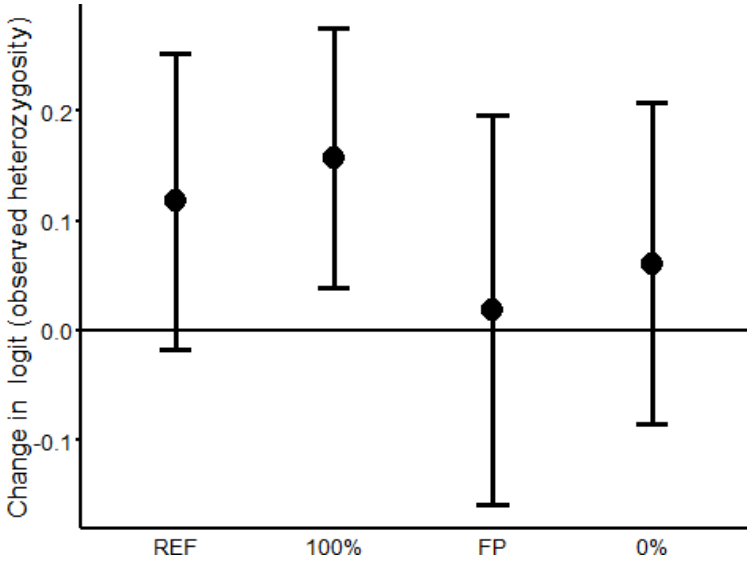

Figure 19. Within-treatment change and 95% CI in logit-transformed observed heterozygosity between pre- and post-harvest sampling for Cope's Giant Salamander.

Table 31. Pairwise contrasts of the between-treatment change (Estimate) in logit-transformed observed heterozygosity between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	0.09	0.15	-0.20	0.38
FP vs. REF	-0.23	0.17	-0.56	0.10
0% vs. REF	-0.13	0.10	-0.33	0.07
100% vs FP	0.32	0.18	-0.03	0.67
100% vs. 0%	0.22	0.17	-0.11	0.55
FP vs. 0%	-0.10	0.18	-0.45	0.25

1-6.1.3.c. Coastal Giant Salamander

Observed heterozygosity ranged from 0.44 to 0.67, with an average of 0.53 (SE = 0.02; **Appendix E**). There was no effect of treatment on change in heterozygosity (F = 0.17; P = 0.92). Overall, there was a small increase in heterozygosity across all treatments relative to the baseline surveys and (**Table 32** and **Table 33**; **Figure 20**).

Table 32. Within-treatment change (Estimate) and 95% CI in observed heterozygosity between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error. The p-value for this model was 0.92, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.05	0.05	-0.10	0.20
100%	0.08	0.02	-0.22	0.37
FP	0.02	0.04	-0.52	0.55
0%	0.02	0.01	-0.05	0.09

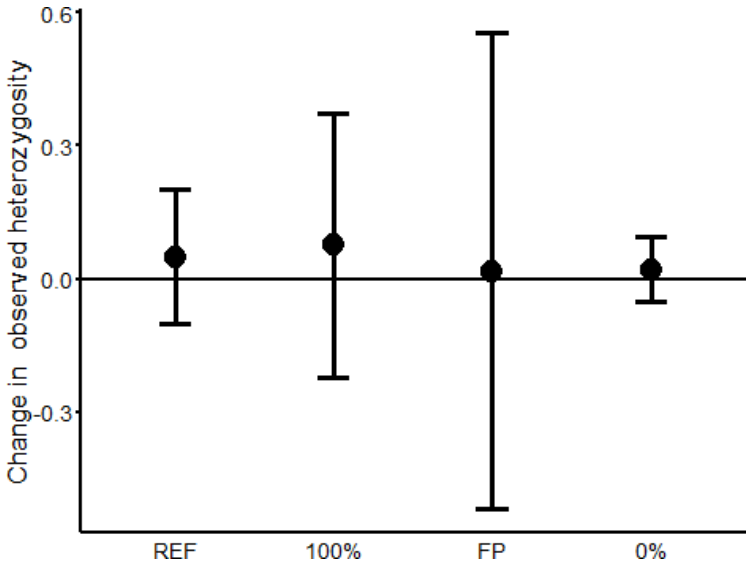

Figure 20. Within-treatment change and 95% CI in observed heterozygosity between pre- and post-harvest sampling for Coastal Giant Salamander.

Table 33. Pairwise contrasts of the between-treatment change (Estimate) in observed heterozygosity between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	0.03	0.08	-0.13	0.19
FP vs. REF	-0.03	0.08	-0.19	0.13
0% vs. REF	-0.03	0.08	-0.19	0.13
100% vs FP	0.06	0.10	-0.14	0.26
100% vs. 0%	0.05	0.10	-0.15	0.25
FP vs. 0%	0.00	0.10	-0.20	0.20

1-6.1.4. Wright's Inbreeding Coefficient

1-6.1.4.a. Coastal Tailed Frog

Average Wright's inbreeding coefficient (F_{IS}) for Coastal Tailed Frog across all sites was 0.02 (SE = 0.01; **Appendix C**) There was no significant treatment effect on the change in F_{IS} (F = 1.61; P = 0.25) and no consistent pattern in the direction of the change in F_{IS} (**Table 34** and **Table 35**; **Figure 21**).

Table 34. Within-treatment change (Estimate) and 95% CI in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Coastal Tailed Frog. SE represents standard error. The p-value for this model was 0.25, which indicated no significant differences based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	-0.03	0.02	-0.07	-0.01
100%	0.03	0.03	-0.11	-0.17
FP	0.00	0.05	-0.59	-0.59
0%	-0.03	0.03	-0.14	-0.09

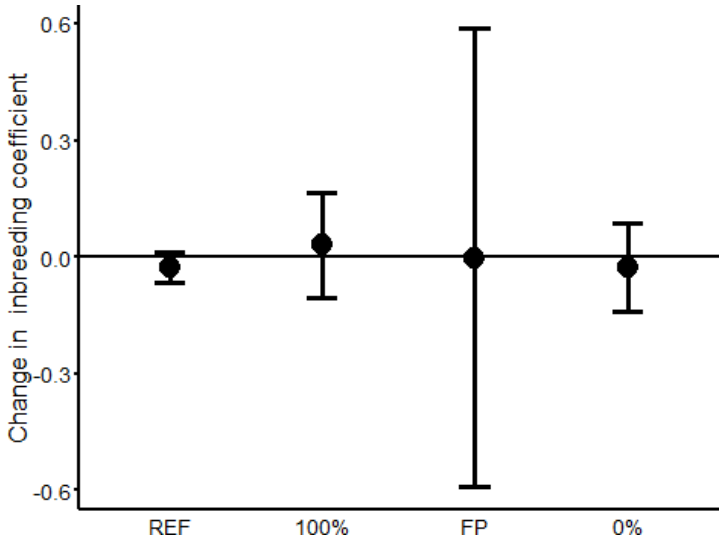

Figure 21. Within-treatment change and 95% CI in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Coastal Tailed Frog.

Table 35. Pairwise contrasts of the between-treatment change (Estimate) in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Coastal Tailed Frog. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first.

Contrast	Estimate	SE	95% CI	
			Lower	Upper
100% vs. REF	0.06	0.03	0.00	0.12
FP vs. REF	0.03	0.03	-0.03	0.09
0% vs. REF	0.00	0.03	-0.06	0.06
100% vs FP	0.03	0.04	-0.05	0.11
100% vs. 0%	0.06	0.03	0.00	0.12
FP vs. 0%	0.03	0.04	-0.05	0.11

1-6.1.4.b. Cope's Giant Salamander

The average F_{IS} for Cope's Giant Salamander across sites was 0.01 (SE = 0.01; **Appendix D**). While we did not observe an overall significant treatment effect on F_{IS} (F = 2.49, P = 0.105; **Table 36**; **Figure 22**), we did observe a pairwise significant difference (p = 0.02) between the FP and 100% treatments (**Table 37**). The F_{IS} for the FP treatment was exactly the same as in the baseline conditions, with all other treatments decreasing in F_{IS}.

Table 36. Within-treatment change (Estimate) and 95% CI in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error. The p-value for this model was 0.105, which approached our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	-0.04	0.02	-0.09	0.01
100%	-0.10	0.02	-0.18	-0.02
FP	0.00	0.03	-0.14	0.15
0%	-0.04	0.02	-0.11	0.03

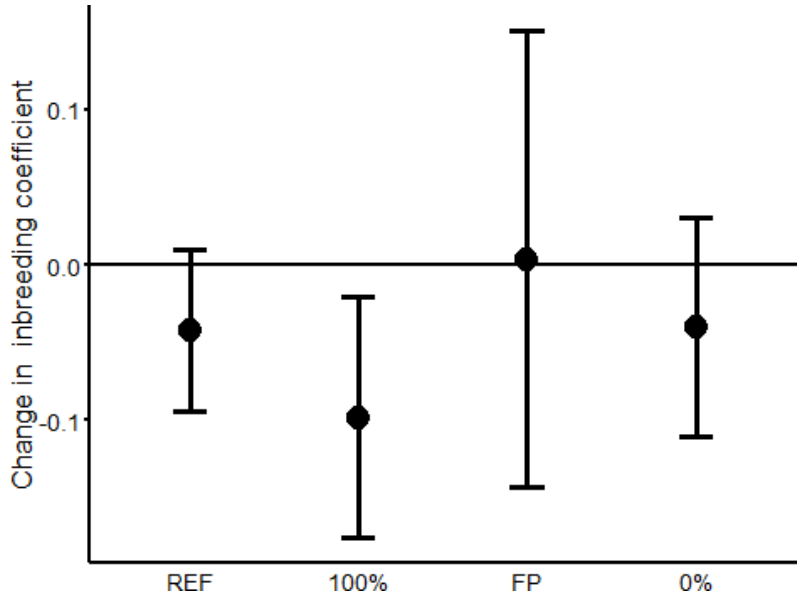

Figure 22. Within-treatment change and 95% CI in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Cope's Giant Salamander.

Table 37. Pairwise contrasts of the between-treatment change (Estimate) in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Cope's Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first. We used an alpha of 0.10 to assess significance based on the p-values.

Contrast	Estimate	SE	P-value	95% CI	
				Lower	Upper
100% vs. REF	-0.06	0.03	0.11	-0.12	0.00
FP vs. REF	-0.05	0.04	0.21	-0.12	0.02
0% vs. REF	0.00	0.03	0.95	-0.06	0.06
100% vs. FP	-0.10	0.04	0.02	-0.18	-0.02
0% vs. 100%	-0.06	0.04	0.12	-0.14	0.02
FP vs. 0%	0.04	0.04	0.27	-0.04	0.12

1-6.1.4.c. Coastal Giant Salamander

The average F_{IS} for Coastal Giant Salamander was 0.01 (SE = 0.02; **Appendix E**). We detected a significant treatment effect on change in F_{IS} (F = 4.23; P = 0.05; **Table 38**; **Figure 23**). The treatment effect was due to significant pairwise contrasts of reference versus both the 100% and

0% treatments (p = 0.02 and p = 0.03; **Table 39**). The average F_{IS} in the 100% and 0% treatments were lower than reference with the FP treatment mean as intermediate.

Table 38. Within-treatment change (Estimate) and 95% CI in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error. The p-value for this model was 0.05, which indicated a significant difference based on our alpha of 0.10.

Treatment	Estimate	SE	95% CI	
			Lower	Upper
REF	0.06	0.03	-0.04	0.15
100%	-0.11	0.01	-0.22	0.00
FP	0.01	0.06	-0.78	0.79
0%	-0.10	0.03	-0.52	0.32

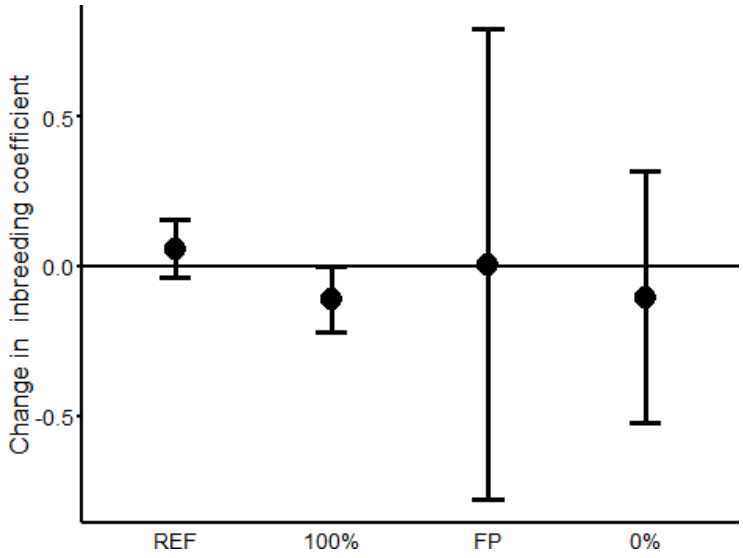

Figure 23. Within-treatment change and 95% CI in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Coastal Giant Salamander.

Table 39. Pairwise contrasts of the between-treatment change (Estimate) in Wright's inbreeding coefficient (F_{IS}) between pre- and post-harvest sampling for Coastal Giant Salamander. SE represents standard error and 95% CI is the 95% confidence interval. For the estimate, the second treatment listed in the contrast is subtracted from the first. We used an alpha of 0.10 to assess significance using the p-values.

Contrast	Estimate	SE	P-value	95% CI	
				Lower	Upper
100% vs. REF	-0.17	0.06	0.02	-0.29	-0.05
FP vs. REF	-0.05	0.06	0.40	-0.17	0.07
0% vs. REF	-0.16	0.06	0.03	-0.28	-0.04
100% vs FP	-0.12	0.07	0.14	-0.26	0.02
100% vs. 0%	-0.01	0.07	0.94	-0.15	0.13
FP vs. 0%	0.11	0.07	0.16	-0.03	0.25

1-6.1.5. Test for Recent Population Size declines

1-6.1.5.a. Coastal Tailed Frog

The heterozygosity excess test provided evidence for recent population size declines for Coastal Tailed Frogs at six sites in the post-harvest period (**Table 40**). Three of these sites (OLYM-REF, WIL1-REF, and OLYM-0%) also had heterozygosity excess in the pre-harvest period, but there were no shifted allelic distributions at any sites. The other three sites only showed heterozygosity excess in the post-harvest period; two of these sites were references (WIL2-REF1 and WIL2-REF2). The only site with a significant (p = 0.02 heterozygosity excess after a treatment was the WIL1-0%. Two sites with significant heterozygosity excess in the pre-harvest period (WIL1-100% and OLYM-FP) had insufficient sample size in the post-harvest period to conduct the tests. Two sites (WIL3-REF and WIL1-FP) had heterozygosity excess before the treatment period but no evidence of heterozygosity excess afterwards.

Table 40. Results of tests for recent population size declines in Coastal Tailed Frogs for both pre- and post-harvest periods. The tests included were the heterozygosity excess tests and shifted allele distributions. H_e represents actual expected heterozygosity and H_{eq} represents heterozygosity expected under equilibrium conditions. A significant p-value (bolded and underlined) indicates a significant heterozygosity excess relative to equilibrium expectations. The Shifted? column indicates whether allelic distributions were normal or skewed. Grayed out cells indicate that sample size was not sufficient to conduct these tests.

	Pre-harvest				Post-harvest			
Site	H_e	H_{eq}	**p-value**	**Shifted?**	H_e	H_{eq}	**p-value**	**Shifted?**
OLYM-REF	0.91	0.88	**0.00**	normal	0.90	0.89	**0.00**	normal
WIL1-REF	0.91	0.89	**0.02**	normal	0.90	0.89	**0.02**	normal
WIL2-REF1	0.90	0.90	0.29	normal	0.90	0.89	**0.00**	normal
WIL2-REF2	0.90	0.89	0.18	normal	0.90	0.88	**0.01**	normal
WIL3-REF	0.92	0.91	**0.02**	normal	0.90	0.90	0.33	normal
CASC-REF	0.85	0.89	0.25	normal	0.87	0.88	0.12	normal
WIL1-100%	0.91	0.90	**0.02**	normal				
WIL2-100%	0.87	0.88	0.75	normal	0.90	0.90	0.37	normal
WIL3-100%	0.90	0.89	0.25	normal				
OLYM-FP	0.88	0.86	**0.02**	normal				
WIL1-FP	0.91	0.90	**0.10**	normal	0.90	0.89	0.82	normal
OLYM-0%	0.90	0.87	**0.00**	normal	0.89	0.87	**0.01**	normal
WIL1-0%	0.87	0.88	0.37	normal	0.88	0.86	**0.02**	normal
WIL2-0%	0.90	0.90	0.18	normal	0.88	0.88	0.46	normal

1-6.1.5.b. Cope's Giant Salamander

Four sites had evidence of population size reductions based on heterozygosity excess for Cope's Giant Salamander (**Table 41**). Two of these sites (WIL2-REF1 and WIL2-0%) had heterozygosity excess in both pre- and post-harvest periods. Of the two sites that had evidence of declines post-harvest, one was a reference (WIL2-REF2) and the other was a FP treatment (WIL1-FP). No sites had shifted allele distributions.

Table 41. Results of tests for recent population size declines in Cope's Giant Salamanders for both pre- and post-harvest periods. The tests included were the heterozygosity excess tests and shifted allele distributions. H_e represents actual expected heterozygosity and H_{eq} represents heterozygosity expected under equilibrium conditions. A significant p-value (bolded and underlined) indicates a significant heterozygosity excess relative to equilibrium expectations. The Shifted? Column indicates whether allelic distributions were normal or skewed. Grayed out cells indicate that sample size was not sufficient to conduct these tests.

	Pre-harvest				Post-harvest			
Site	H_e	H_{eq}	**p-value**	**Shifted?**	H_e	H_{eq}	**p-value**	**Shifted?**
OLYM-REF	0.66	0.72	0.97	normal	0.65	0.73	0.97	normal
WIL1-REF	0.83	0.83	0.38	normal	0.81	0.84	0.14	normal
WIL2-REF1	0.87	0.86	**0.03**	normal	0.86	0.85	**0.07**	normal
WIL2-REF2	0.85	0.84	0.21	normal	0.85	0.83	**0.00**	normal
WIL3-REF	0.85	0.87	0.55	normal	0.88	0.88	0.38	normal
CASC-REF	0.69	0.77	0.99	normal	0.72	0.77	0.97	normal
OLYM-100%	0.58	0.61	0.55	normal	0.51	0.59	0.82	normal
WIL1-100%	0.86	0.88	0.58	normal	0.84	0.85	0.74	normal
WIL2-100%	0.76	0.76	0.42	normal	0.77	0.79	0.21	normal
WIL3-100%								
OLYM-FP	0.67	0.67	0.42	normal	0.65	0.69	0.82	normal
WIL1-FP	0.81	0.81	0.48	normal	0.85	0.84	**0.05**	normal
CASC-FP	0.84	0.85	0.84	normal	0.84	0.83	0.12	normal
OLYM-0%	0.65	0.68	0.45	normal	0.63	0.72	0.75	normal
WIL1-0%	0.74	0.77	0.55	normal	0.73	0.79	0.28	normal
WIL2-0%	0.86	0.83	**0.01**	normal	0.85	0.84	**0.09**	normal
CASC-0%	0.84	0.83	0.16	normal	0.83	0.84	0.71	normal

1-6.1.5.c. Coastal Giant Salamander

There was no evidence for recent reductions in population size for Coastal Giant Salamanders based on either heterozygosity excess or shifted allele distributions (**Table 42**).

Table 42. Results of tests for recent population size declines in Coastal Giant Salamanders for both pre- and post-harvest periods. The tests included were the heterozygosity excess tests and shifted allele distributions. H_e represents actual expected heterozygosity and H_{eq} represents heterozygosity expected under equilibrium conditions. A significant p-value (bolded and underlined) indicates a significant heterozygosity excess relative to equilibrium expectations. The Shifted? Column indicates whether allelic distributions were normal or skewed. Grayed out cells indicate that sample size was not sufficient to conduct these tests.

	Pre-harvest				Post-harvest			
Site	H_e	H_{eq}	**p-value**	**Shifted?**	***H*e**	H_{eq}	**p-value**	**Shifted?**
WIL1-REF	0.59	0.59	0.41	normal	0.58	0.60	0.41	normal
WIL2-REF1					0.60	0.63	0.89	normal
WIL2-REF2								
WIL3-REF	0.66	0.73	0.95	normal	0.69	0.78	0.89	normal
CASC-REF	0.37	0.64	0.91	normal	0.44	0.65	0.94	normal
WIL1-100%	0.66	0.80	0.98	normal	0.71	0.74	0.69	normal
WIL3-100%	0.61	0.78	0.95	normal	0.62	0.70	0.95	normal
WIL1-FP	0.58	0.71	0.92	normal	0.65	0.65	0.31	normal
CASC-FP	0.53	0.60	0.92	normal	0.48	0.56	0.92	normal
WIL2-0%	0.65	0.67	0.69	normal	0.68	0.72	1.00	normal
CASC-0%	0.47	0.70	0.97	normal	0.42	0.56	0.84	normal

1-6.2. GENETIC CLUSTERING

1-6.2.1. Coastal Tailed Frog

The median value of *K* for Coastal Tailed Frog in both pre- and post-harvest was 3 (**Figure 24**and **Figure 25**), although $K = 2$ and $K = 4$ were selected by some of the individual metrics (**Appendix F**). The arrangement of clusters was also similar between the two periods, with sites showing evidence of clustering by region but little evidence of significant genetic structure within each of the three regions. The Olympic region is most clearly differentiated, with a higher degree of admixture between the Willapa Hills and South Cascades.

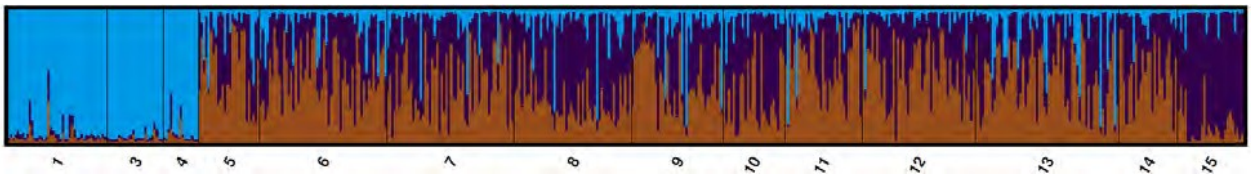

Figure 24. Bayesian genetic clustering results for all samples of Coastal Tailed Frogs pre-harvest assuming *K*=3. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and site 15 in the South Cascades.

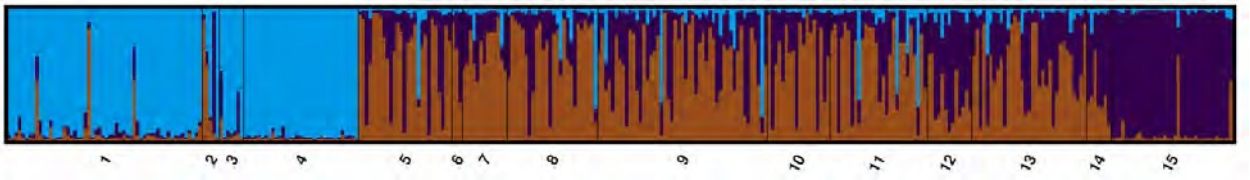

Figure 25. Bayesian genetic clustering results for all samples of Coastal Tailed Frogs post-harvest assuming *K*=3. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

1-6.2.2. Cope's Giant Salamander

There was a difference between estimated values of *K* between periods for Cope's Giant Salamander. The median value of *K* pre-harvest was nine and the median value of *K* post-harvest was between seven and eight (**Figure 26 -28)**. Other values of *K* receiving some support for the pre-harvest period include 7, 8, 10, 11, and 12 (**Appendix G**). In the post-harvest period, *K* = 6, 9, and 10 were selected with some of the individual metrics (**Appendix G**). Despite the different median values of *K*, the pattern of clustering between the periods was very similar. The Olympic and South Cascades regions represented clear clusters and had little substructure within each region. The Willapa Hills region had the greatest subdivision, although the region still had a pattern of high admixture among sites. The major differences were that sites 7 (WIL1-FP) and 11 (WIL2-100%) are differentiated in the pre-harvest period but admixed with other sites post-harvest. Site 2 (OLYM-100%) is completely within the Olympic cluster post-harvest, whereas it is admixed with a separate cluster pre-harvest.

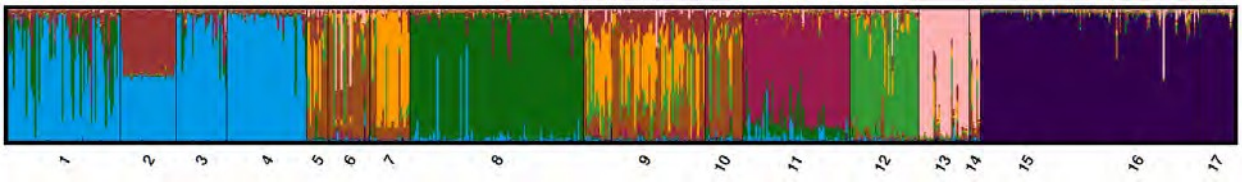

Figure 26. Bayesian genetic clustering results for all samples of Cope's Giant Salamanders pre-harvest assuming $K = 9$. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

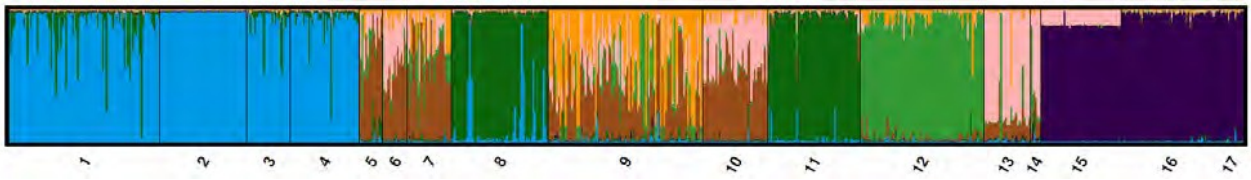

Figure 27. Bayesian genetic clustering results for all samples of Cope's Giant Salamanders post-harvest assuming $K = 7$. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

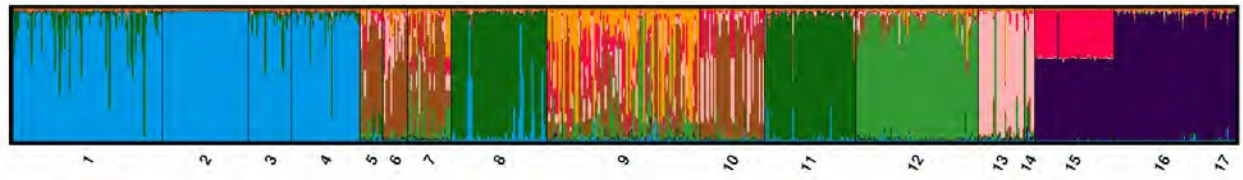

Figure 28. Bayesian genetic clustering results for all samples of Cope's Giant Salamanders post-harvest assuming $K = 8$. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

1-6.2.3. Coastal Giant Salamander

Coastal Giant Salamanders (which are absent from the Olympics and not sampled north of the Willapa River) separate into three clusters across the Willapa Hills and South Cascades in both pre- and post-harvest periods (**Figure 29** and **Figure 30**), although values of $K = 2$, 4, and 5 also received some support (**Appendix H**). The South Cascades form their own cluster with some

limited connectivity to the Willapa sites. The Willapa Hills sites form two clusters, but the composition of the clusters varies between periods. In the pre-harvest period, site 6 (WIL1-100%) represents one cluster (albeit with admixture with other Willapa Hills sites), whereas in the post-harvest period, site 6 clusters more strongly with other members of the Willapa 1 block (sites 5 and 7). Sites 5-7 are in relative close geographic proximity, so genetic connectivity among them is not unexpected, but like the Cope's Giant Salamander, is consistent with increased migration post-harvest.

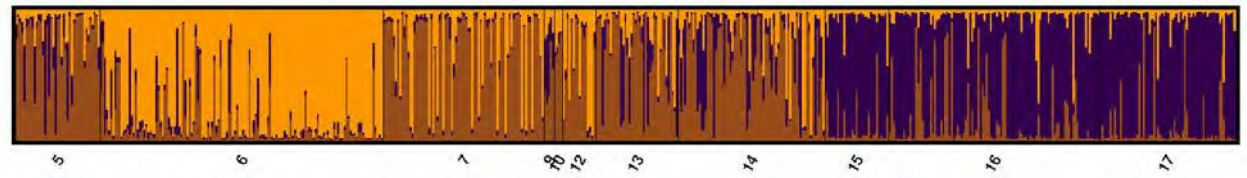

Figure 29. Bayesian genetic clustering results for all samples of Coastal Giant Salamanders pre-harvest assuming $K = 3$. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent site in the order presented in **Table 2**, with sites 5-7, 9, 10 and 12-14 in the Willapa Hills and sites 15-17 in the South Cascades.

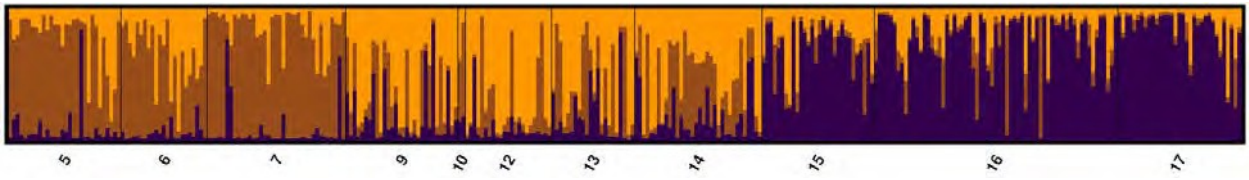

Figure 30. Bayesian genetic clustering results for all samples of Coastal Giant Salamanders post-harvest assuming $K = 3$. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent site in the order presented in **Table 2**, with sites 5-7, 9, 10 and 12-14 in the Willapa Hills and sites 15-17 in the South Cascades.

1-7. **DISCUSSION**

We assumed our samples were representative of individuals born after the study treatments were implemented because less than 1% of post-metamorphic tailed frogs sampled had an SVL ≥ 42 mm, and only <1% of giant salamanders sampled had an SVL ≥ 100 mm.

1-7.1. GIANT SALAMANDER SPECIES IDENTIFICATION AND HYBRIDS

As expected, we identified both species of giant salamanders in sites located in the Willapa Hills and South Cascades. We did not detect Coastal Giant Salamanders in sites located in the Olympics, a finding consistent with previous observations and the documented range limit for the species (Welsh 2005). We also observed only Cope's Giant Salamanders in the two study sites located in the northernmost extent of the Willapa Hills (WIL1-0% and WIL2-100%). Interestingly, we did detect a single Coastal Giant Salamander in WIL2-100% during post-harvest amphibian demographic sampling in 2009 and 2010, which we verified with genetic analysis. We detected only seven hybrid giant salamanders in the post-harvest sampling. While numerically this is fewer than the 33 detected in the pre-harvest period by Spear *et al.* (2011), the sample sizes were too low to statistically compare change between the two periods. However, it certainly does not support the hypothesis of increased opportunities for hybridization due to habitat disturbance (see Mallet 2005 for an in-depth exploration of this issue).

1-7.2. COMPARISON OF GENETIC DIVERSITY MEASURES AMONG TREATMENTS WITHIN SPECIES

Disturbance is considered one of the major impacts on a species' genetic diversity and thus its ability to respond and adapt to future perturbations (Banks *et al.* 2013). There are a number of potential effects of disturbance on genetic diversity and genetic structure. These include direct loss of genetic diversity through population reductions and alteration of genetic connectivity by either facilitating or reducing dispersal. These impacts can interact with each other to further exacerbate disturbance impacts. For instance, the loss of genetic diversity due to population bottlenecks increases the strength of genetic drift, which itself leads to loss of diversity. Loss of genetic connectivity also increases the rate of genetic diversity loss. Conversely, if a disturbances increases migration or dispersal, gene flow could increase maladaptive genotypes if the populations were formerly local adapted. Yet, there are only a handful of studies that have sampled populations both before and after a disturbance (Keyghobadi *et al.* 2005; Peakall and Lindenmayer 2006; Apodaca *et al.* 2013; Vandergast *et al.* 2016). In general, these studies have found a shift in genetic structure with relatively little change in diversity metrics such as allelic diversity and heterozygosity. This is because allele frequency changes (the basis for metrics such as the F-statistics) are more likely to occur than the outright loss of alleles in the absence of strong bottlenecks. In fact, the Baseline Genetic Report (Spear *et al.* 2011) estimated through simulations that effective population size would need to be reduced to as low as 6 to 34 individuals (depending on species) to detect a pattern. We did not detect a strong shift in genetic diversity metrics due to treatment, suggesting that treatments did not cause severe declines for the three species tested in this study.

1-7.2.1. Coastal Tailed Frogs

None of our comparisons were significant at $\alpha\alpha = 0.10$ for Coastal Tailed Frogs. Part of this is likely due to broad confidence intervals across the treatment categories compared to the reference condition due to the uneven number of replicates per treatment. We also had relatively small samples sizes for tailed frogs in some sites and years, including some zeros. This was the case even with a substantial sampling effort that was relatively similar across sites and years. For

instance, the only site with tailed frogs in the Cascades block for either period was the reference site, and no tailed frogs were detected in the 100% treatment in the Olympic block in the pre-treatment period. Standard errors for the reference sites were always smaller than any of the other treatments.

There are a number of potential impacts that harvest treatments could have on stream amphibian populations. These include increased sedimentation in streams (reducing larval habitat), increased stream temperatures, and loss of forest cover constraining terrestrial dispersal by metamorphs and adults (Chelgren and Adams 2017). One of the most immediate effects we thought we might observe if there was a treatment effect was an increase in the number of full siblings relative to total sample size. We hypothesized such an effect because a reduction in population size should lead to fewer breeding individuals and thus fewer family groups represented. The number of unique family groups did numerically decline in the buffer treatments however, this decline mirrored almost exactly a decline in sample number. Therefore, we see no evidence that number of unique family groups had a response to treatment.

While there was an average loss of several alleles for each of the buffer treatment categories, with no change in the reference sites, this finding was not significant and clearly reflected the reduced sample number. Allelic richness, which is independent of sample size, had at most an average change of one allele, and that was an increase in the 100% treatment. Observed heterozygosity was high in tailed frogs (mean = 0.88) and the average change in heterozygosity was small (3%). A high level of heterozygosity is characteristic of tailed frog populations across several watersheds and in both species (Spear and Storfer 2008; Spear and Storfer 2010; Spear *et al.* 2012; Aguilar *et al.* 2013), so high heterozygosity is not surprising. As a result, allelic diversity and heterozygosity are unlikely to be highly sensitive to disturbance in tailed frogs.

F_{IS} can be seen as an evaluation of the extent of random mating given the available genetic diversity. It is easy to see why this might be expected to change quicker than other genetic measures as mating patterns could shift in a single generation. For instance, there is evidence that Coastal Tailed Frog adults move seasonally (Hayes *et al.* 2006), and loss of riparian cover has the potential to inhibit breeding movements and therefore prevent random mating within a population. For the tailed frogs analyzed in this study the mean F_{IS} was close to zero and the confidence intervals for the change in treatments also overlapped zero. Therefore, it appears that tailed frogs did not deviate from random mating regardless of treatment during the period of study. However, the period of study represents a relatively short time for this species, i.e., life span for this species is estimated to be 15 to 20 years (Daugherty and Sheldon 1982). It is entirely possible that the result may differ if populations in our study sites were sampled after a longer time has passed.

The only genetic diversity tests where we did see evidence of a pre- to post-harvest difference in tailed frogs were for the tests for recent reductions in population size, also known as bottleneck tests. In the Baseline Genetic Report (Spear *et al.* 2011) apparent population reductions were detected based on heterozygosity excess tests in multiple sites in the pre-harvest period, especially in the Olympic region. Several of these sites still have the bottleneck signature. Treatment had no consistent effect on bottleneck status, and based on the geographic clustering of several of these sites it is likely a landscape-level effect. Importantly, the ability to detect population declines genetically through these tests is still somewhat uncertain. Peery *et al.*

(2012) and Hoban *et al.* (2013) used simulations to extensively examine the performance of the heterozygosity excess and M-ratio test. Both concluded that heterozygosity excess is characterized by low power and that power is increased with larger bottlenecks, more loci, and more individuals. In our study the number of loci and sample size fall into the low power categories. Therefore, declines may have existed that we did not detect. Peery *et al.* (2012) did demonstrate a low Type I error rate for the heterozygosity excess which increases our confidence in the sites that did have a signature of heterozygosity excess. This is in contrast to the M-ratio which has high Type I error rates and was therefore omitted from post-harvest analysis. The other bottleneck test we used, shifted allele distributions, did not have any deviations from an expected pattern. The research on power of the shifted allele distribution test is lacking, but given that it relies on the loss of rare alleles, it is probably less suitable for detecting bottlenecks within a shorter time representing less than one or even a few generations.

1-7.2.2. Cope's Giant Salamander

The only potential treatment effect we detected in Cope's Giant Salamander was on F_{IS}. The p-value for this variable (0.105) was slightly above our alpha of 0.10, but was close enough that we felt it was worthy of consideration especially since non-random mating is a potential result of population disturbance. The big decrease in the 100% treatment suggests that individuals were more likely to be mating with less related individuals. A F_{IS} decrease of 0.10 represents a large change from non-random mating and is most consistent with migration of individuals from outside genetic populations into the site. Alternatively, it could be due to more dispersal out of the site by related individuals reducing the relatedness of sampled individuals within the site. We do not currently have data to distinguish between these two hypotheses. Regardless of movement direction, the outbreeding seen in the 100% buffer could be due to a combination of harvest disturbance increasing the probability of movement by individuals, with the riparian buffer facilitation such movements. Further research is needed to determine if this hypothesis is likely.

While there was almost no change in allelic diversity across years for Cope's Giant Salamander, we did see an interesting, albeit non-significant, trend in observed heterozygosity. Both the reference and 100% treatment had higher mean heterozygosity than either the FP or 0% treatment. However, heterozygosity did not decrease in any treatment. Finally, there was some limited evidence for significant bottlenecks, although as with tailed frogs, there was no clear pattern by treatment. Two sites had evidence of heterozygosity excess, one reference and one FP. The same caveats as presented for tailed frogs apply for the heterozygosity excess test, although we may have had slightly more power due to more loci and greater sample size.

1-7.2.3. Coastal Giant Salamander

Coastal Giant Salamanders had the fewest number of loci and number of sites and therefore had the lowest power to detect differences. Surprisingly, this was the species with the most effects by treatment; however, some of these differences can be attributed to sample size. For example, Coastal Giant Salamander had a large decrease in the number of unique full-sibling families which, combined with no effect by treatment for the proportion of full siblings, indicates the significant result is due to reduced sample size. Of more immediate relevance to this study was the significant difference by treatment in F_{IS}. Specifically, the 100% and 0% treatments had a decrease in F_{IS} relative to the reference. This was similar to the pattern seen with Cope's Giant

Salamander and likely represents migration in or out of the site. Given the decreased sampling number for the 100% treatment it most likely represents movement out of the site. The hypothesis we proposed for why immigration might increase in a 100% buffer for Cope's Giant Salamander might also apply to Coastal Giant Salamander. However, we hesitate to infer too much from our Coastal Giant Salamander results, as the Type N study was not designed for this species and the species was detected in only 11 of 17 study sites. An increase in sampling intensity and increased number of loci would be warranted in future studies.

The other genetic diversity metrics (allelic number, observed heterozygosity, bottleneck tests) did not show any indication of effect by treatment. The only thing resembling a trend among these three variables was a decrease in allelic number for the 100% treatment which is certainly due to the decrease in sampled individuals. Unlike the other two species there was no evidence for population bottlenecks, although we had poor power to detect any differences if they existed.

1-7.3. INFLUENCE OF THE SPATIAL EXTENT OF GENE FLOW ON POST-HARVEST RESULTS

The Type N Experimental Buffer Treatment study was designed to focus on the individual basin as the experimental unit and does not incorporate landscape level variation. Therefore, a genetic assessment of experimental buffer effects must focus on at-site genetic variables, largely the metrics of genetic diversity, as we discuss in the previous section. Yet genetic diversity is influenced strongly by aspects of genetic connectivity, which requires examining the spatial extent of gene flow across the landscape. Our use of Bayesian clustering analyses is meant to provide context for how we might interpret at-site genetic diversity results. The higher the levels of genetic connectivity among study basins, the greater the magnitude that a treatment effect would need to be to show any changes in genetic diversity within the study period. We can also infer the potential change in genetic diversity by examining genetic clustering patterns between periods; similar clustering patterns means that genetic connectivity is not likely to change rapidly.

Our clustering results across both periods clearly demonstrate a consistent pattern of relative genetic connectivity among the three species, with Coastal Tailed Frogs having the highest genetic connectivity, Coastal Giant Salamanders intermediate, and Cope's Giant Salamander the lowest. This ordering is consistent with previous genetic studies that have been done with the three species (Spear and Storfer 2008; Steele *et al.* 2009; Dudaniec *et al.* 2012; Spear *et al.* 2012; Trumbo *et al.* 2013), although it is important to note that most of these studies included samples from the pre-harvest period and are not completely independent studies. Still, our post-harvest genetic clustering was largely concordant with pre-harvest clustering results, so gene flow patterns are likely stable over short periods to time. With respect to tailed frogs, landscape genetic studies suggests extensive overland gene flow (Spear and Storfer 2008; Spear *et al.* 2012). The gene flow pattern can shift to individuals following stream corridors on managed timberlands (Spear and Storfer 2010). Such versatility in gene flow likely allows tailed frogs to persist under a variety of disturbance regimes as long as the instream environment is suitable for occupancy (e.g., Matsuda and Richardson 2005) and lessens the chance that fine-scale landscape effects (such as the timber harvest treatments included in the Type N study) will significantly impact genetic diversity over short time scales. On the other hand, Cope's Giant Salamander

does show significant substructure within regions, and indeed was the only species to show a difference in the estimated value of *K* between periods.

All of the effects that were significant for giant salamanders are suggestive of movement in and out of study sites; changes in number of individuals sampled without concurrent changes in allelic diversity or heterozygosity and a decrease in F_{IS}. The post-harvest reduction in the estimate of *K* for Cope's Giant Salamander is consistent with this effect, and the most obvious change in clustering was for a site with a 100% harvest treatment, the treatment which had the greatest change in F_{IS}. While the estimate of *K* for Coastal Giant Salamander did not change between periods, the pattern of site clustering also showed increased admixture post-harvest. It is difficult to infer what proportion of these changes were due to within-basin changes relative to the broader landscape, but we can conclude that it is possible to detect some change in genetic structure in giant salamander over a relatively short period, whereas we cannot make that assumption for Coastal Tailed Frogs.

1-8. CONCLUSIONS

Based on our genetic analyses, we did not see any significant evidence that the FP treatment will have a negative effect on future persistence of Coastal Tailed Frogs or giant salamanders at study sites. However, one disadvantage of using genetic metrics to evaluate treatment effects is that a temporal lag frequently exists, unless the effects are extreme (Hoban *et al.* 2014). Despite the possibility of a temporal lag in response, we argue that the genetic portion of this study is critical to fully evaluate the treatments. Larval individuals tend to make up the majority of samples for all three of the species included in these analyses, and relying on demographic estimates of larval density alone is problematic. If a treatment affected factors such as survival through metamorphosis, larval individuals may not represent the total population. One possible outcome of genetically evaluating the larval portion of a population is the possibility of finding that larvae were the offspring of only one or a few adults, which would be an indication of a declining population, a signature that may be missed with a demographic-focused study. We did not see anything resembling this pattern in our genetic results.

There are a number of caveats that should be considered before concluding no genetic impact as a result of harvest treatments. Based on simulation results presented in the Baseline Genetic Report (Spear *et al.* 2011), as well as the more general study of Hoban *et al.* (2014), a severe immediate population decline would be necessary to detect reductions in genetic diversity over the study period, which represented a timeframe of less than one generational turnover. Furthermore, sample sizes of 50 or greater are recommended (Hoban *et al.* 2014). If the harvest treatments did not result in an immediate decline in abundance, but rather resulted in a small but continuous decline over time, several generations would be needed to detect a difference. Overall, we are confident that a severe (> 95%) immediate decline did not occur in our treatment sites. We strongly recommend follow-up genetic investigations in future generations to evaluate the possibility of future declines.

This study represented a rare opportunity to investigate change in genetic diversity and structure immediately after a disturbance. Despite the obvious implications for genetic diversity as a response variable to disturbance (Banks *et al.* 2013), very few empirical examples of how

genetic diversity may respond to altered landscapes exist. This study provides one example of the genetic change we might expect to see in stream-associated amphibian diversity over an eight-year period and contributes further to the understanding of the amphibian population dynamics in Pacific Northwest forests. The dataset also provides an excellent starting point to assess evolutionary dynamics in these three species in the future. We strongly recommend that genetic monitoring be continued at these sites at regular intervals (every 15-20 years) into the future, at least for Cope's Giant Salamander. Sampling each generation would help us understand the population dynamics of stream amphibians following fine-scale disturbance and would likely address some of the uncertainty in previous demographic studies (Kroll 2009). Finally, our study indicates that F_{IS} may be more sensitive than other genetic diversity metrics to short-term changes, a finding that is valuable for future studies.

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Appendix A. Multiplex PCR conditions for Coastal Tailed Frog (locus names per Spear *et al.* 2008)

Multiplexes consist of:

	Per Sample
Qiagen Master Mix	5µl
Q solution	0.5 µl
Primer Mix (5µM stock)	See Below
Water	See Below
Template DNA	1 µl
Total	10 µl

Multiplex TF-A: [96ºC for 10 min, (94ºC for 30 s, 55ºC for 90 s, 72ºC for 1 min) X28, 60ºC for 30 min]
A4-NED- 0.90 µl
A12-6FAM- 0.10 µl
A31-VIC- 0.10 µl
Water- 2.4 µl

Multiplex TF-B: [96ºC for 10 min, (94ºC for 30 s, 55ºC for 90 s, 72ºC for 1 min) X28, 60ºC for 30 min]

A15-6FAM- 0.60 µl
A26-NED- 0.50 µl
A29-PET- 0.20 µl
Water -2.0 µl

Multiplex TF-C: [96ºC for 10 min, (94ºC for 30 s, 60ºC for 90 s, 72ºC for 1 min) X28, 60ºC for 30 min]

A3-PET- 0.90 µl
A13-NED- 0.20 µl
A14-VIC- 0.30 µl
A17-6FAM- 0.30 µl
Water- 1.8 µl

Multiplex TF-D: [96ºC for 10 min, (94ºC for 30 s, 55ºC for 90 s, 72ºC for 1 min) X28, 60ºC for 30 min]

A24-6FAM- 0.26 µl
A1-VIC- 0.14 µl
A2-PET- 0.60 µl
Water- 2.5 µl

Appendix B. Multiplex PCR conditions for giant salamanders (locus names per Steele *et al.* 2009)

Multiplexes consist of:

	Per Sample
Qiagen Master Mix	5µl
Q solution	0.5 µl
Primer Mix (2µM stock; Forward and Reverse, mixed)	1 µl
Water	2.5 µl
Template DNA	1 µl
Total	10 µl

Multiplex DICAMP1: [95ºC for 15 min, (94ºC for 30 s, 53ºC for 90 s, 72ºC for 1 min) X30, 60ºC for 30 min]

D18-6FAM
D13-VIC
D04-PET

Multiplex DICAMP2.1: [95ºC for 15 min, (94ºC for 30 s, 60ºC for 90 s, 72ºC for 1 min) X30, 60ºC for 30 min]

D24-6FAM
D07-NED

Multiplex DICAMP2.2: [95ºC for 15 min, (94ºC for 30 s, 60ºC for 90 s, 72ºC for 1 min) X30, 60ºC for 30 min]

D17-PET

Multiplex DICAMP4: [95ºC for 15 min, (94ºC for 30 s, 60ºC for 90 s, 72ºC for 1 min) X30, 60ºC for 30 min]

D14-6FAM
D05-NED
D06-PET

Multiplex DICAMP5: [95ºC for 15 min, (94ºC for 30 s, 60ºC for 90 s, 72ºC for 1 min) X30, 60ºC for 30 min]

D15-6FAM
D23-VIC

Appendix C. Genetic Diversity Measures of Coastal Tailed Frogs

Pre- and post-harvest estimates for genetic diversity measures of Coastal Tailed Frogs. *N* represents number of unique individuals sampled, Fam represents number of unique family groups, Prop is the proportion of unique family groups relative to total samples, A is total number of alleles, A_r is allelic richness, H_o is observed heterozygosity, and F_{IS} is Wright's inbreeding coefficient.

Period	Site	*N*	Fam	Prop	A	A_r	H_o	F_{IS}
Pre-harvest	OLYM-REF	42.00	41.00	0.98	16.11	16.02	0.86	0.05
	OLYM-FP	24.00	19.00	0.79	12.00	7.67	0.92	0.00
	OLYM-0%	15.00	12.00	0.80	10.22	9.88	0.91	-0.01
	WIL1-REF	26.00	22.00	0.85	15.33	15.22	0.86	0.06
	WIL1-100%	54.00	52.00	0.96	19.22	4.83	0.86	0.06
	WIL1-FP	54.00	51.00	0.94	20.67	12.45	0.84	0.08
	WIL1-0%	50.00	46.00	0.92	15.78	13.79	0.84	0.02
	WIL2-REF1	39.00	34.00	0.87	18.56	18.07	0.88	0.03
	WIL2-REF2	26.00	23.00	0.88	16.67	14.49	0.86	0.04
	WIL2-100%	33.00	27.00	0.82	14.56	13.58	0.85	0.03
	WIL2-0%	48.00	42.00	0.88	18.89	12.26	0.89	0.01
	WIL3-REF	61.00	52.00	0.85	20.89	18.29	0.89	0.03
	WIL3-100%	25.00	24.00	0.96	16.11	7.86	0.90	0.01
	CASC-REF	28.00	24.00	0.86	16.56	16.56	0.82	0.02
	Average	**37.50**	**33.50**	**0.88**	**16.54**	**12.92**	**0.87**	**0.03**
Post-harvest	OLYM-REF	56.00	55.00	0.98	17.89	16.54	0.92	-0.02
	OLYM-FP	7.00	7.00	1.00	8.56	8.56	0.87	0.04
	OLYM-0%	33.00	27.00	0.82	13.56	10.48	0.91	-0.02
	WIL1-REF	27.00	21.00	0.78	15.67	15.28	0.92	-0.02
	WIL1-100%	3.00	3.00	1.00	4.67	4.67	0.85	0.03
	WIL1-FP	13.00	13.00	1.00	12.44	12.05	0.87	0.03
	WIL1-0%	26.00	26.00	1.00	13.78	13.78	0.85	0.03
	WIL2-REF1	49.00	49.00	1.00	19.00	17.47	0.87	0.04
	WIL2-REF2	18.00	18.00	1.00	14.56	14.56	0.87	0.03
	WIL2-100%	28.00	27.00	0.96	16.33	15.86	0.83	0.07
	WIL2-0%	13.00	10.00	0.77	10.67	10.67	0.94	-0.07
	WIL3-REF	33.00	30.00	0.91	17.78	17.78	0.88	0.02
	WIL3-100%	7.00	6.00	0.86	8.67	8.42	0.84	0.09
	CASC-REF	35.00	25.00	0.71	17.11	16.17	0.86	0.01
	Average	**24.86**	**22.64**	**0.91**	**13.62**	**13.02**	**0.88**	**0.02**

Appendix D. Genetic Diversity Measures of Cope's Giant Salamander

Pre- and post-harvest estimates for genetic diversity measures of Cope's Giant Salamander. Column abbreviations are as in **Appendix C**.

Period	Site	*N*	Fam	Prop	A	*A*r	*H*e	*H*o	F_{IS}
Pre-harvest	OLYM-REF	62.00	46.00	0.74	7.64	7.63	0.66	0.66	-0.01
	OLYM-100%	31.00	17.00	0.55	4.00	3.75	0.52	0.45	0.15
	OLYM-FP	28.00	17.00	0.61	5.82	5.59	0.65	0.65	0.01
	OLYM-0%	44.00	29.00	0.66	6.09	6.02	0.64	0.65	-0.02
	WIL1-REF	12.00	11.00	0.92	9.18	8.95	0.83	0.77	0.08
	WIL1-100%	22.00	18.00	0.82	12.00	10.77	0.85	0.78	0.08
	WIL1-FP	22.00	14.00	0.64	10.00	9.92	0.81	0.82	-0.02
	WIL1-0%	96.00	62.00	0.65	9.27	8.58	0.74	0.68	0.07
	WIL2-REF1	67.00	41.00	0.61	15.00	14.93	0.86	0.84	0.03
	WIL2-REF2	21.00	17.00	0.81	11.09	10.98	0.84	0.79	0.07
	WIL2-100%	59.00	47.00	0.80	9.36	9.21	0.75	0.66	0.12
	WIL2-0%	37.00	33.00	0.89	13.00	12.83	0.85	0.75	0.12
	WIL3-REF	28.00	23.00	0.82	12.18	11.91	0.84	0.76	0.10
	WIL3-100%	6.00	6.00	1.00	6.36	5.84	0.80	0.74	0.09
	CASC-REF	52.00	37.00	0.71	8.18	7.96	0.68	0.67	0.02
	CASC-FP	69.00	62.00	0.90	11.91	11.56	0.83	0.77	0.08
	CASC-0%	19.00	16.00	0.84	8.82	8.04	0.83	0.77	0.08
	Average	**39.71**	**29.18**	**0.76**	**9.41**	**9.09**	**0.76**	**0.72**	**0.06**
Post-harvest	OLYM-REF	90.00	86.00	0.96	7.55	7.20	0.64	0.63	0.01
	OLYM-100%	52.00	41.00	0.79	4.00	3.61	0.48	0.49	-0.02
	OLYM-FP	26.00	23.00	0.88	6.00	5.85	0.65	0.66	-0.01
	OLYM-0%	41.00	38.00	0.93	5.91	5.88	0.63	0.63	-0.01
	WIL1-REF	14.00	12.00	0.86	9.18	8.48	0.81	0.83	-0.02
	WIL1-100%	15.00	13.00	0.87	10.27	10.27	0.84	0.84	0.00
	WIL1-FP	26.00	17.00	0.65	10.73	10.20	0.85	0.80	0.05
	WIL1-0%	58.00	53.00	0.91	8.45	8.43	0.73	0.70	0.03
	WIL2-REF1	92.00	55.00	0.60	15.18	14.50	0.86	0.85	0.01
	WIL2-REF2	39.00	25.00	0.64	11.82	10.63	0.85	0.86	-0.01
	WIL2-100%	55.00	53.00	0.96	9.64	9.59	0.76	0.72	0.05
	WIL2-0%	74.00	56.00	0.76	14.18	12.71	0.84	0.78	0.08
	WIL3-REF	28.00	23.00	0.82	12.55	12.28	0.87	0.86	0.01
	WIL3-100%	6.00	6.00	1.00	7.09	6.35	0.85	0.83	0.02
	CASC-REF	48.00	41.00	0.85	8.18	8.16	0.72	0.70	0.03
	CASC-FP	59.00	55.00	0.93	10.91	10.81	0.83	0.80	0.04
	CASC-0%	14.00	14.00	1.00	8.36	8.20	0.82	0.84	-0.02
	Average	**43.35**	**35.94**	**0.85**	**9.41**	**9.01**	**0.77**	**0.75**	**0.01**

Appendix E. Genetic Diversity Measures of Coastal Giant Salamander

Pre- and post-harvest estimates for genetic diversity measures of Coastal Giant Salamander. Column abbreviations are as in **Appendix C**.

Period	Site	*N*	Fam	Prop	A	*A*r	*H*o	F_{IS}
Pre-harvest	WIL1-REF	41.00	14.00	0.34	5.60	5.14	0.57	-0.11
	WIL1-100%	137.00	53.00	0.39	10.80	6.84	0.57	0.09
	WIL1-FP	78.00	20.00	0.26	6.80	5.17	0.53	-0.04
	WIL2-REF1	5.00	4.00	0.80	2.00	1.53	0.28	-0.22
	WIL2-REF2	4.00	4.00	1.00	3.20	2.27	0.55	-0.12
	WIL2-0%	16.00	10.00	0.63	5.40	4.75	0.55	0.14
	WIL3-REF	40.00	23.00	0.58	7.20	5.82	0.53	0.17
	WIL3-100%	71.00	43.00	0.61	9.20	6.94	0.45	0.17
	CASC-REF	31.00	14.00	0.45	4.40	4.38	0.40	-0.09
	CASC-FP	102.00	53.00	0.52	6.60	6.34	0.49	0.07
	CASC-0%	66.00	35.00	0.53	6.80	5.64	0.41	0.11
	Average	**53.73**	**24.82**	**0.55**	**6.18**	**4.98**	**0.48**	**0.02**
Post-harvest	WIL1-REF	30.00	21.00	0.70	5.20	4.91	0.53	-0.08
	WIL1-100%	23.00	19.00	0.83	6.40	6.19	0.67	-0.01
	WIL1-FP	37.00	27.00	0.73	5.60	5.16	0.59	0.03
	WIL2-REF1	30.00	22.00	0.73	5.60	2.16	0.53	-0.06
	WIL2-REF2	2.00	2.00	1.00	2.20	2.20	0.50	0.00
	WIL2-0%	23.00	16.00	0.70	6.60	5.27	0.56	0.07
	WIL3-REF	22.00	20.00	0.91	7.80	7.22	0.53	0.16
	WIL3-100%	34.00	29.00	0.85	7.20	6.63	0.50	0.05
	CASC-REF	30.00	21.00	0.70	5.20	5.20	0.48	-0.09
	CASC-FP	65.00	55.00	0.85	6.00	5.99	0.46	0.02
	CASC-0%	33.00	27.00	0.82	4.20	4.20	0.44	-0.03
	Average	**29.60**	**23.20**	**0.80**	**5.78**	**5.09**	**0.54**	**0.01**

Appendix F. Genetic clustering of Coastal Tailed Frogs

STRUCTURE bar plots for $K = 2$ and $K = 4$ for both pre- and post-harvest samples of Coastal Tailed Frogs.

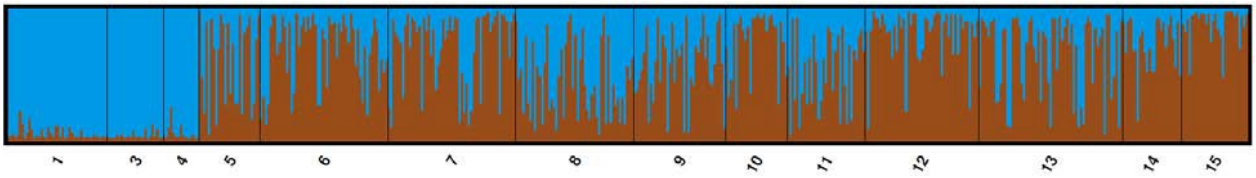

Figure F1. Bayesian genetic clustering results for all samples of Coastal Tailed Frogs pre-harvest assuming K=2. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and site 15 in the South Cascades.

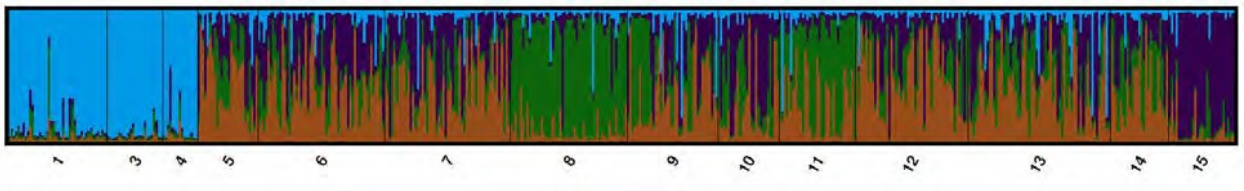

Figure F2. Bayesian genetic clustering results for all samples of Coastal Tailed Frogs pre-harvest assuming K=4. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and site 15 in the South Cascades.

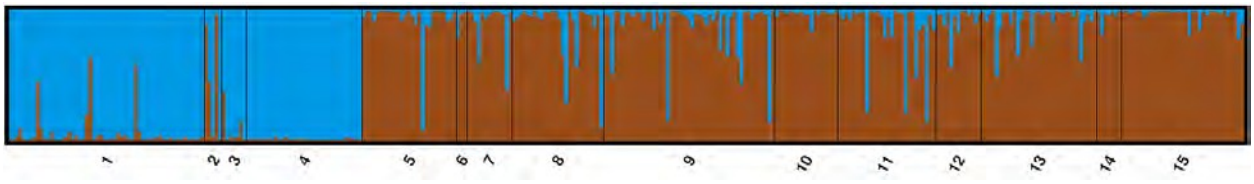

Figure F3. Bayesian genetic clustering results for all samples of Coastal Tailed Frogs post-harvest assuming K=2. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and site 15 in the South Cascades.

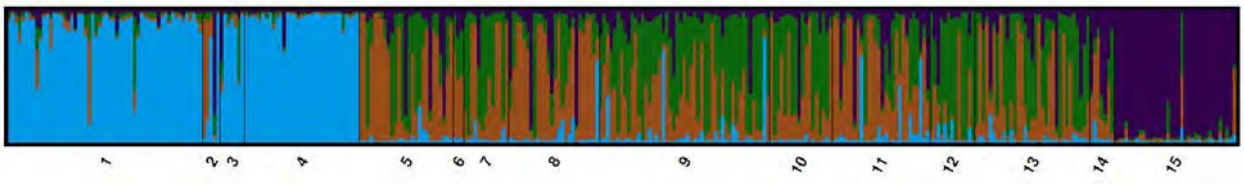

Figure F2. Bayesian genetic clustering results for all samples of Coastal Tailed Frogs post-harvest assuming *K*=4. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**.

Appendix G. Genetic clustering of Cope's Giant Salamander

STRUCTURE bar plots for K = 7, 8, 10, 11, and 12 for pre-treatment period and K = 6, 9, and 10 for the post-harvest period for Cope's Giant Salamander.

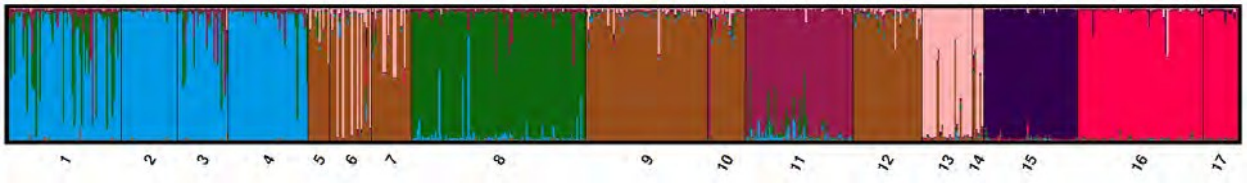

Figure G1. Bayesian genetic clustering results for all samples of Cope's Giant Salamander pre-harvest assuming K=7. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

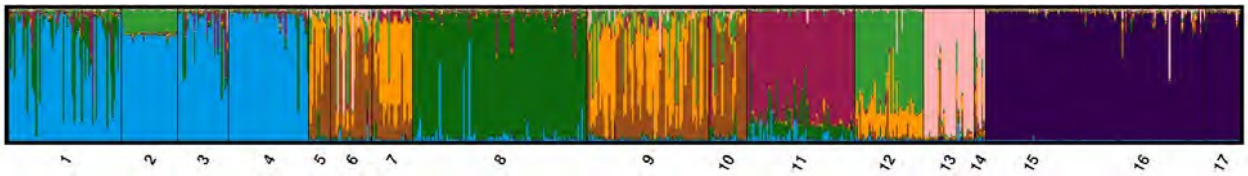

Figure G2. Bayesian genetic clustering results for all samples of Cope's Giant Salamander pre-harvest assuming K=8. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

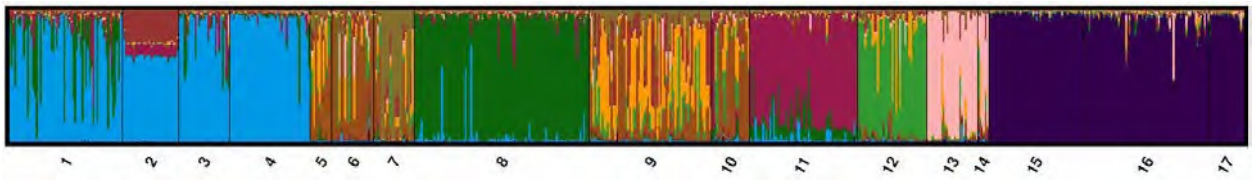

Figure G3. Bayesian genetic clustering results for all samples of Cope's Giant Salamander pre-harvest assuming K=10. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

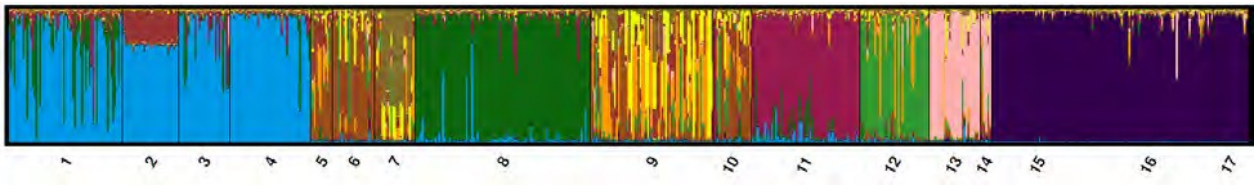

Figure G4. Bayesian genetic clustering results for all samples of Cope's Giant Salamander pre-harvest assuming *K*=11. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

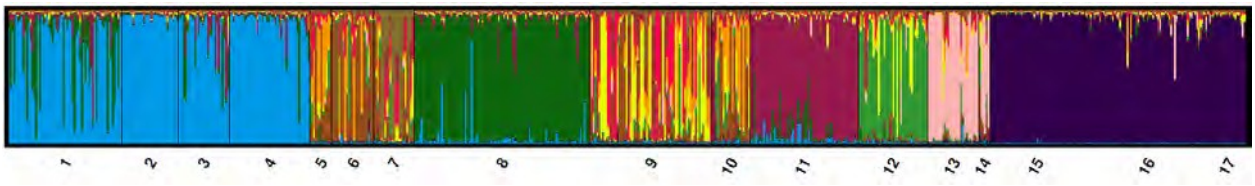

Figure G5. Bayesian genetic clustering results for all samples of Cope's Giant Salamander pre-harvest assuming *K*=12. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

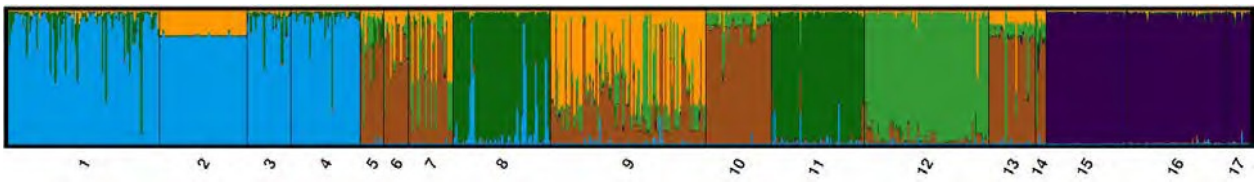

Figure G6. Bayesian genetic clustering results for all samples of Cope's Giant Salamander post-harvest assuming *K*=6. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

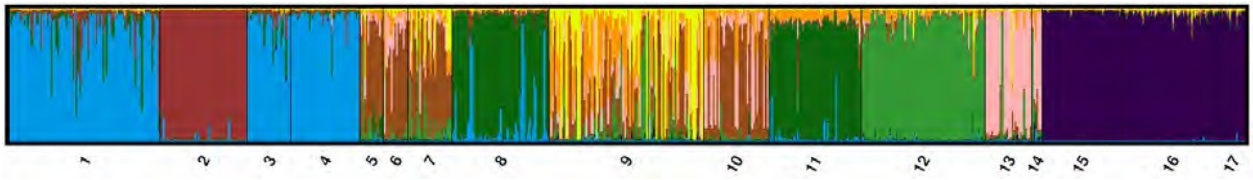

Figure G7. Bayesian genetic clustering results for all samples of Cope's Giant Salamander post-harvest assuming *K*=9. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

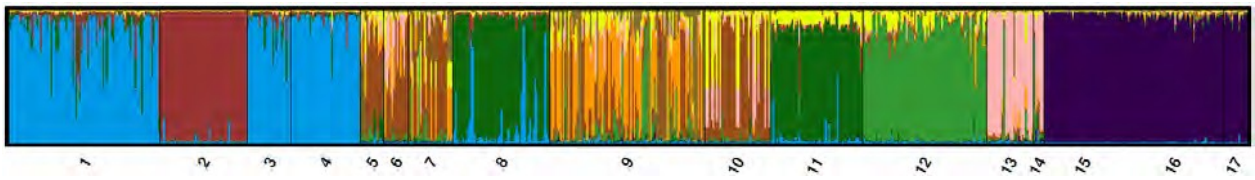

Figure G8. Bayesian genetic clustering results for all samples of Cope's Giant Salamander post-harvest assuming *K*=10. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 1-4 located in the Olympics, sites 5-14 in the Willapa Hills and sites 15-17 in the South Cascades.

Appendix H. Genetic clustering of Coastal Giant Salamander

STRUCTURE bar plots for K = 2, 4, and 5 for pre-treatment period and K = 2 and 5 for the post-harvest period for Coastal Giant Salamander.

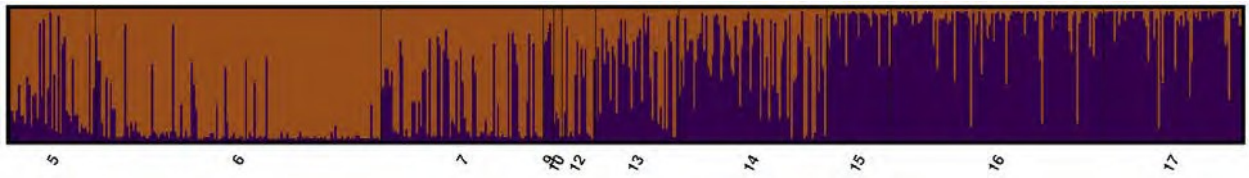

Figure H1. Bayesian genetic clustering results for all samples of Coastal Giant Salamander pre-harvest assuming K=2. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 5-7, 9, 10 and 12-14 in the Willapa Hills and sites 15-17 in the South Cascades.

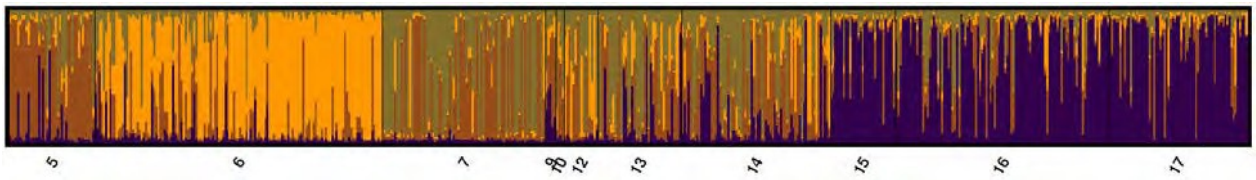

Figure H2. Bayesian genetic clustering results for all samples of Coastal Giant Salamander pre-harvest assuming K=4. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 5-7, 9, 10 and 12-14 in the Willapa Hills and sites 15-17 in the South Cascades.

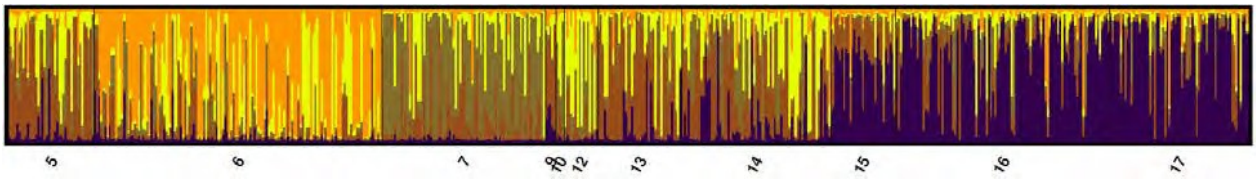

Figure H3. Bayesian genetic clustering results for all samples of Coastal Giant Salamander pre-harvest assuming K=5. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 5-7, 9, 10 and 12-14 in the Willapa Hills and sites 15-17 in the South Cascades.

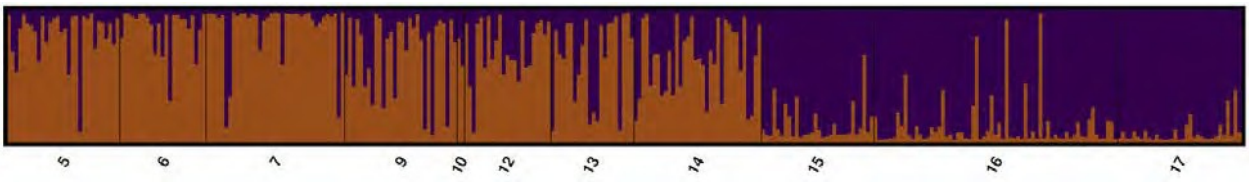

Figure H4. Bayesian genetic clustering results for all samples of Coastal Giant Salamander post-harvest assuming *K*=2. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 5-7, 9, 10 and 12-14 in the Willapa Hills and sites 15-17 in the South Cascades.

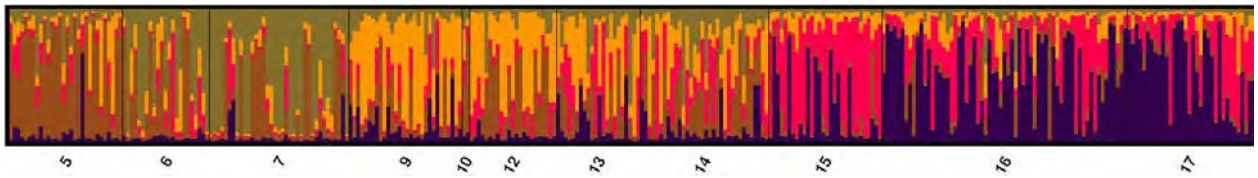

Figure H5. Bayesian genetic clustering results for all samples of Coastal Giant Salamander post-harvest assuming *K*=5. The y-axis represents proportion of individual membership to each cluster. Colors indicate different clusters. Numbers on the x-axis represent sites in the order presented in **Table 2**, with sites 5-7, 9, 10 and 12-14 in the Willapa Hills and sites 15-17 in the South Cascades.