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Predation on Larval Suckers in the Williamson River Delta Revealed by Molecular Genetic Assays—A Pilot Study

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U.S. Department of the Interior
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By Danielle M. Hereford, Carl O. Ostberg, and Summer M. Burdick

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Conversion Factors

International System of Units to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
	Area	
square kilometer (km^2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
microliter (µL)	3.381×10^{-5}	ounce, fluid (fl. oz)
gallon (gal)	3.785	liter (L)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as °F = (1.8 × °C) + 32.Abbreviations

Abbreviations

Ct	threshold cycles
DNA	deoxyribonucleic acid
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
SL	standard length
SNP	single nucleotide polymorphism
USGS	U.S. Geological Survey

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Abstract

Predation of endangered Lost River suckers (*Deltistes luxatus*) and shortnose suckers (*Chasmistes brevirostris*) during larval egress to Upper Klamath Lake from the Williamson River is poorly understood but may be an important factor limiting recruitment into adult spawning populations. Native and non-native piscivores are abundant in nursery wetland habitat, but larval predation has not been directly studied for all species. Larvae lack hard body structures and digest rapidly in predator digestive systems. Therefore, traditional visual methods for diet analysis may fail to identify the extent of predation on larvae. The goals of this study were to (1) use quantitative polymerase chain reaction (qPCR) and single nucleotide polymorphism (SNP) assays developed for Lost River and shortnose suckers to assay predator stomach contents for sucker DNA, and (2) to assess our ability to use this technique to study predation. Predators were captured opportunistically during larval sucker egress. Concurrent feeding trials indicate that most predators—yellow perch (*Perca flaverscens*), fathead minnow (*Pimephales promelas*), blue chub (*Gila coerulea*), Klamath tui chub (*Siphatales bicolor bicolor*), Klamath Lake sculpin (*Cottus princeps)*, slender sculpin (*Cottus tenuis*)—preyed on sucker larvae in the laboratory. However, sucker DNA was not detected in fathead minnow stomachs. Of the stomachs screened from fish captured in the Williamson River Delta, 15.6 percent of yellow perch contained sucker DNA. This study has demonstrated that the application of qPCR and SNP assays is effective for studying predation on larval suckers. We suggest that techniques associated with dissection or detection of sucker DNA from fathead minnow stomachs need improvement.

Introduction

Lost River sucker (*Deltistes luxatus*) and shortnose sucker (*Chasmistes brevirostris*) are federally listed endangered fishes endemic to the Upper Klamath Basin. Both suckers are long-lived, lake obligate species that spawn in rivers or at shoreline springs (National Research Council, 2004). These suckers have adult populations in Upper Klamath Lake—a large, shallow, hypereutrophic lake in southern Oregon. Survival of adult suckers is high, but populations continue to decline because recruitment of young suckers does not compensate for adult mortality (Hewitt and others, 2014). Poor juvenile survival is limiting the recovery of these species, and limited larval survival could be contributing to low juvenile abundance (Burdick and others, 2009). Several factors may be contributing to poor survival at early life stages, including inadequate rearing habitat, poor water quality, and predation by piscivorous fishes in sucker nursery habitat.

The recovery plan for Lost River sucker and shortnose sucker includes restoring wetland nursery habitat to support larvae and juvenile life stages (U.S. Fish and Wildlife Service, 2013). In 2007 and 2008, The Nature Conservancy breached levees and flooded approximately 20 km^2 (5,000 acres) of land that had been diked and drained in the 1940s. This created the Williamson River Delta Preserve (hereinafter "the Delta"), which effectively restored the hydrologic function between Agency Lake and Upper Klamath Lake to some semblance of its natural condition (Erdman and others, 2011). The restored habitat in the Delta supports larval and juvenile suckers at least as well as Upper Klamath Lake (Burdick and Hewitt, 2012).

River spawning sucker populations in Upper Klamath Lake spawn in the Williamson and Sprague Rivers between March and May, when water temperatures are greater than 10 °C (Martin and others, 2013; Hewitt and others, 2014). In May and June, larvae drift downstream and can be found in the Williamson River, Williamson River Delta, surrounding wetlands, and throughout Upper Klamath Lake (Burdick and Brown, 2010; Hewitt and others, 2011; Ellsworth and Martin, 2012).

There are many piscivorous and potentially piscivorous fishes in Upper Klamath Lake, the Delta, and the Williamson River. Non-native species include yellow perch (*Perca flaverscens*), fathead minnow (*Pimephales promelas*), brown bullhead (*Ameiurus nebulosus*), pumpkinseed (*Lepomis gibbosus*), largemouth bass (*Micropterus salmoides*). Native species (excluding native suckers) include blue chub (*Gila coerulea*), Klamath tui chub (*Siphatales bicolor bicolor*), Klamath Lake sculpin (*Cottus princeps*), Upper Klamath marbled sculpin (*Cottus klamathensis klamathensis*), slender sculpin (*Cottus tenuis*), Klamath redband trout (*Oncorhynchus mykiss* subsp.), and Klamath speckled dace (*Rhinichthys osculus klamathensis*). Piscivores that are known to prey on larval sucker include blue chub, fathead minnow, Klamath redband trout, Klamath tui chub, largemouth bass, pumpkinseed, and yellow perch (Markle and Dunsmoor, 2007; Burdick and Hewitt, 2012). Of these larval sucker predators, blue chub, fathead minnow, Klamath tui chub, and yellow perch are abundant in the Williamson River, the Delta, and adjacent lake habitat (Burdick and Hewitt, 2012). The extent to which predator species prey upon larval suckers is unknown.

Larvae are digested quickly because their small, soft bodies are not protected by more resilient body structures such as scales or exoskeletons. Controlled laboratory studies report larvae are visually unidentifiable 15–90 minutes post-consumption; some visual assays fail to observe larvae while others underrepresent the proportion of larvae relative to hard-bodied prey (such as invertebrates) (Folkvord, 1993; Symondson, 2002; Schooley and others, 2008). To reduce bias associated with unequal digestion, molecular techniques have been developed to study predation (Symondson, 2002). Prey can be identified by extracting DNA from stomach contents and using species-specific primers to target known single nucleotide polymorphisms (SNPs) for the species of interest (King and others, 2008). In laboratory studies, molecular techniques have detected larvae up to 48 hours after consumption, although consistent (more than 50 percent positive assay) amplification occurs less than 10–12 hours post-consumption (Carreon-Martinez and others, 2011; Ley and others, 2014). Other factors that influence digestion rates are temperature, stomach fullness, larvae size, and predator feeding mechanics (Folkvord, 1993; Handeland and others, 2008; Schooley and others, 2008; Legler and others, 2010; Carreon-Martinez and others, 2011). When prey is rapidly digested, the window of opportunity to identify prey is substantially larger using molecular techniques. Short fragments and more abundant mitochondrial DNA are often used in predator-prey studies because these fragments persist longer after ingestion (Rosel and Kocher, 2002; Dunshea, 2009; Carreon-Martinez and others, 2011). Another advantage of using molecular tools for gut analysis is sampling flexibility; overnight sets are often

required to catch target predator species, but larvae digest too quickly to be identified using visual techniques (Carreon-Martinez and others, 2011). Moreover, a variety of preservation methods can be employed for gut samples; most often fish are rapidly frozen whole, but some studies inject ethanol into the stomach to halt digestion (Barnett and others, 2010; Legler and others, 2010; Carreon-Martinez and others, 2011). Stomach lavage can be used on rare species that can not be sacrificed (Hartleb and Moring, 1995; Barnett and others, 2010). Molecular assays are commonly used to study predation because methods are widely applicable, more affordable, and quantitative assessment is, to some degree, possible.

Molecular assays enable quantitative analysis of prey DNA in predator gut contents. Predator gut contents typically contain unknown and variable quantities of prey DNA; therefore, standard curves must be developed for each DNA segment in order to estimate the quantity of prey DNA in predator guts. Standard curves utilize known concentrations of DNA, allowing the quantity of DNA in a sample to be estimated from the number of polymerase chain reaction (PCR) cycles required to detect amplification of the target region (Wong and Medrano, 2005; Ley and others, 2014). The number of cycles at which a PCR product is first detected is called the cycle threshold (Ct) value, and the threshold cycles value is inversely related to the amount of DNA in a sample. Therefore, low quantities of DNA have high Ct values because more PCR cycles are required for a product to be detected (Wong and Medrano, 2005). In addition, more concentrated forms of DNA, such as mitochondrial DNA, will amplify in fewer cycles than less concentrated forms, like nuclear DNA. When a DNA sequence from non-target species is similar to the primer sequences, non-specific binding of primers may occur, resulting in cross-amplification where DNA from the non-target species is amplified. Non-specific binding may be more typical for abundant mitochondrial DNA, and cross-amplification of the non-target species may be distinguished by substantially higher Ct values. Therefore, careful assessment of Ct values at different concentrations of DNA is essential for establishing a baseline for each primer (Wong and Medrano, 2005).

Before predation can be confirmed, DNA from predators, other prey items, and the water-column needs to be screened to verify the specificity of primers and to screen for environmental DNA (eDNA; King and others, 2008; Dunshea, 2009). Cross-amplification of DNA from non-target species or eDNA can cause false-positives and erroneous conclusions. Another potential pitfall is PCR inhibition or amplification failure, which can cause false negatives (Ley and others, 2014). PCR inhibition can be caused by a variety of agents including non-target DNA, body fluids, organic compounds, bacteria, pollen, soil, and humic acid, among others (Wilson, 1997; Gonzalez and others, 2005; Juen and Traugott, 2006). The pervasiveness of false negatives can be identified using controlled feeding trials (Admassu and others, 2006; Ley and others, 2014).

One way to assess the frequency of larval sucker predation in the Williamson River Delta is to identify gut contents of predators in the Delta using quantitative PCR (qPCR) and SNP assays developed for suckers. Hoy and Ostberg (2015) developed 20 SNP assays that distinguish Lost River suckers from other native suckers. These assays have been used to identify larval and juvenile suckers in Upper Klamath Lake, Oregon (Burdick and others, 2015; Hoy and Ostberg, 2015). To assess the prevalence of sucker DNA in the guts of predators and potential predators (hereinafter predators), we opportunistically collected fish from the Williamson River, the Delta, and surrounding wetlands (hereinafter Williamson River Delta) in spring 2015 when sucker larvae were drifting downstream. Some of these predators were used in positive-control laboratory feeding trials while others were sacrificed in the field. We screened all predator stomachs for sucker DNA using four sucker SNP assays.

The specific objectives of this pilot study were to (1) assess sucker SNP assays for cross-amplification of predator DNA, (2) assess amplification of four SNP assays in larvae collected from the Williamson River Delta, (3) assess our ability to detect sucker DNA in the guts of predators fed larvae in laboratory trials, and (4) assess the frequency of sucker DNA in the guts of predators captured in the Williamson River Delta.

Methods

Field Sampling

Habitat suspected of supporting sucker larvae in the Williamson River Delta was opportunistically sampled for sucker larvae from May 4 to May 19, 2015. Sucker larvae were captured using 30 cm dip nets with 1 mm mesh. When larvae were present, a small subsample (n= 6–48) were preserved in 95 percent ethanol for identification. When many larvae were present, another subsample was kept alive and returned to the laboratory for feeding trials. Larvae were identified as suckers using pigmentation (dorsal melanophores) and a subsample of larvae were measured to total length.

We set 1–3 trap nets in areas we observed and captured sucker larvae in the Williamson River and Delta (fig. 1). Initially trap nets were set for 2–4 hours to increase the likelihood of capturing predators with prey in their digestive system; however, zero predators were captured in these nets so all nets were set overnight. Upon retrieval, predators were immediately put on dry ice and stored at -80°C until gut contents could be extracted. Up to seven individuals of each opportunistically available predator species (table 1) were kept alive and transported in coolers with aerators to the laboratory for feeding trials. All predators were measured to standard length after sacrifice. Yellow perch and sculpins used in the feeding trials were collected from Hagelstein Pond, a spring fed pond connected to the eastern side of Upper Klamath Lake because we captured few specimens of these fishes from the Williamson River Delta.

Table 1. Fishes, excluding suckers, present in Williamson River Delta, Hagelstein Park, or Upper Klamath Lake, Oregon.

Common name	Scientific name	Native or introduced	Captured in this study
brown bullhead	*Ameiurus nebulosus*	I	X
Upper Klamath marbled sculpin	*Cottus klamathensis klamathensis*	N	X
Klamath Lake sculpin	*Cottus princeps*	N	X
slender sculpin	*Cottus tenuis*	N	X
blue chub	*Gila coerulea*	N	X
pumpkinseed	*Lepomis gibbosus*	I	-
largemouth bass	*Micropterus salmoides*	I	-
Klamath redband trout	*Oncorhynchus mykiss subsp.*	N	-
yellow perch	*Perca flaverscens*	I	X
fathead minnow	*Pimephales promelas*	I	X
Klamath speckled dace	*Rhinichthys osculus klamathensis*	N	-
Klamath tui chub	*Siphateles bicolor bicolor*	N	X

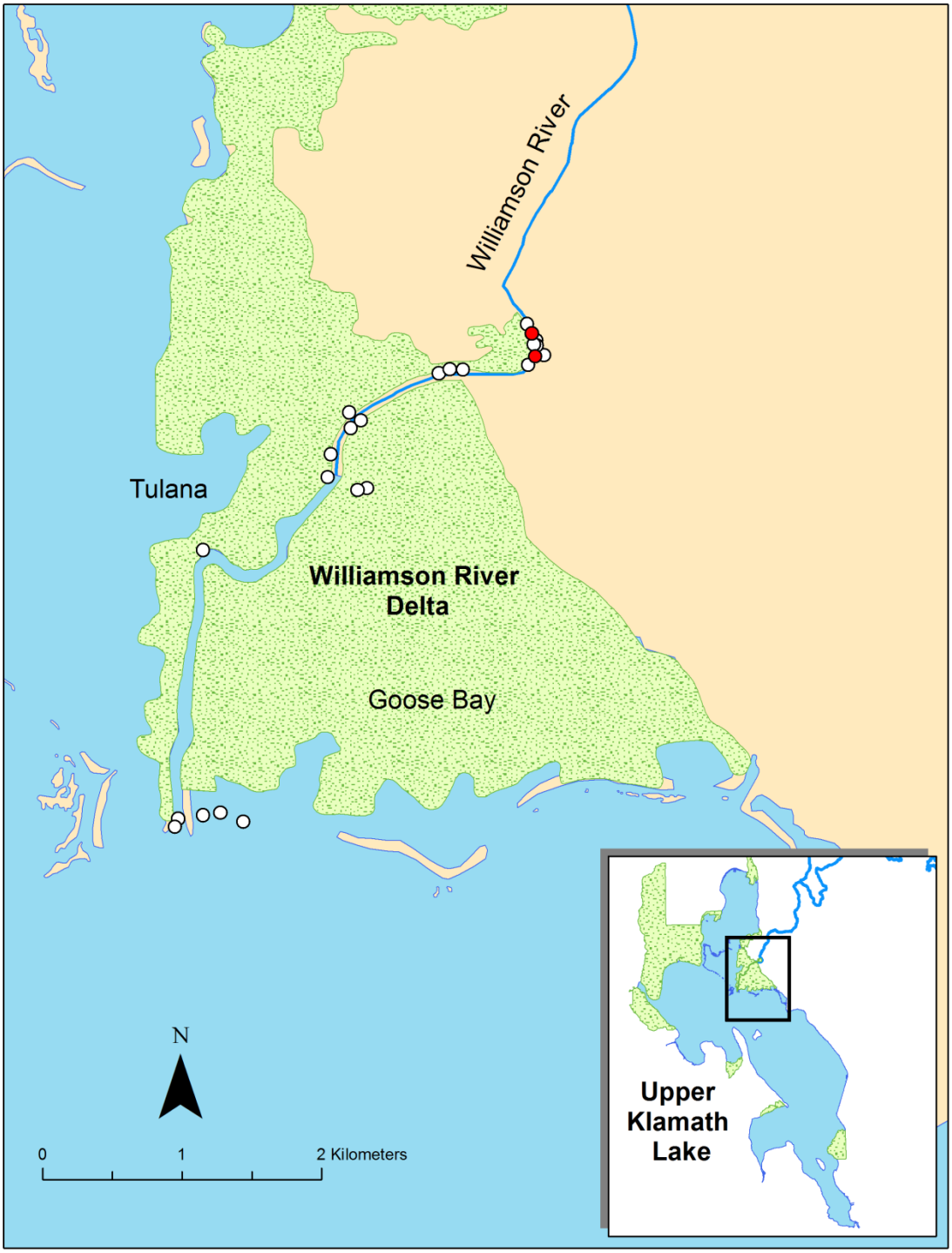

Figure 1. Sample locations where larvae were present (white and red circles) and capture locations of predators with positive molecular assays for sucker predation (red circles) in the Williamson River Delta, Oregon.

Feeding Trials and Dissection

Predators were starved for 16–48 hours prior to feeding trials. Feeding chambers were constructed out of a white 2-gal bucket, but feeding chambers for larger brown bullheads were 12-gal coolers. Each feeding chamber was filled with dechlorinated water, equipped with an aerator, and covered with white 1-mm mesh to prevent predators from jumping out of the feeding chamber. Water was dechlorinated using Instant Ocean Marine Conditioner (United Pet Group, Inc., Cincinnati, Ohio); 0.5 mL Marine Conditioner: 1 galof water. Water temperatures ranged from those of field conditions (10–15 °C) to ambient room temperature (20 °C). A single predator and two live, wild-caught larvae were introduced to each feeding chamber. Predators and larvae were intermittently monitored to determine relative (not exact) time of larvae consumption. Predators were sacrificed, measured to standard length, and immediately frozen (-80 °C) after both larvae had been consumed. In some instances (n= 6), predators were sacrificed after one larva was consumed. In these instances, predators were sacrificed several hours after consuming a single larva because we wanted the predator to eat the second larvae. In instances when the predator failed to consume any larvae, the predator was either sacrificed (n=3 [2 bullheads, 1 yellow perch]) and DNA was extracted from any remnant stomach contents from the field, or if the predator was native, the predator was released (n=2 sculpins).

To extract gut contents from field captured predators and laboratory feeding trial predators, all specimens were thawed, and the stomachs were dissected using clean and flame-sterilized tools, as recommended by King and others (2008). Stomachs (and in some cases intestines) were scraped, and DNA was extracted from sample. Subsamples from the fore, mid, and hind stomach were combined for DNA extraction in large (>1 mL) stomach samples. Fathead minnow stomachs were difficult to open and dissect after the freeze-thaw, so contents were squeezed (rather than scraped) out. Stomach samples were preserved on 1 × 1 cm filter paper and stored at -20 °C until DNA was extracted.

Extraction, Amplification, and SNP Assessment

DNA was extracted from predator fin clips, whole larvae, and stomach/intestine contents of predators using DNeasy extraction kits (QIAGEN, Inc., Valencia, California) according to the manufacturer's instructions for animal tissue. All samples were screened using four SNP assays developed for Lost River and shortnose suckers; one mitochondrial DNA assay (Dlux434) and three nuclear DNA assays (Dlux6487, Dlux9136, and Dlux55662). PCR conditions followed the methods described in Hoy and Ostberg (2015). PCRs were run for 40 cycles, and all DNA samples were run in triplicate. Internal controls were included with each run and consisted of a negative DNA extraction control (no tissue included during DNA extraction) and a no-template control (water in place of DNA template in qPCR). Standard curves were generated for each SNP assay using 10-fold serial dilutions (10,000; 1,000; 100; 10; 1 pg/µL) of juvenile sucker DNA in triplicate (fig. 2).

To determine whether the four sucker assays cross-amplified predator DNA, we performed qPCR using predator DNA. We assessed the cycle number at which PCR products were first detected for 10,000 pg/ µL of predator DNA and larval sucker DNA. When cross-amplification was detected, sequences of predator DNA (archived at GenBank) were analyzed and compared to sucker primer and probe sequences. All stomach samples (feeding trials and field collected) were screened in triplicate using the four SNP assays. For all stomach sample assays, we used 1 µL of DNA extract per PCR. Sucker DNA was considered present in stomach samples when the majority of replicates and markers had positive assays.

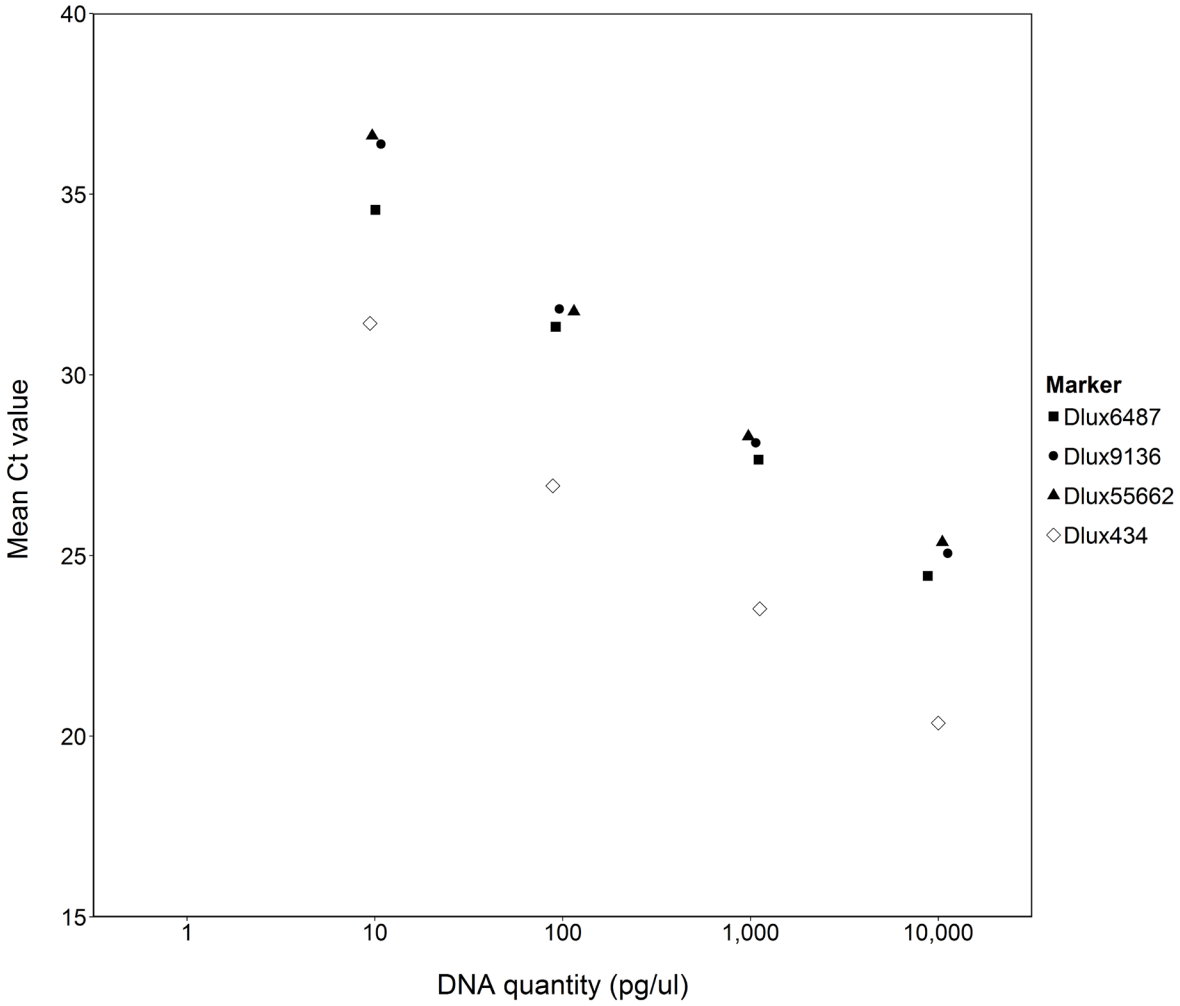

Figure 2. Standard curves for real-time qPCR assays of four Lost River and shortnose sucker SNP assays. Standard curves were developed using the mean number of PCR cycles when exponential fluorescence was first detected (Ct value) for three replicates at each serial dilution. The 1 pg/µL standard was not amplified after 40 cycles for any SNP assay.

Results

DNA Extraction and Standard Curves

Approximately 300 sucker larvae and 262 predators were collected from 30 locations (fig. 1) in the Williamson River and Williamson River Delta. DNA was extracted from 22 predator fin clips, 20 whole larvae, and 253 (228 field samples, 25 feeding trials) predator stomach samples. Extractions failed for three predator fin clips (two blue chub, one yellow perch) and four field-captured stomach samples (four fathead minnows). The standard curves for all SNPs were developed without the lowest serial dilution (1 pg/µL) because these dilutions failed to amplify (fig. 2). Using these methods, our level of detection is between 1 and 10 pg/µL.

Amplification of Larvae and Predator DNA

For the 20 larvae screened, the cycle number at which a PCR product was first detected ranged from 17.99 to 20.89 for mitochondrial SNP Dlux434 and from 26.93 to 29.33 for nuclear SNP Dlux55662 (fig. 3). Mitochondrial DNA was detected in fewer cycles because it is more concentrated than nuclear DNA. None of the nuclear DNA SNP assays cross-amplified with predator DNA (table 2, fig. 3). The mitochondrial SNP Dlux434 cross-amplified after 35.15 to 39.94 PCR cycles in one or more qPCR from each predator species (table 2). On average, 18.55 more PCR cycles were required to amplify predator DNA than sucker larvae DNA at Dlux434 (fig. 3). Primer and probe sequences at the cytochrome b region (Dlux434 for suckers) mismatched predator DNA by 12–15 cumulative bases (table 3).

Table 2. DNA extractions and instances of cross-amplification of predator DNA using three nuclear SNP assays and one mitochondrial SNP (Dlux434) assay developed for Lost River and shortnose suckers.

[U, Product undetected after 40 qPCR cycles]

Predator	DNA extractions		SNP cross-amplification			
	Success	Fail	Dlux434	Dlux6487	Dlux9136	Dlux55662
marbled sculpin	5	-	2	U	U	U
blue chub	3	2	2	U	U	U
yellow perch	4	1	4	U	U	U
fathead minnow	5	-	2	U	U	U
tui chub	5	-	1	U	U	U

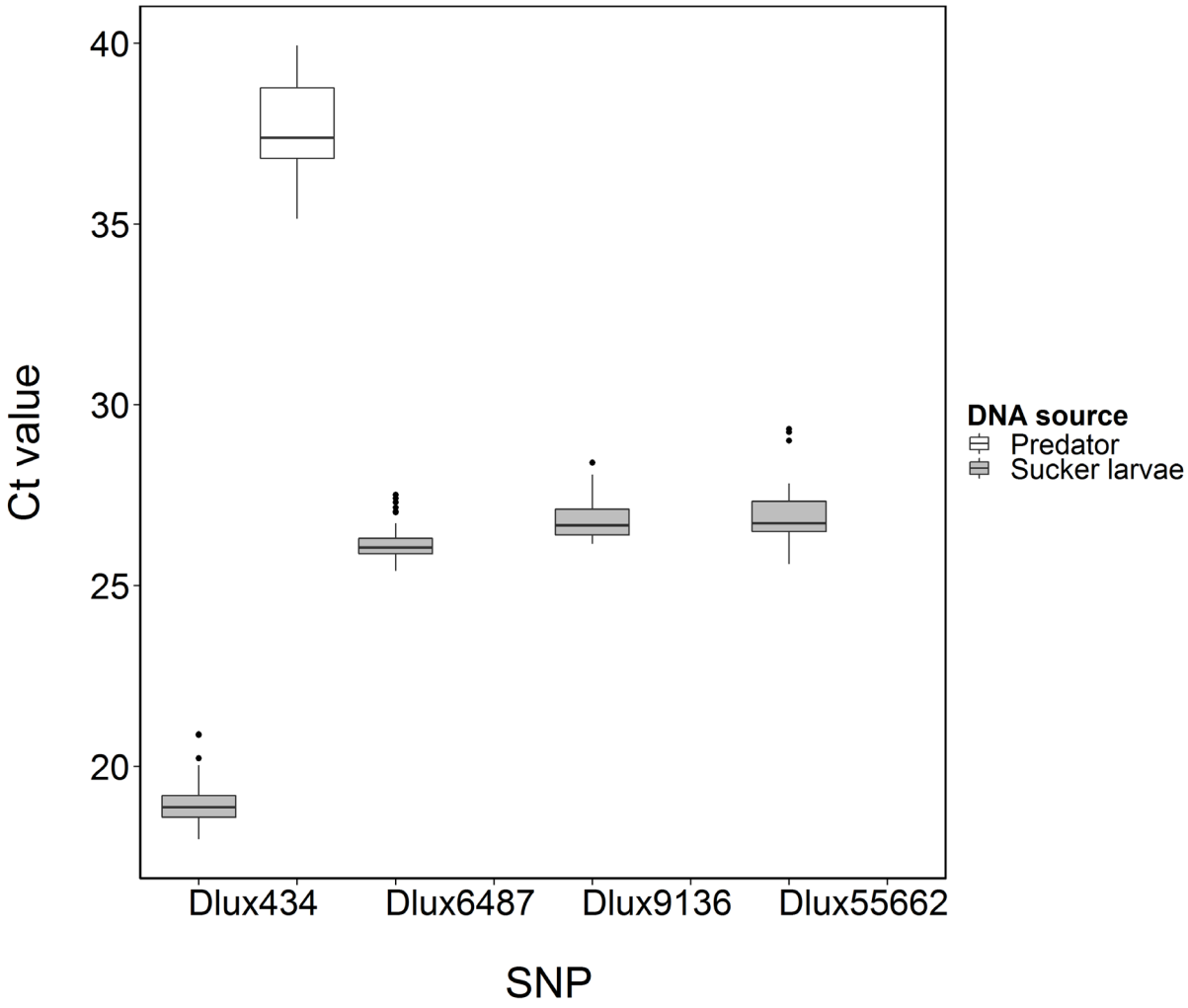

Figure 3. The cycle number (Ct value) at which a PCR product was first detected for 10,000 pg/µL of sucker larvae and predator DNA at four SNPs developed for Lost River and shortnose suckers. Amplification of predators at Dlux6487, Dlux9136, and Dlux5562 is not shown because predators did not cross-amplify.

Table 3. Dlux434 primer and probe sequences for shortnose and Lost River sucker cytochrome b region and DNA mismatches (M) in predators for this gene region.

[Mismatches in predators are indicated by nucleotide. Nucleotides that are conserved between suckers and predators are indicated by a dot (.). Shortnose sucker and Lost River sucker sequence data are from Hoy and Ostberg (2015). GenBank accession number for each predator species is in parentheses]

Species	Forward primer																									M
shortnose sucker	G	G	A	T	A	C	G	T	A	C	T	C	C	C	A	T	G	A	G	G	A	C	A	A		-
Lost River sucker	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			-
marbled sculpin (JX484674)	.	.	G	.	.	.	.	.	C	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	G		5
slender sculpin (JX484673)	.	.	.	.	.	T	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G		4
blue chub (KF514155)	.	.	C	.	.	T	.	.	.	.	.	T	.	.	.	.	.	G	.	.	C	.	.	.			5
yellow perch (GQ184520)	.	.	G	.	.	T	.	.	C	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.			5
fathead minnow (AF045357)	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	G			4
tui chub (JX443083)	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.			3
	Probe																									
shortnose sucker (allele A)	A	G	G	T	G	C	T	A	C	G	G	T	A	A	T	T										-
Lost River sucker (allele B)	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.										-
marbled sculpin	.	.	.	A	.	.	C	.	.	T	.	.	C	.	.	C										5
slender sculpin	.	.	.	.	.	.	C	.	.	T	.	.	C	.	.	C										4
blue chub	.	.	.	C	.	.	C	.	.	A	.	.	.	.	.	.										2
yellow perch	.	.	.	G	.	.	A	.	.	T	.	.	T	.	.	.										4
fathead minnow	.	.	.	.	.	.	C	.	.	A	.	.	C	.	.	.										2
tui chub	G	.	.	G	.	.	C	.	.	A	.	.	.	.	.	.										3
	Reverse primer																									
shortnose sucker	A	C	T	A	A	C	C	T	T	T	T	A	T	C	C	G	C	A	G	T	C	C	C			-
Lost River sucker	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			-
marbled sculpin	.	.	A	.	.	.	.	.	.	C	.	T	.	.	T	.	.	.	.	.	T	.	.			5
slender sculpin	.	.	A	.	.	.	.	.	.	C	.	T	.	.	T	.	.	.	.	.	T	.	.			5
blue chub	.	.	A	.	.	.	T	.	A	.	.	G	.	.	A	.	.	.	.	.	A	.	.			6
yellow perch	.	.	C	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	A	.	.			4
fathead minnow	.	.	A	.	.	T	.	.	A	C	.	.	.	.	A	.	.	G	.	.	A	.	.			7
tui chub	.	.	A	.	.	.	.	.	A	C	.	G	.	.	A	.	.	.	.	.	G	.	.			6

Feeding Trials

Laboratory feeding trials ranged in length from 1 hour to as long as 2 days, and we observed no instances of regurgitation. Of the predators that consumed larvae, 68 percent were sacrificed within approximately 2 hours. The longest times between consumption and sacrifice were for the predators that only ate one larva. For example, one sculpin was sacrificed 8–23 h after it had consumed one larva and sucker DNA was not detected in this fish. Eighty-three percent (25 of 30) of predators in laboratory feeding trials consumed at least one sucker larva. Most (63.3 percent) predators consumed both larvae although 16.7 percent of all specimens (two bullheads, one yellow perch, and two sculpins) did not consume any larvae (table 4). Sucker DNA was not detected in 2 of the 6 predators that ate a single larva and 9 of the 19 predators that ate two larvae (table 4). Of the successful feeding trials, sucker DNA was detected in 100 percent of yellow perch, 50 percent of tui chub, 100 percent of blue chub, and 80 percent of sculpins (table 4). We failed to detect sucker DNA in 40 percent of predators that consumed larvae, including all (seven) fathead minnows, two tui chub, and one slender sculpin (table 4). Larvae were visually observed in 2 of 6 yellow perch stomachs, but were not observed in any other specimens. Sample sizes were too small to thoroughly investigate the relationship of predator size and their ability to consume larvae however no predators in feeding trials were too small to consume larvae, although bullheads may have been too large (see table 4 for size range). Similarly, excluding fatheads, we were able to extract DNA from predators of all sizes we tested and thus there was no apparent relationship between predator size and our ability to detect larvae.

Table 4. Collection site, standard length (millimeter), number of feeding trials, successful trials (predators that consumed larvae), and positive assays (sucker DNA detected) for predators in feeding trials collected May 4–19, 2015.

Species	Collection site	Size (SL)	Number of feeding trials			Percent
		Mean (range)	Total trials	Successful trials	Positive assay	Positive assay
brown bullhead	Upper Klamath Lake	235 (210–260)	2	0	-	-
sculpin[1]	Hagelstein Park	65 (54–83)	7	5	4	80
blue chub	Williamson River	85 (78–95)	4	4	4	100
yellow perch	Hagelstein Park	99 (87–113)	6	5	5	100
fathead minnow	Williamson River	47 (41–52)	7	7	0	0
tui chub	Williamson River	101 (74–120)	4	4	2	50

[1]A single Klamath Lake sculpin (*Cottus princeps*) was included in the feeding trials; the other six specimens were slender sculpins (*Cottus tenuis*).

Field-Collected Predators

Of the 232 wild-caught predators assayed, sucker DNA was not detected in fathead minnow, tui chub, blue chub, or sculpin stomachs (table 5). However, sucker DNA was detected in 15.6 percent of yellow perch (table 5). Yellow perch with positive assays for sucker DNA ranged in size from 55 to 75 mm SL and were captured at two sites near "The Bend" in the Williamson River (red circles, fig. 1). The distribution and abundance of larvae throughout the study area was beyond the scope of this project; however, larvae appeared to be in high densities and were easily captured at sites near "The Bend" throughout the study period, relative to some other sampled locations.

Table 5. Collection site, standard length (millimeter), sample size, successful extractions, and positive assays (sucker DNA detected) for field-captured predators collected May 4–19, 2015 in the Williamson River Delta, Oregon.

Species	Collection site	Size (SL)	Number of predators			Percent
		Mean (range)	Sample size	Successful extraction	Positive assay	Positive assay
brown bullhead	Williamson River	-	0	-	-	-
marbled sculpin	Williamson River	68 (55–83)	16	16	0	0
blue chub	Williamson River	73 (41–107)	75	75	0	0
yellow perch	Williamson River	75 (55–170)	32	32	5	15.6
fathead minnow	Williamson River	48 (40–55)	35	31	0	0
tui chub	Williamson River	94 (45–154)	74	74	0	0

Discussion

This study is the first to demonstrate that qPCR can be an effective tool to study predation of larval suckers in the Williamson River Delta using sucker-specific markers developed by Hoy and Ostberg (2015). Molecular techniques found sucker DNA in 15.6 percent of wild-caught yellow perch, verifying yellow perch in the Williamson River prey on suckers (presumably larvae) during larval egress. This study did not detect predation by other fishes in the area, including blue chub, tui chub, sculpin, or fathead minnow. However, all of these fishes preyed on larval suckers in laboratory feeding trials and other factors may have caused the negative results that were observed in the wild.

Others have hypothesized that fathead minnow predation limits sucker recruitment in Upper Klamath Lake (Markle and Dunsmoor, 2007). This study was unable to determine if fathead minnows prey on larval suckers in the wild. Sucker DNA was not detected in any fathead minnow stomachs, including positive-control feeding trials where fathead minnows were observed preying on larvae. It is possible that the methods we employed to dissect fathead minnows were inadequate and caused the false-negative result. Other studies have verified rapid freezing and thawing as an effective method to halt digestion but future studies should test alternative methods for extracting and preserving gut contents for fathead minnows (Legler and others, 2010). For example, extracting contents immediately after sacrifice could improve dissection ease, or injecting ethanol into the stomach could improve DNA preservation (Carreon-Martinez and others, 2011). Alternatively, PCR inhibition could be the cause of the false-negative result. Non-target DNA, digestive enzymes, biological molecules, soil, many organic and inorganic compounds have been identified as PCR inhibitors (Wilson, 1997; Tichopad and others, 2004; Gonzalez and others, 2005; Juen and Traugott, 2006). Combining fathead minnow DNA or digestive enzymes with sucker DNA at different stages of the extraction and PCR process is a practical next step for identifying the cause of this false-negative result.

Feeding mechanics and digestion rates vary among predator species. In contrast to predators like yellow perch that consume prey whole, fathead minnows masticate prey, which leaves larvae visually unidentifiable and accelerates digestion (Schooley and others, 2008). Fathead minnows and other predators with false-negative results from feeding trials (two tui chubs, one sculpin) may have rapid digestion rates relative to other species, resulting in DNA too degraded for detection. The false negatives observed in tui chub and sculpin samples appear to be caused by prolonged digestion; that is, more time passed between consumption and sacrifice than in other conspecific samples. Rapid digestion could be one reason we observed no sucker predation in wild-caught blue chub, tui chub, or sculpin although few (n=16) wild-caught sculpins were assayed. Other factors that affect digestion rates include temperature, gut-fullness, and prey size (Folkvord, 1993; Schooley and others, 2008; Legler and others, 2010; Carreon-Martinez and others, 2011). Experiments controlling these factors could identify why sucker DNA was not detected in some samples.

Bullheads did not consume any sucker larvae during feeding trials. Bullhead specimens were large in comparison to other species (table 4), and larval prey may have been too small for that size class. Additionally, bullheads may require prolonged starvation before trials. Yellow bullheads (*Ameiurus natalis*; TL 58–113 mm) readily ate razorback sucker (*Xyrauchen texanus*) larvae during feeding trials conducted by Schooley and others (2008) after being starved for 72 hours. In our study, bullheads were starved for 24 hours prior to feeding trials and both specimens regurgitated about 50 mm fish during transport to the laboratory. One trial was terminated after 3 days, the second was terminated after 24 hours because larvae condition was deteriorating. Future studies could benefit by withholding feed for at least 72 hours or targeting smaller bullheads (Schooley and others, 2008).

Tui chub, blue chub, and sculpins readily ate sucker larvae during feeding trials (table 4). Piscivory has been documented for blue chub in Upper Klamath Lake, but not for tui chub or sculpin, although few stomachs have been evaluated for these species (Bond and others, 1968; Markle and Clauson, 2006). Piscivory has been documented for other subspecies of tui chub and sculpins so it is possible these species prey on suckers in Upper Klamath Lake (Koch, 1973; Henkanaththegedara and Stockwell, 2013; Swain and others, 2014). Larger sample sizes could better explore piscivory among tui chubs, blue chubs, and sculpins.

The feeding trial design could be improved to better assess digestion rates and factors that contribute to detecting DNA. In addition to withholding feed for more than 72 hours before feeding trials, future studies could benefit by giving predators a short amount of time to consume larvae, sacrifice predators at specific intervals (for example, 1h, 2h, 4h, 8h, etc.) after consuming larvae, and include more replicates (King and others, 2008; Schooley and others, 2008; Ley and others, 2014). Temperature and other parameters that may affect digestion rates should be held constant (King and others, 2008).

Future studies should also test the specificity of primers by screening prey items (other fishes, macroinvertebrates, algae, cyanobacteria) and other predators (brown bullhead, Klamath redband trout) to further evaluate the risk of false-positive results. Similarly, water samples should be screened for sucker DNA because eDNA could cause false-positives (King and others, 2008). This pilot study screened 2–5 samples from some predator species but more robust sample sizes are necessary to exclude the possibility of cross-amplification.

We observed cross-amplification of mitochondrial marker Dlux434 after 35-39 PCR cycles in at least one sample from each predator species screened (table 2). Identical concentrations (10,000 pg/ µL) of larval sucker DNA amplified after an average of 19.26 cycles, and low concentrations (10 pg/µL) amplified after an average of 31.41 PCR cycles (figs. 2and 3). The apparent cross-amplification observed at Dlux434 was likely due to non-specific amplification, which can occur in high concentrations of DNA when primers misalign and amplify non-target DNA (Shigemori and others,

2005; Juen and Traugott, 2006). Hence, it is critical to investigate amplification of each marker at different DNA concentrations so cross-amplification can be differentiated from real detection (especially for detecting low levels of DNA). Mitochondrial markers are advantageous in studies where DNA may be present in low levels because mitochondrial DNA is more abundant than nuclear DNA. Therefore, developing a more specific mitochondrial DNA marker may be necessary to replace Dlux434 in gut studies.

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References Cited

Admassu, B., Juen, A., and Traugott, M., 2006, Earthworm primers for DNA-based gut content analysis and their cross-reactivity in a multi-species system: Soil Biology and Biochemistry, v. 38, p. 1308–1315.

Barnett, A., Redd, K.S., Frusher, S.D., Stevens, J.D., and Semmes, J.M., 2010, Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information: Journal of Experimental Marine Biology and Ecology, v. 393, p. 188–192.

Bond, C.E., Hazel, C.R., and Vincent, D., 1968, Relations of nuisance algae to fishes in Upper Klamath Lake–Terminal Progress Report: U.S. Federal Water Pollution Control Administration, 126 p.

Burdick, S.M., and Brown, D.T., 2010, Distribution and condition of larval and juvenile Lost River and shortnose suckers in the Williamson River Delta restoration project and Upper Klamath Lake, Oregon—2009 annual data summary: U.S. Geological Survey Open-File Report 2010-1216, 78 p.

Burdick, S.M., and Hewitt, D.A., 2012, Distribution and condition of young-of-year Lost River and shortnose suckers in the Williamson River Delta restoration project and Upper Klamath Lake, Oregon, 2008–10—Final Report: U.S. Geological Survey Open-File Report 2012-1098, 52 p.

Burdick, S.M., Elliott, D.G., Ostberg, C.O., Conway, C.M., Dolan-Caret, A., Hoy, M.S., Feltz, K.P., and Echols, K.R., 2015, Health and condition of endangered juvenile Lost River and shortnose suckers relative to water quality and fish assemblages in Upper Klamath Lake, Oregon, and Clear Lake Reservoir, California: U.S. Geological Survey Open-File Report 2015-1217, 56 p.

Burdick, S.M., Ottinger, C., Brown, D.T., VanderKooi, S.P., Robertson, L., and Iwanowicz, D., 2009, Distribution, health, and development of larval and juvenile Lost River and shortnose suckers in the Williamson River Delta restoration project and Upper Klamath Lake, Oregon—2008 annual data summary: U.S. Geological Survey Open-File Report 2009-1287, 76 p.

Carreon-Martinez, L., Johnson, T.B., Ludsin, S.A., and Heath, D.D., 2011, Utilization of stomach content DNA to determine diet diversity in piscivorous fishes: Journal of Fish Biology, v. 1170, p. 1170–1182.

Dunshea, G., 2009, DNA-based diet analysis for any predator: PLOS One, v. 4, e5252, p. 1–9.

Ellsworth, C.M., and Martin, B.A., 2012, Patterns of larval sucker emigration from the Sprague and lower Williamson Rivers of the Upper Klamath Basin, Oregon, after the removal of Chiloquin Dam – 2009–10 Annual Report: U.S. Geological Survey Open-File Report 2012–1037, 34 p.

Erdman, C.S., Hendrixson, H.A., and Rudd, N.T., 2011, Larval sucker distribution and condition before and after large-scale restoration at the Williamson River Delta, Upper Klamath Lake, Oregon: Western North American Naturalist, v. 71, p. 472–480.

Folkvord, A., 1993, Prey recognition in stomachs of cannibalistic juvenile cod (*Gadus morhua L.*): Sarsia, v. 78, p. 97–100.
Gonzalez, J.M., Portillo, M.C., and Salz-Jimenez, C., 2005, Multiple displacement amplification as a pre-polymerase chain reaction (pre-PCR) to process difficult to amplify samples and low copy number sequences from natural environments: Environmental Microbiology, v. 7, p. 1024–1028.
Handeland, S.O., Imsland, A.K., and Stefansson, S.O., 2008, The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts: Aquaculture, v. 283, p. 36–42.
Hartleb, C.F., and Moring, J.R., 1995, An improved gastric lavage device for removing stomach contents from live fish: Fisheries Research, v. 24, p. 261–265.
Henkanaththegedara, S.M., and Stockwell, C.A., 2013, The role of gape-limitation in intraguild predation between endangered Mohave tui chub and non-native western mosquitofish: Ecology of Freshwater Fish, v. 22, p. 11–20.
Hewitt, D.A., Hayes, B.S., Janney, E.C., Harris, A.C., Koller, J.P., and Johnson, M.A., 2011, Demographics and run timing of adult Lost River (*Deltistes luxatus*) and shortnose (*Chasmistes brevirostris*) suckers in Upper Klamath Lake, Oregon, 2009: U.S. Geological Survey Open-File Report 2011-1088, 38 p.
Hewitt, D.A., Janney, E.C., Hayes, B.S., and Harris, A.C., 2014, Demographics and run timing of adult Lost River (*Deltistes luxatus*) and shortnose (*Chasmistes brevirostris*) suckers in Upper Klamath Lake, Oregon, 2012: U.S. Geological Survey Open-File Report 2014-1186, 44 p.
Hoy, M.S., and Ostberg, C.O., 2015, Development of 20 TaqMan assays differentiating the endangered shortnose and Lost River suckers: Conservation Genetic Resources, v. 7, p. 673–676.
Juen, A., and Traugott, M., 2006, Amplification facilitators and multiplex PCR—Tools to overcome PCR-inhibition in DNA-gut-content analysis of soil-living invertebrates: Soil biology and Biochemistry, v. 38, p. 1872–1879.
King, R.A., Read, D.S., Traugott, M., and Symondson, W.O.C., 2008, Molecular analysis of predation—A review of best practice for DNA-based approaches: Molecular Ecology, v. 17, p. 947–963.
Koch, D.L., 1973, Reproductive characteristics of the cui-ui lakesucker (*Chasmistes cujus Cope*) and its spawning behavior in Pyramid Lake, Nevada: Transactions of the American Fisheries Society, v.102, p. 145–149.
Legler, N.D., Johnson, T.B., Heath, D.D., and Ludsin, S., 2010, Water temperature and prey size effects on the rate of digestion of larval and early juvenile fish: Transactions of American Fisheries Society, v. 139, p. 868–875.
Ley, G., Saltzgiver, M.J., Dowling, T.E., Karam, A.P, Kesner, B.R., and Marsh, P.C., 2014, Use of a molecular assay to detect predation on an endangered fish species: Transactions of the American Fisheries Society, v. 143, p. 49–54.
Markle, D.F., and Clauson, K., 2006, Ontogenetic and habitat-related changes in diet of late larval and juvenile suckers (*Catostomidae*) in Upper Klamath Lake, Oregon: Western North American Naturalist, v. 66, p. 492–501.
Markle, D.F., and Dunsmoor, L.K., 2007, Effects of habitat volume and fathead minnow introduction on larval survival of two endangered sucker species in Upper Klamath Lake, Oregon: Transactions of the American Fisheries Society, v. 136, p. 567–579.
Martin, B.A., Hewitt, D.A., and Ellsworth, C.M., 2013, Effects of Chiloquin Dam on spawning distribution and larval emigration of Lost River, shortnose, and Klamath largescale suckers in the Williamson and Sprague Rivers, Oregon: U.S. Geological Survey Open-File Report 2013-1039, 32 p.

National Research Council, 2004, Endangered and threatened fishes in the Klamath River Basin—Causes of decline and strategies for recovery: Washington, D.C., The National Academies Press, 397 p.

Rosel, P.E., and Kocher, T.D., 2002, DNA-based identification of larval cod in stomach contents of predatory fishes: Journal of Experimental Marine Biology and Ecology, v. 267, p. 75–88.

Schooley, J.D., Karam, A.P., Kesner, B.R., Marsh, P.C., Pacey, C.A., and Thornbrugh, D.J., 2008, Detection of larval remains after consumption by fishes: Transactions of the American Fisheries Society, v. 137, p. 1044–1049.

Shigemori, Y., Mikawa, T., Shibata, T. and Oishi, M., 2005, Multiplex PCR—Use of heat-stable *Thermus thermophiles* RecA protein to minimize non-specific PCR products: Nucleic Acids Research, v. 33, p. e126, 1–9.

Symondson, W.O.C., 2002, Molecular identification of prey in predator diets: Molecular Ecology, v. 11, p. 627–641.

Swain, N.R., Hocking, M.D., Harding, J.N., and Reynolds, J.D., 2014, Effects of salmon on the diet and condition of stream-resident sculpins: Canadian Journal of Fisheries and Aquatic Sciences, v. 71, p. 521–532.

Tichopad, A., Didier, A., and Pfaffl, M.W., 2004, Inhibition of real-time RT-PCR quantification due to tissue-specific contaminants: Molecular and Cellular Probes, v. 18, p. 45–50.

U.S. Fish and Wildlife Service, 2013, Revised recovery plan for the Lost River sucker (*Deltistes luxatus*) and shortnose sucker (*Chasmistes brevirostris*): U.S. Fish and Wildlife Service, xviii + 122 p.

Wilson, I.G., 1997, Inhibition and facilitation of nucleic acid amplification: Applied and Environmental Microbiology, v. 63, p. 3741–3751.

Wong, M.L., and Medrano, J.F., 2005, Real-time PCR for mRNA quantification: BioTechniques, v. 39, p. 75–85.

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U.S. Geological Survey
6505 NE 65th Street
Seattle, Washington 98115
http://wfrc.usgs.gov/

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