2016 Chehalis Intensive Study in Off-Channel Habitats 3rd (31 December 2016) Progress Report

Marc Hayes, Julie Tyson, Keith Douville, Jacob Layman, Tara Newman, and Kevin Young Washington Department of Fish and Wildlife, Habitat Program Science Division, Aquatic Research Section

EXECUTIVE SUMMARY

Introduction: The purpose of the Intensive Study was to determine the seasonal patterns for stillwater-breeding amphibians and other Aquatic Species Restoration Plan (ASRP) nonsalmonid target species in off-channel habitats in the mainstem Chehalis floodplain over a twoyear period. We focused on six off-channel habitats selected across exotic predator abundance and hydroperiod gradients, which are the conditions thought to most strongly influence the native aquatic biota in these habitats. This report updates the intensive survey effort through the water year October 2015-September 2016; this effort represents one half of the planned overall effort. This study, part of the larger effort addressing off-channel habitats, contributes directly and indirectly to the ASRP. Its goals are to contribute to identifying seasonal aquatic biota habitat utilization patterns in off-channel habitats in the Chehalis floodplain, to support the Programmatic Environmental Impact Statement (PEIS) development process, to evaluate potential changes in off-channel habitats in the Chehalis floodplain as a consequence of flood control alternatives via coupling to inundation modeling, and to help inform and prioritize restoration efforts in the Chehalis floodplain. We conducted this work with the generous permission of one public and nine private landowners: Thomas Christin, Darryl Dick and Darlene Toland, Wayne Gray, Roy and Joyce Osborn, Andrew and Linda Styger, Weyerhaeuser Natural Resources Company, and Washington Department of Fish and Wildlife (WDFW).

Methods: We typically surveyed each site on two consecutive day intervals on a roughly monthly rotation beginning the first week of October 2015. We used a single overnight trap set of collapsible minnow traps, fyke nets and turtle traps coupled with dipnet surveys as the focal sampling methods. High water conditions required additional days to fully survey sites in a few circumstances. Beginning with either the 7th or 8th sampling round (April or May 2016), we also began conducting one electrofish sampling effort on an every other survey rotation; this was done to verify which aquatic taxa may have been missed or underestimated in abundance.

Results: Concordant with the water year, we sampled the six target sites through 12 monthly rounds. At each site, we found 3-5 native stillwater-breeding amphibians from the suite of: Long-toed salamander (*Ambystoma macrodactylum*), Northern red-legged frog (*Rana aurora*), Northwestern salamander (*Ambystoma gracile*), Pacific treefrog (*Pseudacris regilla*), and Roughskin newt (*Taricha granulosa*). We also detected at least 5-9 native fish species at all but the upstream-most intensive site (Weyerhaeuser) from the suite of: Coho salmon (*Oncorhynchus kisutch*), Pacific lamprey (*Entosphenus tridentatus*), Largescale sucker (*Catostomus macrocheilus*), Northern pikeminnow (*Ptychocheilus oregonensis*), Olympic mudminnow (*Novumbra hubbsi*), Redside shiner (*Richardsonius balteatus*), sculpin (*Cottus* sp.),

Speckled dace (*Rhinichthys osculus*), and Three-spine stickleback (*Gasterosteus aculeatus*). At the four sites located furthest downstream, we also detected the exotic American bullfrog (*Lithobates catesbeianus*), and at all except the upstream-most site, at least 3-9 exotic fish species from the suite of: Black crappie (*Pomoxis nigromaculatus*), Bluegill (*Lepomis macrochirus*), Brown bullhead (*Ameiurus nebulosus*), Common carp (*Cyprinus carpio*), Largemouth bass (*Micropterus salmoides*), Pumpkinseed (*Lepomis gibbosus*), Rock Bass (*Ambloplites rupestris*), Smallmouth bass (*Micropterus dolomieu*), and Yellow perch (*Perca flavescens*). Some uncertainty in species richness (= number of species) reflects currently unidentified lamprey, sculpin, and juvenile sunfishes; their genetic verification is pending.

Aquatic species composition among the intensively surveyed sites was variable, but several patterns were evident. Native amphibian seasonal breeding phenology resulted in the appearance of eggs in the February-April 2016 sampling rounds at all sites, consistent with the typical post-winter thaw oviposition of the native amphibian species suite. Both native and exotic fish species richness increased with increasing downstream position of the intensive sites, but native amphibian richness showed no change with stream position. Additionally, one important feature suggests that presence of exotic amphibians and fishes affects native amphibian reproduction. Evidence of production (eggs and larval stages) following oviposition and recruitment (appearance of juveniles) was generally limited or non-existent at sites with significantly more aquatic exotics than natives. This pattern was most evident at the permanent hydroperiod sites such as Osborn, which was the site with the highest exotic-to-native-species ratio and had an abundance index of aquatic exotic species that was over 70-fold greater than that of native aquatic species; a less pronounced pattern was evident at less exotic-loaded sites. Sites where native amphibian production is greater are either isolated or have hydroperiods that appear to either limit successful bullfrog production, eliminate fish seasonally from the habitat, or a combination of these. However, apparent recruitment failure of native amphibians was not universally attributable to exotics. In particular, at the fish and exotic-free Weyerhaeuser site, and perhaps also at the exotic fish-occupied Christin site, recruitment failure of some native amphibians may reflect the dominance of the native Roughskin newt, a predator well known to focus seasonally on amphibian eggs.

Several patterns have emerged that are advisory for restoration. For amphibians, an intermediate hydroperiod appears to be required to ensure regular production and simultaneously limit exotics. For fishes such as Coho salmon, adequate seasonal connectedness, which is likely to depress warmwater exotics, may be necessary to maximize local production. The second year of sampling and the coupling of data from the 28-year timeline of Hydrologic Engineering Center—River Analysis System (HEC-RAS) inundation modeling to the data from the existing sites will solidify our understanding of the long-term seasonal connectedness (and perhaps hydroperiod) of the intensive sites, allow us to better understand these emerging patterns, and, in particular, determine whether or not they may be in conflict (i.e., are different types of off-channel sites needed to effectively restore habitat for native amphibians versus native fishes). Regardless of the outcome, enough uncertainties exist in the response to any

restoration actions performed in off-channel habitats to require an experimental approach (meaning a comparison to unmanipulated [control or reference] sites) in order for our findings to be useful in guiding future actions.

An emerging pattern advisory to monitoring for the ASRP is that, in floodplain off-channel habitats, five native amphibian species (hereafter referred to as core species) represent the current best condition, though one additional species, the Western toad, may be restorable to those habitats. Although all core species have potential for monitoring because they are all widespread, the species most likely to behave as an umbrella for the core species suite is the Northern red-legged frog. The latter conclusion is based on the fact that Northern red-legged frogs require a hydroperiod of intermediate length to successfully recruit. That hydroperiod requirement encompasses the hydroperiod requirements of most life history pathways needed by the other four native amphibians for successful recruitment. Importantly, the focus on hydroperiod intermediacy, as noted above, will be necessary for effective restoration in an exotic-occupied landscape until effective solutions (currently lacking) for exotic removal are found. With regard to sampling effectiveness, electrofishing proved important for capturing exotic aquatic species, especially centrarchid fishes thought to be problematic for native species. If reduction of aquatic exotics as a result of restoration efforts is important to evaluate, electrofishing will be indispensable.

Next steps: The Intensive Study field effort will extend through another water year (September 2017) to obtain basic information on inter-year variability. It will be Important to determine how water year differences may change recruitment of native amphibians and fish occupancy and abundance; and how changes in hydroperiod and the exotic species suite may alter recruitment patterns for native aquatic species. When biotic data from both years are coupled to data from the 28-year HEC-RAS modeled inundation timeline, it will refine our understanding of variation in seasonal connectedness for the Intensive Study sites, and provide an indication of the pattern of connectedness that allows native species success. We will also strategically deploy temperature dataloggers to improve our resolution of hydroperiod. Lastly, we will use salmon roe-baited collapsible minnow traps to improve our detection of Coho and other salmonids.

INTRODUCTION: Intensive Study surveys were designed to identify **seasonal** changes in the aquatic biota of off-channel habitats in the mainstem Chehalis River floodplain that could not be obtained with the more temporally restricted egg mass and extensive surveys in those habitats. This refers mainly to patterns of use by aquatic biota not present in those habitats year-round, such as selected life stages of stillwater-breeding amphibians (e.g., adult northern red-legged frogs [*Rana aurora*]) and fishes (e.g., juvenile Coho salmon [*Oncorhynchus kisutch*]). As a consequence, surveys were focused on six off-channel habitats that were sampled at a monthly resolution. This report summarizes progress of the Intensive Study survey efforts through September 2016.

SITE SELECTION: We selected six sites (Figure 1) from the 324-site pool of off-channel habitats spanning the entire floodplain of the Chehalis mainstem from the proposed dam location (just above Pe Ell) to the 101 bridge in Aberdeen; this is the same pool of sites used to structure site selection for the egg mass and extensive surveys of floodplain off-channel habitats. We defined the mainstem floodplain as the FEMA-specified 100-year floodplain plus an additional 100 meters drawn perpendicular to that line.

Figure 1. Location of the six intensively surveyed sites (red dots) in the Chehalis mainstem floodplain. For perspective, a portion of the proposed flood control reservoir at full pool is shown south of the Weyerhaeuser site location.



We selected the six sites based on three criteria: 1) proximity to the proposed dam and reservoir; 2) connectedness to the Chehalis mainstem; and 3) relative abundance of exotic aquatic species. The first criterion reflected the need to have some sites with a greater likelihood of being influenced by proposed flood control alternatives, because a key focus was to inform evaluation of those alternatives. The latter two criteria reflected a need to have some understanding of the behavior of aquatic biota in off-channel habitats that were more versus less connected to the mainstem and had a greater versus lesser loading of exotic aquatic

species. Information on connectedness and relative abundance of exotic species was based on data from a combination of egg mass and extensive surveys during the 2015 season, as all six of the intensive survey sites had been surveyed during at least one of those efforts (all completed prior to 1 October 2015). Data used to select these sites based on the aforementioned three criteria are provided in **Table 1**.

Table 1. Criteria upon which selection was based for the six Intensive sites surveyed and their variation based on 2015 pre-survey data. Sites listed by proximity to proposed dam site (RM 108.3 [RKm 173.9]).

		Selection	Criteria		
	Site Name	Proximity – River Miles (RM) below	Connectedness to	Exotic Aq	uatic Species
		Proposed Dam Site (PDS)	Chehalis mainstem	Bullfrogs	Warmwater Fishes
1	007_Weyerhaeuser	0.7 RM [1.1 RKm] below PDS @107.6 RM [172.8 RKm]	Never	None	None
2	004_Christin	14.5 RM [23.3 RKm] below PDS @ 93.8 RM [150.6 RKm]	Limited Seasonally	None	Some
3	020_Styger_N	31.7 RM [51.0 RKm] below PDS @ 76.6 RM [123.0 RKm]	Limited Seasonally	Some	None
4	068_Osborn	31.7 RM [51.0 RKm] below PDS @ 76.6 RM [123.0 RKm]	Substantial Seasonally	Some	Many
5	025_Dick	64.0 RM [103.0 RKm] below PDS @ 44.3 RM [71.1 RKm]	Substantial Seasonally	Some	Some
6	086_Hoxit 2	71.8 RM [115.6 RKm] below PDS @ 36.5 RM [58.6 RKm]	Substantial Seasonally	Some	Some

SAMPLING: We sampled the six Intensive Study sites on an approximately monthly rotation; **Table 2** shows the sampling dates through the September 2016. All surveys were conducted with at least three people over at least two days. We established 10 relatively evenly spaced transects at each site, at which we recorded vegetation composition, percent cover, water temperatures, deepest depth, visibility, wetted widths and distance between transects. Biotic surveys employed dipnets (25 dipnet samples/site), minnow traps (3 traps/transect; 30 total per site), and at least one fyke and one turtle net. We placed all traps on the first sampling day each month, left them overnight, and retrieved them the next day, at which time we processed then released animals. Processing meant identifying, photographing, and/or measuring species caught in traps, and recording their location and capture method. We also noted other relevant data (e.g., bird or mammal activity or sign). Lastly, beginning in the 7th or 8th sampling round, we added electrofishing to the sampling schedule on every other visit. The purpose of this addition was to determine if we had either missed important fish species or severely underestimated the relative abundance of selected aquatic taxa. Because this effort was a late addition, we present those data separate from the balance of the information.

We also conducted amphibian egg mass surveys during the most effective interval for this type of survey (mid-January—end of April). These surveys included a visual encounter survey (VES) for amphibian egg masses of the entire site (up to approximately 1 m in water depth) and 50 additional dipnet samples. Each site was surveyed for egg masses at least three times, with approximately one month between surveys. Data from these surveys are combined with the data from the Intensive Study surveys when reporting results.

Table 2. Location, elevation and survey dates for the six Extensive Survey Off-Channel Sites over the first 12 surveys spanning the 1st water year (October 2015 through September 2016). Latitude and longitude (in decimal degrees) are from a relatively central point within each off-channel site.

	Site Name	Location			Elevation		Survey	Round	
	Site Name	Chehalis River Segment	Latitude	Longitude	feet (meters)	1st	2nd	3rd	4th
1	007_Weyerhaeuser	Elk Creek to Proposed Dam	46.550690	-123.304193	450 ft (137 m)	14-Oct	9-Nov	7-Dec	19-Jan
2	004_Christin	South Fork Chehalis River to Elk Creek	46.635691	-123.166147	235 ft (72 m)	20-Oct	16-Nov	14-Dec	25-Jan
3	020_Styger_N	Newaukum River to South Fork Chehalis River	46.647589	-123.001875	170 ft (52 m)	22-Oct	23-Nov	16-Dec	13-Jan
4	068_Osborn	Newaukum River to South Fork Chehalis River	46.644339	-122.997943	165 ft (50 m)	26-Oct	30-Nov	27-Dec	27-Jan
5	025_Dick	Porter Creek to Black River	46.826396	-123.257333	50 ft (15 m)	2-Nov	2-Dec	5-Jan	8-Feb
6	086_Hoxit 2	Porter Creek to Black River	46.911953	-123.302853	40 ft (12 m)	6-Oct	4-Nov	21-Dec	11-Jan

	Site Name				Survey	Round			
	Site Name	5th	6th	7th	8th	9th	10th	11th	12th
1	007_Weyerhaeuser	10-Feb	9-Mar	11-Apr	11-May	14-Jun	12-Jul	11-Aug	13 Sep
2	004_Christin	22-Feb	14-Mar	13-Apr	16-May	16-Jun	14-Jul	16-Aug	22 Sep
3	020_Styger_N	24-Feb	23-Mar	20-Apr	18-May	21-Jun	19-Jul	17-Aug	19-Sep
4	068_Osborn	29-Feb	28-Mar	25-Apr	25-May	28-Jun	28-Jul	25-Aug	27-Sep
5	025_Dick	2-Mar	29-Mar	27-Apr	23-May	30-Jun	26-Jul	30-Aug	29-Sep
6	086_Hoxit 2	1-Feb	7-Mar	5-Apr	2-May	8-Jun	7-Jul	4-Aug	7-Sep

RESULTS: At this time, we have sampled all six Intensive Study sites through 12 monthly rounds (**Table 2**). High water conditions in late November and December required minor shifts in the schedule (see dates in **Table 2**), but these shifts only slightly altered our timeline. Reduced visibility during the sixth sampling round (mostly in March) resulted from rain during the surveys and increased turbidity due to elevated levels of suspended material. The latter was particularly prominent at the Christin and Weyerhaeuser off-channel sites. All results are presented in site order of proximity to the proposed dam site, from up- to downstream (see **Table 1**).

The Weyerhaeuser Site: Weyerhaeuser, the site most proximate to the proposed dam and the highest elevation of the six intensively surveyed sites (**Figure 1**, **Table 1**), is the only intensive site that is probably never connected to the Chehalis mainstem during high water, as a consequence of its elevated position (vertically about 45 ft [13.7 m]) above ordinary high water in the Chehalis mainstem. Sampling through the 12th round has not revealed either exotic vertebrates or native fish at this site. This human-created pond, originally built to receive stormwater runoff from the adjacent Weyerhaeuser management facility, likely has a permanent hydroperiod; it retained water in late summer during the extreme drought year of 2015. Other than the aforementioned runoff pattern, one 1.8-km unnamed stream flows toward this pond, but bypasses it via a conveyance ditch just west of the Weyerhaeuser facility. A mixed forest of Douglas-fir (*Pseudotsuga menziesii*), Red alder (*Alnus rubra*) and Western red cedar (*Thuja plicata*) surrounds most of this pond.

Aquatic vertebrates in the Weyerhaeuser pond were exclusively native amphibians. Roughskin newts (*Taricha granulosa*) were the prominently dominant species (**Figure 2A and 2B**). All native amphibians except newts exhibited direct evidence of reproduction (eggs)¹ in this pond, but Northwestern salamanders (*Ambystoma gracile*) laid the greatest numbers of eggs (see especially April in **Figure 2B**). Only Northwestern salamanders had modest larval numbers and their larvae or neotenes² were detected in every month of the year (**Figure 2A** and **2B**). However, small numbers of larval newts were observed in every month of the year except for March (**Figure 2A**) and May (**Figure 2B**); larval newt numbers appeared slightly higher in July and August than during the rest of the year. Very few Long-toed salamander (*Ambystoma macrodactylum*) and Pacific treefrog (*Pseudacris regilla*) egg packets were found in the Weyerhaeuser pond (see March in **Figure 2A** and April in **Figure 2B**). No larval Long-toed

¹ Newts lay single eggs concealed in soft aquatic vegetation and as a consequence, their oviposition patterns were impractical to track. However, based on the larval newts observed, they probably successfully reproduce in this pond.

² Northwestern salamanders have two life-history pathways. The familiar pathway is to metamorphose into a form capable of utilizing terrestrial habitats. However, an alternative pathway is to mature into a larviform adult; this life stage looks like an oversized larva (possesses gills) but is reproductive. Neotenes occur in reliably permanent-water habitats where food resources are rarely limited. We scored neotenes as larvae in counts on figures.

Figure 2A. Time series of native amphibian life stages for first half of the 2015-2016 Water Year for the Weyerhaeuser Intensive Site – includes all sampling.



Figure 2B. Time series of native amphibian life stages for second half of the 2015-2016 Water Year for the Weyerhaeuser Intensive Site – includes all sampling except the electrofish sampling begun on 11 May 2016 on an every two-month rotation.



Figure 3. Comparison between electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for different amphibians at the Weyerhaeuser intensive site.



salamanders were detected, and few Pacific treefrog larvae were found during the summer months (see July-September in **Figure 2B**). Northern red-legged frogs deposited a fair number of egg masses in February and March (see **Figure 2A**), but we detected very few of their larvae (see May-July in **Figure 2B**). Except for newts, December numbers for all amphibian species were depressed. Adult newt numbers increased rather than declining during the winter period.

During the three samplings in which it was applied, electrofishing at Weyerhaeuser produced few amphibians (Figure 3). Electrofishing detected fewer individuals than non-electrofishing methods for three of the four amphibian species recorded. This was prominently evident for Northwestern salamanders and Roughskin newts, where the numbers of individuals recorded were large (Figure 3). Numbers of Northern red-legged frogs were so small that any conclusion about differences between methods was uncertain. We recorded adult or larval Pacific treefrogs on two electrofishing sampling dates, but their numbers were low enough that any conclusions about patterns are also uncertain.

The Christin Site: Christin, the intensive site next in proximity to the proposed dam (Figure 1, Table 1), is largely separated from the Chehalis mainstem by a berm for the old railroad grade used for part of the Willapa Hills Trail system. At least one culvert connects the Christin off-channel wetland to the mainstem through this berm, but that culvert appears partially debris obstructed, so this may affect changes in water levels in the wetland during high water periods. Three drainages also feed this wetland from upslope, and only one of these had yearround water input. The Christin site is largely surrounded by riparian forest dominated by Bigleaf maple (Acer macrophylum), though a fenced pasture approaches the northwest margin. Four of the five native amphibian species found in the Weyerhaeuser pond were also present in the Christin wetland (Figures 4A and 4B). Pacific treefrog, the fifth native amphibian, was recorded at Christin based on one adult incidentally observed while obtaining vegetation data, but not during regular sampling. Also similar to the Weyerhaeuser pond, some adult newts were observed at every sampling round, though in much lesser numbers than in the Weyerhaeuser pond (compare Figures 2A, 2B to Figures 4A, 4B). Further, similar to the Weyerhaeuser pond, all amphibians except newts (see footnote 1) laid some eggs in the Christin wetland (see February-March in Figure 4A and April in Figure 4B). Low numbers of Northwestern salamanders eggs recorded in March (see Figure 4A) was coincident with a high turbidity high water event that markedly reduced visibility. However, we subsequently recorded no larvae of Long-toed and Northwestern salamanders and few larvae of Northern red-legged frogs over the balance of the season (see Figure 4B). Moreover, unlike the Weyerhaeuser pond, we recorded no larval newts during the October-February interval (see Figure 4A). We found amphibian numbers to be generally depressed in the November-January interval (see Figure 4A).

We also recorded at least 11 fish species in the Christin wetland, including at least six native species (**Figure 5**) and at least five exotic species (**Figure 6**). Based on non-electrofish sampling,

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Figure 4A. Time series of native amphibian life stages for first half of the 2015-2016 Water Year for the Christin Intensive Site – includes all sampling.



Figure 4B. Time series of native amphibian life stages for second half of the 2015-2016 Water Year for the Christin Intensive Site – includes all sampling except the electrofish sampling begun on 17 May 2016 on an every two-month rotation.



Figure 5. Time series of native fishes for the Christin Intensive Site – includes all sampling except the electrofish sampling begun on 17 May 2016 on an every two-month rotation.



Figure 6. Time series of exotic fishes for the Christin Intensive Site – includes all sampling except the electrofish sampling begun on 17 May 2016 on an every two-month rotation.



we recorded exotic fish species (n = 81) almost four times as frequently as native fishes (n = 22; compare **Figures 5** and **6**). Except for one bullhead catfish recorded on 14 July, all exotic fishes were centrarchids (**Figure 6**), including Bluegill (*Lepomis macrochirus*), Largemouth bass (*Micropterus salmoides*), Pumpkinseed (*Lepomis gibbosus*), and Rock bass (*Ambloplites rupestris*). Of the exotic fishes, Largemouth bass (n = 47) was the most frequently recorded and represented over half of the observations of exotic fishes (**Figure 6**). The eight individuals of unknown sunfishes (**Figure 6**), all juveniles, are likely Bluegill or Pumpkinseed, but we cannot exclude a possible third sunfish species or a hybrid of these two species. Species verification of these juveniles based on genetic evaluation of their sampled tissues is pending.

On the three dates sampled using electrofishing, the lamprey species were recorded only with electrofishing (**Figure 7A**). Further, sculpins were recorded only with electrofishing on two of the three dates (**Figure 7A**), and all exotic species (all centrarchids) were recorded more frequently with electrofishing except for pumpkinseed on one date (**Figure 7B**). We did not record any taxa with electrofishing that had not already been recorded with non-electrofishing methods on other dates. Notably, no amphibians were recorded with electrofishing.

The next two intensive sites, Osborn and Styger, are located nearly the same distance downstream from the proposed dam and are both at approximately the same elevation (**Figure 1**, **Table 1**). However, Osborn is located on the north bank of Chehalis River, whereas Styger is located on its south bank.

The Osborn Site: The Osborn site is a horseshoe-shape oxbow within an agricultural landscape. Its margin is lined with mostly herbaceous vegetation; a few Douglas-fir, Big-leaf maple, and Oregon ash (*Fraxinus latifolia*) trees occur along its margin. The <10-m wide herb and shrub margin forms the interface between the oxbow and pastures or plowed fields of the surrounding landscape. The Osborn oxbow was seasonally connected to the Chehalis River mainstem at high water in each year of our observations (2015-2016). It became disconnected for over half the year annually during low flow, but maintained a permanent hydrology. Most of the Osborn oxbow is steep-sided and it has a slowly graded shallowing only on its ends and in one small side arm.³

Osborn differed from all the other intensive sites by having the most limited evidence of native amphibian reproduction: six eggs masses or packets collectively laid by three species (see the March dates in **Figure 8A**). In contrast, we recorded numerous observations of exotic American bullfrogs (*Lithobates catesbeianus*) at Osborn, mostly as larvae, and they were captured in every month of the year except for December (**Figures 8A** and **8B**). Based on non-electrofishing methods, we also recorded a modest number of observations (n = 32) of at least four native fish species, mostly Northern pikeminnow (*Ptychocheilus oregonensis*) and sculpins (*Cottus* sp.; **Figure 9A**). In contrast, we recorded seven times as many observations of exotic fishes with non-electrofishing methods (n = 243; **Figure 9B**), representing at least six species,

³ The small side arm of the Osborn oxbow, located on its north side, appears excavated.

Figure 7A. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for different native fish species at the Christin Intensive Site.



Figure 7B. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for different native fish species at the Christin Intensive Site.



Figure 8A. Time series of amphibian life stages for the first half of the 2015-2016 water year at the Osborn Intensive Site – includes all sampling.



Figure 8B. Time series of amphibian life stages for the second half of the 2015-2016 water year at the Osborn Intensive Site – includes all sampling except the electrofish sampling begun on 26 April 2016 on an every two-month rotation.



Figure 9A. Time series of native fishes for the Osborn Intensive Site – includes all sampling except the electrofish sampling begun on 26 April 2016 on an every two-month rotation. One native fish species, Largescale sucker (*Catostomus macrocheilus*), recorded as only two individuals during electrofishing is not shown here; see **Figure 9A** for those data.



Figure 9B. Time series of exotic fishes for the Osborn Intensive Site – includes all sampling except the electrofish sampling begun on 26 April 2016 on an every two-month rotation.



but bullhead catfishes (*Ameiurus sp.*) were numerically dominant (n > 211; **Figure 9B**).⁴ Similar to the Christin site, we found two juvenile sunfish that could not be visually identified (**Figure 9B**); these may be Bluegill or Pumpkinseed, but the same aforementioned uncertainty about a third species or hybrids cannot be excluded; their genetic verification is pending.

For the eight taxa that could be compared, electrofishing performed uniformly better than non-electrofishing methods for American bullfrog larvae (Figure 10A), Brown bullhead and Yellow perch (Figure 10B), and also generally better for Largemouth bass and Pumpkinseed (Figure 10B). Numbers of adult and juvenile American bullfrogs, Largescale sucker, Pacific lamprey (Figure 10A) and Bluegill (Figure 10B) were too low to compare methods. Largescale sucker was the only species recorded with electrofishing that was not recorded with non-electrofishing methods. Sculpins were the only taxon for which more individuals were recorded with non-electrofishing methods than with electrofishing (Figure 10A),⁵ but low numbers qualify this comparison. No native amphibians were recorded with electrofishing.

The Styger Site: The Styger intensive site is near the opposite of Osborn in its characteristics. In particular, Styger is topographically complex. Based on data from the combination of fall 2015 and late summer 2016, Styger has a seasonal hydroperiod, but is at least partially fed by decades-old drain tiles from adjacent fields. In the extreme drought year of 2015, Styger came extremely close to drying completely in October; 2016 was wetter than 2015, but Styger had dried completely by 17 August 2016. However, Styger's regular seasonal spring connection with the Chehalis mainstem at high water appears to last longer than the connection at Osborn. Styger is imbedded in a landscape that is a mosaic of agricultural fields and riparian forest. Black cottonwoods (*Populus trichocarpa*) and Big-leaf maples dominate the riparian forest.

We recorded all five native amphibians at Styger that we documented at the Weyerhaeuser site (compare Figures 11A and 11B and Figures 2A and 2B). However, based on non-electrofish sampling, the aquatic vertebrate assemblage at Styger differed from that at Weyerhaeuser in four important ways: first, Roughskin newts were infrequent (19 observations overall: 3 adults and 16 larvae); second, all native amphibians showed evidence of modest to high levels of larval production, and in the case of Pacific treefrogs, larval production was quite high (see April in Figure 11B); third, native fishes were seasonally abundant, especially Three-spine stickleback (*Gasterosteus aculeatus*) (Figure 12A); and fourth, exotic species were observed in only modest numbers (American bullfrogs; Figures 11A and 11B) or were observed extremely infrequently (all exotic fishes; Figure 13B). Observations of American bullfrog post-metamorphic stages were few (Figures 11A and 11B), but the species displayed a larval pulse in July (Figure 11B). Long-toed salamander adults appeared in relatively large numbers in the November-January interval, peaking in December (Figure 11A). No amphibians were found in October of 2015 (Figure 11A)

⁴ The 200 value of unknown bullhead on Figure 9B is a conservative estimate, the actual number was likely larger.

⁵ Sculpins lack a swim bladder, which affects how they react to the electrofisher.

or in August and September of 2016 (Figure 11B), when, respectively, almost no water was present. Lastly, dead and dying American bullfrog larvae and Three-spine sticklebacks were evident in July, when the aquatic footprint of Styger was drying.

Non-electrofishing methods were approximately equal to or more effective for sampling of amphibian species at Styger (Figure 13A). Non-electrofishing methods were also equal or superior to electrofishing for sampling of American bullfrogs and fishes, especially Three-spined sticklebacks (Figure 13B).

Figure 10A. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for American bullfrogs and native fishes at the Osborn intensive site.



Figure 10B. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for American bullfrogs and native fishes at the Osborn intensive site.



Figure 11A. Time series of amphibian life stages for the first half of the 2015-2016 water year for the Styger Intensive Site – includes all sampling.



Figure 11B. Time series of amphibian life stages for the second half of the 2015-2016 water year for the Styger Intensive Site – includes all sampling except electrofishing begun on 19 May 2016 on an every two-month rotation.



Figure 12A. Time series of native fish species for the Styger Intensive Site – includes all sampling except electrofishing begun on 19 May 2016 on an every two-month rotation.



Figure 12B. Time series of exotic fish species for the Styger Intensive Site – includes all sampling except electrofishing begun on 19 May 2016 on an every two-month rotation.



Figure 13A. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for different native amphibians at the Styger Intensive Site. Thirteen larvae of the Unknown *Ambystoma* (mole salamander) could have been either Long-toed or Northwestern salamanders. If a sampling date does not appear, it indicates that the life stage of that amphibian was not recorded by either sampling program (electrofish and non-electrofish sampling) for that particular date.



Figure 13B. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for the exotic American bullfrog and different fish species at the Styger Intensive Site. If a sampling date does not appear, it indicates that the life stage of that amphibian or fish was not recorded by either sampling program (electrofish and non-electrofish sampling) for that particular date.



Dick, the fifth Intensive Study site, is a crescent-shaped oxbow located on the southwest bank of the Chehalis River, somewhat downstream of the confluence with the Black River (**Figure 1, Table 1**). The Dick oxbow is largely surrounded by a broad band of riparian forest dominated by Big-leaf maple, Black cottonwood, and Oregon ash; outside this band is a mosaic of agriculture and more riparian forest.⁶ The Dick site appears to have a near-permanent hydroperiod and a regular seasonal hydrological connection with the Chehalis mainstem.

We recorded all five native amphibian species at Dick that we found at Styger and Weyerhaeuser (compare Figures 14A and 14B to Figures 11A and 11B and 2A and 2B). Similar to Styger, Roughskin newts were observed infrequently. Production of larval native amphibians was more substantial than at other sites, though not quite as great as at Styger. Also similar to Styger, bullfrog numbers were modest and larval bullfrog production had a July pulse, with juvenile bullfrogs not recorded later during the summer. Unlike Styger, Long-toed salamanders appeared to be a less important part of the native amphibian assemblage (compare Figures 14A and 14B and 11A and 11B). However, based on non-electrofishing sampling methods, Dick diverged markedly from Styger in its high species richness of both native (Figure 15A) and exotic fishes (Figure 15B). In particular, Dick harbored at least nine native fish species; Threespine stickleback was by far the most frequently observed (Figure 15A). In addition, Darryl Dick (personal communication), the landowner of this site and an avid fisherperson, has observed juvenile Coho salmon utilizing this oxbow. The Dick site also harbored at least seven exotic fish species recorded in modest numbers with the exception being Largemouth bass, for which large numbers of juveniles were recorded in May (Figure 15B). However, the exotic fish assemblage showed a sharp increase in observations during the summer months when contrasted with winter months (Figure 15B).

Non-electrofishing methods uniformly outperformed electrofishing methods for native amphibians and fishes at the Dick Intensive Site with the possible exceptions of Northern pikeminnow and Largescale sucker, but the numbers for the latter two species are too small for unambiguous determination (**Figures 16A** and **16B**). In sharp contrast, electrofishing generally outperformed non-electrofishing methods for exotic aquatic species (American bullfrogs and warmwater fishes; Figure 16C).

The Hoxit Site: The sixth and last site, Hoxit 2, was the downstream-most Intensive Study site (Figure 1, Table 1). A Washington Department of Fish and Wildlife-managed site, the hydrology of Hoxit 2 appears to be partially influenced by an adjacent wetland containing a small dam that helps maintain its aquatic footprint. The culvert in this dam has a water control structure, and its water level is drawn down seasonally to a smaller pool, in part to enable

⁶ We did not survey a large portion of the Dick site that consists of inundated forest in sections of the old river channel that flood/connect during high water. This is because stillwater amphibians typically do not deposit eggs in areas of shaded closed canopy.

Figure 14A. Time series of amphibian life stages for the first half of the 2015-2016 water year at the Dick Intensive Site – includes all sampling.



Figure 14B. Time series of amphibian life stages for the Dick Intensive Site – includes all sampling except electrofishing, which began on 28 Apr 2016 date on an every two-month rotation.



Figure 15A. Time series of native fishes for the Dick Intensive Site – includes all sampling except electrofishing, which began on 28 Apr 2016 date on an every two-month rotation.



Figure 15B. Time series of exotic fishes for the Dick Intensive Site – includes all sampling except electrofishing, which began on 28 Apr 2016 date on an every two-month rotation.



Figure 16A. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for different native amphibians at the Dick Intensive Site. AMsp indicates unidentified *Ambystoma* (mole) salamanders; these could be either Long-toed or Northwestern salamanders.



Figure 16B. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for native fishes at the Dick Intensive Site.



Figure 16C. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for exotic aquatic species at the Dick Intensive Site.



haying of the adjacent pasture on its west side and in part to maintain vegetated waterfowl habitat within the aquatic footprint. The outflow below Hoxit 2 has a direct connection to the Chehalis River mainstem, albeit through a relict beaver dam (not actively maintained by beavers). During seasonal high water in most years, the entire area inundates in a manner that allows the Chehalis River mainstem flow to move through the north end of the site.

We recorded the same five native amphibian species at Hoxit 2 that we found at the Weyerhaeuser, Styger, and Dick Intensive Sites (compare Figure 17A and 17B to Figures 2A and 2B, 11A and 11B, and 14A and 14B). Hoxit 2 appears to experience modest native amphibian egg and larval production, but no juveniles of native amphibians were observed and newts were observed infrequently. Most amphibian egg masses at Hoxit 2 were observed in an arm of the pond that is seasonal and at least partially isolated by berms. We also made a fair number (n = 217) of observations of American bullfrogs at Hoxit 2 (Figure 17A and 17B). Based on non-electrofishing methods, Hoxit 2 also had the highest fish species richness of any of the intensive sites, both for native fishes (at least seven taxa; Figure 18A) and exotic fishes (at least nine taxa; Figure 18B). Among native fishes, Northern pikeminnow was dominant (Figure 18B). Northern pikeminnow was most frequently recorded fall to spring (Figure 18A), whereas exotics were most frequently recorded in late spring and summer (Figure 18B).

Electrofishing data, which began during the 8th (3 May 2016) sampling round revealed that in comparison to non-electrofishing methods, it provided no advantage for sampling native amphibians. Further, its benefit for bullfrogs seemed limited, though this conclusion reflects comparisons made only for larval stages (**Figure 19A**). Similarly, except possibly for lamprey and sculpins, electrofishing seemed to provide little benefit over non-electrofishing methods for sampling native fishes (**Figure 19B**); the small sample size for lamprey and sculpins justifies broader evaluation. With some small sample size exceptions on selected days, electrofishing seems generally advantageous over non-electrofishing methods for exotic fishes (**Figure 19C**).

Figure 17A. Time series of amphibian life stages for first half of water year 2015-2016 at the Hoxit 2 Intensive Site – includes all sampling.



Figure 17B. Time series of amphibian life stages for first half of water year 2015-2016 at the Hoxit 2 Intensive Site – includes all sampling.except the electrofish sampling, which began 3 May 2016 on a two-month rotation.



Figure 18A. Time series of native fishes over the 2015-2016 water year for the Hoxit 2 Intensive Site – includes all sampling except the electrofish sampling, which began 3 May 2016 on a two-month rotation.



Figure 18B. Time series of exotic fishes over the 2015-2016 water year for the Hoxit 2 Intensive Site – includes all sampling except the electrofish sampling, which began 3 May 2016 on a two-month rotation.



Figure 19A. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for different amphibians at the Hoxit 2 Intensive Site. Species are not partitioned by life stage because all comparison involve only larvae.



Figure 19B. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for different native fishes at the Hoxit 2 Intensive Site.



Figure 19B. Comparison of electrofish and non-electrofish sampling (dip net, collapsible minnow trap and fyke net sampling combined) for different exotic fishes at the Hoxit 2 Intensive Site.



Discussion: We found considerable variability in the aquatic biota across the Intensive Study sites. We collectively recorded five different native stillwater-breeding amphibian species at Intensive Study sites: Long-toed salamander, Northern red-legged frog, Northwestern salamander, Pacific treefrog, and Roughskin newt. All five native amphibian species were found at all sites, except Osborn, which lacked Northern red-legged frog and Roughskin newt (**Table 3**), and native amphibian species richness showed no obvious trends in floodplain position. We found only one exotic amphibian species, the American bullfrog, at any of the six Intensive Study sites, and all American bullfrog observations were made at the four most downstream locations (**Table 3**). Importantly, major differences among sites with amphibians, exotic or native, were not differences in species richness patterns, but differences in seasonal abundance patterns.

				Amphik	oian Spe	ecies		
	Site Name	Long-toed salamander	Northern red-legged frog	Northwestern salamander	Pacific treefrog	Roughskin newt	Native Amphibian Richness	American bullfrog
1	007_Weyerhaeuser	Х	Х	Х	Х	х	5	
2	004_Christin	Х	Х	Х	Х	х	5	
3	068_Osborn	Х		Х	Х		3	х
4	020_Styger_N	Х	Х	Х	Х	х	5	Х
5	025_Dick	Х	Х	Х	Х	х	5	Х
6	086_Hoxit 2	х	Х	Х	Х	х	5	х

Table 3. Summary of amphibian species composition across Intensive Study sites. This includesdata collected from all sampling methods plus incidental observations.

We collectively recorded at least 10 different native fish species at Intensive Study sites: Coho salmon, Largescale sucker, Northern pikeminnow, Olympic mudminnow, Pacific lamprey, Redside shiner, Sculpin (species undetermined), Specked dace, Three-spine stickleback and Western Brook lamprey (**Table 4**). A third lamprey species may be represented among the unidentified lamprey recorded, and as many as three sculpin taxa may be represented among the undetermined sculpins; genetic verification of these taxa is pending. The Weyerhaeuser site lacked fish entirely. For the sites with native fish species, Osborn had the fewest (n = 3), and Dick (n = 9) and Hoxit 2 (n = 8) had the most (**Table 4**). Northern pikeminnow was the only native fish species recorded at all five sites where fish were present. Olympic mudminnow was the only native fish species recorded at only one site (Dick) (**Table 4**). Except for the Osborn site, native fish species richness generally increased with downstream position (**Table 4**).

We collectively recorded at least nine different exotic fish species at four Intensive Study sites: Black crappie, Bluegill, Brown bullhead, Common carp, Largemouth bass, Pumpkinseed, Rock bass, Smallmouth bass, and Yellow perch (**Table 5**). Unknown bullhead and sunfishes were

Table 4. Summary of native fish species composition across Intensive Study sites. Richness is counted based on the minimum number of possible species excluding pending genetic differentiation of lamprey and sculpin taxa.

						Na	ative Fish	Species					
	Site Name	Coho salmon	Largescale sucker	Northern pikeminnow	Olympic mudminnow	Pacific lamprey	Westem Brook lamprey	Unknown Iamprey	Redside shiner	Speckled Dace	Three-spine stickleback	Unknown sculpin	Richness
1	007_Weyerhaeuser												0
2	004_Christin		х	х		Х	х			х		х	6
3	068_Osborn		х	х		Х			Х			х	5
4	020_Styger_N	х		х				х		х	х		5
5	025_Dick		х	х	х	Х	х	х	Х	х	х	х	9
6	086_Hoxit 2	Х	х	х		Х		х	Х	х	х	х	8

Table 5. Summary of exotic fish species composition across Intensive Study sites. Richness is counted based on the minimumnumber of possible species excluding pending genetic differentiation of bullhead and sunfish taxa.

							Exotic Fis	sh Spe	cies				
	Site Name	Black crappie	Bluegill	Brown bullhead	Common carp	Largemouth bass	Pumpkinseed	Rock bass	Smallmouth bass	Yellow perch	Unknown bullheads	Unknown sunfishes	Richness
1	007_Weyerhaeuser												0
2	004_Christin		х			х	х	х			х	х	5
3	068_Osborn		х	х		х	х	х		Х	х	х	6
4	020_Styger_N			х			х	х					3
5	025_Dick	Х	х	х		х	х	х		Х		х	7
6	086_Hoxit 2	Х	х	x	х	х	х	х	х	х		х	9

juveniles that could not be visually identified to species. These sunfishes may be Bluegill or Pumpkinseed, but a third sunfish species or a hybrid may also be represented. We also cannot exclude the possibility that the unknown bullhead represents a species other than Brown bullhead. As with the lamprey and sculpin, genetic verification of these taxa is pending.

Of the six sites, only Weyerhaeuser lacked fish. Of the remaining sites, we recorded the fewest exotic fish species at Styger (n = 3), and Hoxit 2 had the most (n = 9); exotic fish species richness generally increased with downstream position (**Table 5**). We recorded two centrarchid fishes (Pumpkinseed, and Rock Bass) at all five sites with some exotic fish species present, and Bluegill, Brown bullhead and Largemouth bass were also present at four of the five sites with exotic fishes (**Table 5**).

Examining the aquatic biota collectively, we recorded at least 25 different amphibian and fish species across Intensive Study sites. The general increase in overall aquatic species richness with progressive downstream position reflects the more or less parallel trends for native and exotic fish species richness previously noted (**Table 6**). Exotic species contribution to the overall species richness of amphibians and fishes (ratio of exotic to native species [i.e., the number of exotic species divided by the number of native species]) varied from zero (Weyerhaeuser) to 0.88 (Osborn; **Table 6**).

	Site	A Spec	mphibi ies Ricł	an nness	Spec	Fish ies Ricł	nness	&	Overall Fish Spe	Amphik ecies Ric	bian Chness
	0.00	Native	Exotic	Totals	Native	Exotic	Totals	Native	Exotic	Totals	Exotic/Native Ratio
1	007_Weyerhaeuser	5	0	5	0	0	0	5	0	5	0.00
2	004_Christin	5	0	5	6	5	11	11	5	16	0.45
3	068_Osborn	3	1	4	5	6	11	8	7	15	0.88
4	020_Styger_N	5	1	6	5	3	8	10	4	14	0.40
5	025_Dick	5	1	6	9	7	16	14	8	22	0.57
6	086_Hoxit 2	5	1	6	8	9	17	13	10	23	0.77

Table 6. Summary of overall aquatic species richness across Intensive Study sites. Speciesrichness is again counted based on the minimum number of possible species excluding pendinggenetic differentiation of selected taxa.

We also found large variability across the Intensive Sites in relative abundance. Using a relative abundance index,⁷ species-specific relative abundance indices for native amphibians varied from zero (several species at different sites) to as high as 129 (Roughskin newt at the

⁷ The abundance index was based on summing a species' life stages over each survey round and averaging these across the 12 survey rounds. Egg masses or egg packets were each counted as a single unit in this index despite their containing varied numbers of individuals (embryos).

Weyerhaeuser site; **Table 7**). This analysis reveals that though the Weyerhaeuser site had the highest mean relative abundance index (30.1), the Dick and Styger sites were also native amphibian productive (20.2-22.1). In contrast, the Christin, Hoxit 2, and Osborn sites all had low mean relative abundance indices for native amphibians (0.1-2.9). The American bullfrog relative abundance index was similarly variable across sites, ranging from a high of 77.2 at Osborn to zero at Christin and Weyerhaeuser (**Table 7**). Mean relative abundance index for American bullfrog had no clear relationship to the mean relative abundance index for native amphibians across the six intensive sites ($\rho = -0.522$; $\rho_{critical} = -0.830$), but the number of units (sites) for this comparison are too few for adequate evaluation.

				Abu	ndance In	dices ^ª		
	Site Name	Long-toed salamander	Northern red-legged frog	Northwestern salamander	Pacific treefrog	Roughskin newt	Mean Native Abundance Index	American bullfrog
1	007_Weyerhaeuser	0.2	15.1	32.3	1.1	102.0	30.1	0.0
2	004_Christin	0.8	1.8	8.3	0.3	8.8	2.9	0.0
3	068_Osborn	0.1	0.0	0.3	0.1	0.0	0.1	77.2
4	020_Styger_N	85.3	12.8	8.2	97.4	1.6	22.1	18.8
5	025_Dick	4.2	28.8	19.8	46.6	1.8	20.2	16.7
6	086_Hoxit 2	1.5	6.7	5.0	0.1	0.8	2.8	18.1

Table 7. Summary of amphibian species relative abundance indices across intensive study sites.

^a See footnote 3

Relative abundance indices for native fishes also varied. Values ranged from zero (several species at several sites) to as high as 176.5 (Three-spine stickleback at the Styger site; **Table 8**). This analysis revealed that the Styger site had the highest mean abundance index for native fish species (35.6); Dick, the second ranked site for native fishes, had a mean relative abundance index of slightly over one-seventh that value (4.7) and Hoxit 2, the third ranked site for native fishes, had a relative abundance index of less than a third the latter (**Table 8**). Dick and Styger were also notable due to their relatively high relative abundance indices for Three-spine stickleback, whereas Hoxit 2 was notable for its relatively high Northern pikeminnow relative abundance index.

Relative abundance indices for exotic fishes were also variable. Values ranged from zero (several species at several sites; **Table 9**) to as high as 17.6 for bullhead catfishes (combined Brown bullhead and unknown bullhead at the Osborn site; **Table 9**). Of the sites with exotic fish species, Styger had the lowest mean relative abundance index for exotic fish species (**Table 9**). This analysis also revealed that the Dick site had the highest mean abundance index for exotic fishes (3.8) and Osborn (3.3) was a relatively close second (**Table 9**). Largemouth bass had a relatively high relative abundance index (\geq 3.9) at four of the five sites at which they were recorded; Pumpkinseed, Bluegill, and Unknown sunfishes were the only other fish species with

							Abundan	ce Indice	S				
	Site Name	Coho salmon	Largescale sucker	Northern pikeminnow	Olympic mudminnow	Pacific lamprey	Western brook lamprey	Lamprey sp.	Redside shiner	Sculpin sp.	Speckled dace	Three-spine stickleback	Mean Abundance Index
1	007_Weyerhaeuser	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	004_Christin	0.0	0.3	0.3	0.0	0.1	0.1	0.0	0.0	0.6	0.5	0.0	0.3
3	068_Osborn	0.0	0.0	1.1	0.0	0.2	0.0	0.0	0.1	2.6	0.0	0.0	1.0
4	020_Styger_N	0.2	0.0	1.2	0.0	0.0	0.0	0.1	0.0	0.0	0.1	176.5	35.6
5	025_Dick	0.0	0.2	2.2	1.4	0.9	0.1	0.1	0.6	1.8	0.1	40.0	4.7
6	086_Hoxit 2	0.1	0.0	7.7	0.0	0.2	0.0	0.3	0.3	0.4	0.7	1.2	1.4

Table 8. Summary of native fish species relative abundance indices across Intensive Study sites.

Table 9. Summary of exotic fish species relative abundance indices across Intensive Study sites.

							Abundar	nce Ind	lices				
	Site Name	Black crappie	Bluegill	Brown bullhead	Common carp	Largemouth bass	Pumpkinseed	Rock bass	Smallmouth bass	Yellow perch	Unknown bullhead	Unknown sunfishes	Mean Abundance Index
1	007_Weyerhaeuser	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	004_Christin	0.0	0.5	0.0	0.0	3.9	0.9	0.3	0.0	0.0	0.1	0.7	1.1
3	068_Osborn	0.0	0.3	0.9	0.0	8.0	4.6	0.4	0.0	1.0	16.7	0.3	3.3
4	020_Styger_N	0.0	0.0	0.1	0.0	0.0	0.1	<0.1	0.0	0.0	0.0	0.0	0.1
5	025_Dick	0.3	3.3	0.3	0.0	17.3	2.1	0.3	0.0	1.1	0.0	6.0	3.8
6	086_Hoxit 2	0.1	1.0	0.1	0.1	7.9	1.5	1.3	0.1	0.1	0.0	6.8	1.9

relative abundance indices > 3 at any sites (**Table 9**). Notably, all the fishes with relative abundance indices > 3, except for bullheads, are centrarchid species.

Similar to the relative abundance index between native and exotic amphibians, the relative abundance index between native fishes showed no clear relationship to that for exotic fishes ($\rho = 0$; see fishes columns in **Table 10**). Considering amphibians and fishes together also did not improve the relationship between native and exotic species ($\rho = -0.486$; see totals columns in **Table 10**). Similar to amphibians, the number of sites for this comparison is too few for adequate evaluation.

	Sita Nama	Amph	ibians	Fish	nes	Tot	tals
	Site Name	Native	Exotic	Native	Exotic	Native	Exotic
1	007_Weyerhaeuser	30.1	0.0	0.0	0.0	30.1	0.0
2	004_Christin	2.9	0.0	0.3	1.1	3.2	1.1
3	068_Osborn	0.1	77.2	1.0	3.3	1.1	80.5
4	020_Styger_N	22.1	18.8	11.3	0.0	33.4	18.8
5	025_Dick	20.2	16.7	3.3	2.7	23.5	19.4
6	086_Hoxit 2	2.8	18.1	1.6	1.0	4.4	19.1

Table 10. Overall summary of species relative abundance indices across Intensive Study sites.

Native amphibian and fish recruitment patterns likely have different explanations across sites. At the apparently fishless Weyerhaeuser site, the dominance of predatory salamanders, especially the Roughskin newt, is the likely reason for suppression of recruitment in Long-toed salamanders, Northern red-legged frogs, and Pacific treefrogs. Post-metamorphic Roughskin newts are well known amphibian egg consumers (Chivers and Mizra. 2001, Kiesecker et al. 2002, Lehman 2006), and all three of these amphibians deposit their eggs in soft jellies (Jones et al. 2005), making their deposited eggs highly accessible to post-metamorphic newts. In contrast, Northwestern salamanders possess extraordinarily tough jelly, which is the firmest of any Pacific Northwest amphibian (Jones et al. 2005). Roughskin newts have been observed to excavate into this firm jelly to access eggs and developing embryos (M. Hayes, personal observation), but differential accessibility because of jelly toughness may partly explain why Northwestern salamanders were the only native amphibian with some larval recruitment. The fact that larval Northwestern salamanders were present in every sampling month (Figure 2A and **2B**) agrees with this hypothesis. Part of the aforementioned explanation may also be that the Northwestern salamander may be one amphibian in the assemblage of five native species recorded at Intensive Study sites with the greatest potential to significantly deter predators because of unpalatable granular glands that develop early in the larval stage (Larson and Hoffman 2002), though recent data on larval Roughskin newts suggest that the long-held assumption about their lack of toxicity may not be correct (Gall et al. 2011). However, in the

case of the Weyerhaeuser site, the predators are either Roughskin newts or other Northwestern salamanders, and the interaction between Northwestern salamander larvae and their unpalatability/toxicity to these potential predators is largely unstudied. Roughskin newts may have also influenced the extraordinarily low native amphibian recruitment at Christin because Christin was the only other Intensive Study site where post-metamorphic newts were common and appeared in every month (**Figures 4A** and **4B**). However, Christin also has exotic fishes may have also contributed to the pattern (**Figure 6**, **Table 9**) and the steep-sided structure of the Christin site may limit the degree to which native amphibians can lay eggs at this site.

Exotics may influence differential recruitment at the remaining four Intensive Study sites. Support for this hypothesis comes from Osborn, the site at which native amphibians had the lowest species richness (Table 6), the lowest abundance index (Table 9), and the most limited native amphibian oviposition among Intensive Study sites (compare Figures 8A and 8B to Figures 2A and 2B, 4A and 4B, 11A and 11B, 14A and 14B, and 17A and 17B), as well as no evidence of native amphibian larval recruitment (Figures 8A and 8B). Further, Osborn had the highest exotic to native species ratio (Table 6) and also had the highest abundance indices among intensive sites for both exotic fishes and bullfrogs (Table 9). At the other extreme is Styger, which had the best native amphibian egg and larval production across all sites with at least some fish present, few newts, and modest bullfrog numbers (Figures 11A and 11B). The remaining two sites (Dick and Hoxit 2) also seemed somewhat consistent with this pattern, displaying intermediate levels of both exotic abundance indices and recruitment among native amphibians, though other subtleties may influence details of these patterns. Although not mentioned in the results, both these sites have areas that become isolated and dry during the summer months; for Dick, the isolated sections seem to have more native amphibian adults, whereas for Hoxit 2, the isolated sections seem to have more native amphibian egg masses. Exotic-influenced recruitment patterns are in general agreement with published information indicating that exotics can suppress native aquatic species recruitment, and an influence from exotic fishes may be more likely to have a greater impact than influence from bullfrogs (Hayes and Jennings 1985, Adams 2000, Pearl et al. 2005). Moreover, the fact that centrarchid fishes were an important exotic element at all four sites where we suspect an exotic influence suggests that this species assemblage may be the important causal factor, as has been suggested elsewhere (Adams et al. 2003).

Hydroperiod, by affecting isolation and suppressing exotic recruitment, likely plays a significant role in amphibian recruitment, as Hayes and Jennings (1985) and Adams (1999) have previously suggested. The Intensive Study sites of Dick and Styger, which appear to have dried in both 2015 and 2016, support this hypothesis. At Styger, larval American bullfrog and fish mortality observed in July 2016 suggests that bullfrog production at this site was eliminated, as no juvenile American bullfrogs were observed following this mortality event and the site had

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dried completely by August 2016. Though we did not observe Styger when completely dried out, we suspect that it also dried in the summer of 2015 because our first observations in October 2015 revealed extraordinarily limited water in which no American bullfrog life stages were found, and unusual precipitation in August 2015 (**Figure 20**) was enough to have created the low water levels observed at our first 2015 visit in October. What is not clear about Styger is whether bullfrog production actually occurred in situ or bullfrog larvae gained entry to this site during high water; additional data will be needed to distinguish between the two.

Both the parallel pulse in larval American bullfrog and the near-complete lack of juvenile American bullfrogs at the portion of the Dick site that dried (Figure 14A and 14B) strongly resembles the pattern at Styger. In contrast, little if any suppression of exotics appears to have occurred at the three sites that had a permanent hydroperiod: Christin, Hoxit 2, and Osborn. The implication of this pattern is large because exotics exist in a large majority of Chehalis floodplain sites. If reduction of exotics is desired, two general options exist. The first is exotic elimination. This option is impractical because American bullfrogs can disperse overland to unoccupied aquatic habitats unless they are eliminated from the whole system (an unlikely possibility), and high water events will re-introduce exotic fishes into aquatic habitats from which they were previously removed. Moreover, elimination efforts themselves are impractically costly. The second option is to suppress exotics by altering habitat to manipulate hydroperiod or create habitat with a desirable hydroperiod. The aforementioned observations suggest that an intermediate hydroperiod can be successful in effecting native amphibian recruitment and suppressing exotic survival and recruitment. However, precise understanding of the hydroperiod, or rather the hydroperiod variation (among year variation must be considered), is necessary to effect a positive (successful) result. We cannot overemphasize that gaining such an understanding is tricky because of the interplay among the contribution of high water events, precipitation, ground inputs, soil characteristics and local topography contributing to inter-year variation that has complex local time lags. Further, though hydroperiod manipulation may benefit native amphibians, it may not benefit native fishes such as Coho salmon, so the impacts on the entire native aquatic assemblage must be understood when considering different options, because any one option may not be a universal benefit. At this juncture, it suffices to state that measurements of a number of these variables will be necessary to successfully restore habitat using such an option, and measurement will require some understanding of how different water years structure variation. A high degree of uncertainty unquestionably underlies such an effort, so restorations that elect this option must have an experimental structure (using control or reference sites) so that their results can usefully (adaptively) feed into future efforts.

One final pattern that needs mention is the limited observation of juvenile Coho salmon in the five Intensive Study sites that are at least seasonally connected. Our expectation was that

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Figure 20. Monthly variation in precipitation from the Centralia Meteorological Station (Station ID: USC00451276) for the water years 2014-2015 and 2015-2016. This station (latitude, longitude [in decimal degrees]: 46.7200, -122.9528; elevation: 185 ft [56.4 m]) is 5.6 mi (9.0 km) NNW of the Styger Intensive Study site.



we would see significant use of Intensive sites by Coho salmon during the fall to spring, as has been reported in the lower Chehalis (Henning et al. 2006, 2007). Very limited coho salmon use was observed only at Styger and Hoxit 2, and Darryl Dick, one of the Dick site landowners, indicated he had previously observed Coho salmon use of this site. We added electrofishing to our sampling efforts in part to improve Coho salmon detection, but that improvement was not realized. However, delayed receipt of equipment delayed electrofishing application in 2016 until the tail of the period when we would expect Coho salmon use of off-channel sites. But two thirds of our few observations of juvenile Coho salmon were obtained with non-electrofishing methods, so making ambiguous how much we may have actually missed. Regardless, several possibilities need consideration. First, earlier electrofish sampling may more reliably detect Coho salmon use at Intensive Study sites. Understanding that an adequate inundation season is always a caveat, conducting earlier electrofishing can be addressed in the second water year.

Second, Coho salmon use of off-channel habitats in the more upstream areas of the basin may be more limited. If electrofishing occurs earlier seasonally and few juvenile Coho salmon are found, this possibility would be supported. Electrofishing coincident with the companion Egg Mass Study can evaluate this alternative. Third, the 2015-2016 water year lacked adequate inundation for Coho salmon to make extensive use of off-channel habitat. The 2015-2016 water year, best described as modest in floodplain inundation, immediately followed an extreme drought year (2014-2015). The current water year gives all indications of improved water conditions and perhaps connectivity. Earlier electrofish sampling this year will provide an indication of the likelihood of this possibility, and HEC-RAS modeling being completed may also inform key between-year differences. Fourth, Intensive Sites we currently monitor may not facilitate much off-channel use during the fall-spring juvenile Coho utilization season because their seasonal connection is poor. This cannot be evaluated without restoring connectivity deemed inadequate via comparison to other sites. Future restoration efforts may provide insight. Fifth, electrofishing across the approximately 1 m (3 ft) depth range may miss most Coho salmon if depths outside that range dominate Coho salmon habitat utilization patterns in off-channel habitats. Both the work of Henning and colleagues (2006, 2007) and differential sampling deep versus shallow sampling in off-channel habitats for Coho salmon (Swales et al. 1987) contradict this possibility. Lastly, sampling for juvenile Coho using salmon roe-baited collapsible minnow traps indicates that this approach would be successful in off-channel habitats (Rosenfeld et al. 2008). We will evaluate this alternative this year.

Results from addition of electrofishing to our sampling suggest that it provides an advantage, but only for some taxa. Electrofishing provided no advantage for sampling native amphibians. In particular, non-electrofishing methods captured twice (Pacific treefrog) to over 18 times (Long-toed salamander) as many individuals as electrofishing over comparable intervals (Table 11). However, electrofishing appears to be about 1.6 times more effective than non-electrofishing methods in sampling American bullfrogs (Table 11). In contrast, electrofishing appears to provide a clear advantage for the sampling of at least one native fish, the Largescale sucker, and may provide some advantage for the sampling of lamprey and sculpins (**Table 12**). It is worth noting that the electrofishing advantage for selected lamprey and sculpins may be site-specific. More data are needed to determine the conditions where electrofishing may be advantageous for these two taxa. However, electrofishing seems clearly disadvantageous for the sampling of Northern pikeminnow, Olympic mudminnow and Threespine stickleback. Lastly, electrofishing was equal or better than non-electrofishing methods for all exotic fish taxa compared, except Rock bass (Table 13). In fact, two exotic taxa would not have been detected at two different sites if electrofishing had not been used. This detection failure pattern may be an underestimate because of the uncertainty about what the unknown sunfish category actually represents.

							Spec	ies					
	Site Name	Long-to salamai	oed nder	Northe legge	ern red- d frog	Northv Salarr	vestern nander	Pacific t	reefrog	Roughsk	in newt	America	n bullfrog
		E	NE	E	NE	E	NE	E	NE	E	NE	E	NE
1	007_Weyerhaeuser	0	0	1	3	27	81	7	2	15	180	0	0
2	004_Christin	0	0	0	0	0	0	0	0	0	19	0	0
3	068_Osborn	0	0	0	0	0	0	0	0	0	0	411	238
4	020_Styger_N	3	21	4	25	2	37	165	176	1	7	135	169
5	025_Dick	0	18	2	40	0	32	4	174	0	13	218	69
6	086_Hoxit 2	0	17	0	14	0	9	0	0	0	4	47	47
	Totals	3	56	7	83	29	159	176	352	16	223	811	523

Table 11. Summary of electrofishing (E) versus non-electrofishing (NE) results across Intensive Study sites for amphibians.

Table 12. Summary of electrofishing (E) versus non-electrofishing (NE) results across Intensive Study sites for native fishes. Electrofishing results for the Weyerhaeuser Intensive Study site are not included since no fishes were obtained at that site.

Site Name			Species																
		Coho salmon		Largescale sucker		Northern pikeminnow		Olympic mudminnow		Pacific lamprey		Western brook lamprey		Redside shiner		Three-spine stickleback		Unknown sculpin	
		Е	NE	Е	NE	Е	NE	Е	NE	Е	NE	Е	NE	E	NE	Е	NE	E	NE
2	004_Christin	0	0	1	2	0	1	0	0	1	0	3	1	0	0	0	0	8	2
3	068_Osborn	0	0	2	0	2	0	0	0	0	2	0	0	0	0	2	11	7	15
4	020_Styger_N	0	0	0	0	0	1	0	0	0	0	0	0	0	0	83	1092	0	0
5	025_Dick	0	0	1	0	0	1	0	12	4	8	0	1	0	2	0	224	1	7
6	086_Hoxit 2	0	1	2	0	2	17	0	0	2	2	0	0	0	0	1	9	3	1
	Totals	0	1	6	2	4	20	0	12	7	12	3	2	0	2	86	1336	19	77

Table 13. Summary of electrofishing (E) versus non-electrofishing (NE) results across Intensive Study sites for exotic fishes. Electrofishing results for the Weyerhaeuser Intensive Study site is not included since no exotic fishes were obtained with at those sites.

Site Name		Exotic Fish Species															
		Black crappie		Bluegill		Brown bullhead		Largemouth bass		Pumpkinseed		Rock bass		Yellow perch		Unknown sunfish	
		E	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E	NE
2	004_Christin	0	0	0	0	0	0	27	12	1	2	0	3	0	0	6	3
3	068_Osborn	0	0	1	1	8	4	55	54	31	4	0	0	16	6	3	0
4	020_Styger_N	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
5	025_Dick	1	0	16	17	0	0	48	13	4	1	1	1	50	5	37	22
6	086_Hoxit 2	0	0	4	4	12	0	67	53	10	7	0	3	0	0	41	24
	Totals	1	0	21	22	20	4	197	132	46	14	1	8	30	0	30	8

In summary, we sampled six Chehalis floodplain off-channel habitats with a suite of methods designed to detect both amphibians and fishes on a monthly rotation from October 2015 through May 2016. This effort revealed of the following patterns:

 Five different native stillwater-breeding amphibian species (Long-toed salamander, Northern red-legged frog, Northwestern salamander, Pacific treefrog, and Roughskin newt) were found at five of the six Intensive Study sites. The Northern red-legged frog and the Northwestern salamander were the only two species not detected at the sixth Intensive Study site: Osborn.

Two additional native stillwater-breeding amphibians with some possibility of occurring in Chehalis floodplain, the Oregon spotted frog and Western toad, were not found at any Intensive Study site. This pattern agrees with the Egg Mass and Extensive Studies, which involved much broader sampling of off-channel habitats in the Chehalis mainstem floodplain. In that study, Oregon spotted frogs were not detected, respectively, at any of the 86 (Egg Mass) and 98 (Extensive) different off-channel sites sampled across the length of Chehalis mainstem floodplain. In the Extensive Study, the Western toad was found at only two different off-channel locations on the lower Chehalis mainstem close to the Satsop and Wynoochee Rivers. In the Egg Mass Study, Western toads were recorded at three off-channel sites. In this case, one location was the same off-channel location close to the Satsop River, a second location was near Elk Creek and the third location was near the confluence of the South Fork Chehalis River with the Chehalis mainstem. However, all five detections of Western toads at these four sites involved older post-metamorphic juveniles or adults; no evidence of eggs, larvae or recently metamorphosed juveniles that might indicate breeding in off-channel habitats was found. These findings imply that Western toads may not breed in off-channel habitats in the Chehalis floodplain, and that the life stages found in these surveys dispersed from nearby locations in the mainstem where we have found breeding.

- 2) One exotic amphibian, the American bullfrog, was found at four of the six sites; the four sites at which American bullfrogs were found are the more downstream in position of the six sites. American bullfrogs were not detected at the two upstream-most sites; this pattern agrees with findings from the Egg Mass and Extensive Studies, where American bullfrogs were found to occur less frequently at the upstream end of the floodplain. Off-channel habitats are both less frequent and more distant from one another in the upstream tail of the floodplain, where the floodplain is more limited and a more incised channel exists.
- 3) The most upstream site and most disconnected from the floodplain, Weyerhaeuser, appears to be fishless. This pond, which was intentionally selected for its isolation, is a constructed pond that is perched about 45 ft (13.7 m) above the Chehalis mainstem at

normal high water. It may never have the opportunity for a water connection with the mainstem, enabling fish to enter, even during the most extreme high water events.

- 4) Except for Northwestern salamanders, native amphibian recruitment (as reflected by low larval stage numbers and lack of juveniles) appears to be extremely poor at the upstream-most site, the fishless Weyerhaeuser site. Recruitment limitation at this site may reflect the presence of post-metamorphic Roughskin newts, renowned amphibian egg predators that seem more abundant at this site than at the remaining five sites. Newts may also influence native amphibian recruitment at Christin, but Christin also has several exotic fishes that could influence amphibian recruitment (see point number 6 below). How much depression of native amphibian recruitment is due to newts versus exotic fishes at Christin is unclear. However, the steep Christin shoreline is also not ideal for amphibian oviposition, which could intrinsically limit native amphibian reproduction.
- 5) At sites where we recorded fishes, we found at least 5-9 native fish species from the suite of native fishes that included the following: Coho salmon, Lamprey (species not identified), Largescale sucker, Northern pikeminnow, Olympic mudminnow, Pacific lamprey, Redside shiner, sculpin (species not identified), Speckled dace, Three-spine stickleback, and Western brook lamprey. We also found 3-9 exotic species from the suite of exotic fishes that included: Black crappie, Bluegill, Brown bullhead, Common carp, Largemouth bass, Pumpkinseed, Rock bass, Smallmouth bass, Sunfish (species not identified), and Yellow perch. Tissue samples from unidentified lamprey and sunfish may or may not represent species already found; genetic identification of these samples is pending. Preliminary analysis of tissue samples from sculpin collected in off-channel habitat indicate that more than one taxon is represented; final results from further genetic analysis to determine the actual number of sculpin taxa are pending.
- 6) Exotic fishes, likely interacting with American bullfrogs, appear to have varying influence on native amphibian recruitment at the five sites where at least some exotic fishes are present because a) the one site with few exotic fishes, but American bullfrogs in modest numbers, Styger, has the best native amphibian recruitment; b) the one site with the highest exotic fish and American bullfrog abundance index, Osborn, has the most limited amphibian recruitment; and c) the three other sites, Dick, Hoxit, and Christin, with intermediate levels of exotic fishes and American bullfrogs have intermediate native amphibian recruitment levels.
- 7) All four sites with at least modest numbers of exotic fishes (all except Styger) have at least four exotic centrarchid fishes present: Bluegill, Largemouth bass, Pumpkinseed and Rock bass. Except for Osborn, which has Brown bullhead in abundance, these four fishes may be responsible for the native amphibian recruitment pattern noted in number 6 above with the possible exception of Christin, where native Roughskin newts may also contribute to the pattern.

- 8) Species richness of both native and exotic fishes generally increases with downstream position. Such a pattern is not evident for native amphibians. Both these patterns also agree with the identical patterns found across the entire Chehalis floodplain during the Egg Mass and Extensive Studies, and may reflect some combination of greater area of off-channel habitat and progressively greater contribution from the alluvial floodplain of major tributaries.
- 9) Electrofishing was useful for the detection of exotic fishes, particularly most centrarchid species, and the native Largescale sucker. It may also have some value for lampreys and sculpins, though more data are needed to clearly understand the pattern.
- 10) The influence of hydroperiod on the relative success of native amphibians and fishes and the relative failure of exotics, as exemplified in the patterns at Styger and Dick and in contrast to the exotic-occupied sites, represents a high-interest pattern for restoration simply because the elimination of exotics from the Chehalis basin is not a viable option given current technology. However, better understanding of the precise hydroperiod range that is needed to ensure the success of natives will be necessary to provide the best-considered approach for restoration.
- 11) Low levels of juvenile Coho salmon use of Intensive Site off-channel habitats remains a somewhat of a puzzle that is a confound between habitat conditions and sampling issues. Earlier electrofishing sampling and addition of salmon-roe baited collapsible minnow trap sampling should help clarify this problem.

The Intensive Study field effort will continue into 2017 through its second water year. We expect that the remaining months will continue to confirm or alter the conclusions we suggest here. However, particularly important will be the determination of the following: a) how a different water year may change recruitment of native amphibians across Intensive Study sites; b) how changes in hydroperiod and the exotic species suite may alter recruitment patterns in the native species suite. When biotic data from both years are coupled to data from the 28-year HEC-RAS modeled inundation timeline, it will refine our understanding of variation in seasonal connectedness (and perhaps hydroperiod) for the Intensive Study sites, and provide an indication of the pattern of connectedness that allows native species success. To improve our resolution in hydroperiod patterns, we will deploy temperature dataloggers at appropriate locations at all sites to identify temperature spikes that identify habitat drying. Lastly, sampling in the coming water year and HEC-RAS modeling associated with Intensive Sites can help sort out the reality of low levels of juvenile Coho salmon use.

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