Project: Environmental DNA sampling for the detection of chum salmon by the Oregon Department of Fish and Wildlife

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Introduction

Environmental DNA (eDNA) is DNA extracted from an environmental sample, such as soil, water, or air, without directly sampling the target organism (Lodge et al. 2012, Taberlet et al. 2012). Researchers have demonstrated that detection of target organisms using eDNA is more sensitive, efficient, and cost-effective than traditional sampling methods, particularly when the organism of interest has a limited distribution or is rare (Dejean et al. 2012). For example, the eDNA-based detection probability of a single trout in 100 m of stream is ~0.85, substantially greater than that of electrofishing (Wilcox et al. 2016). As a result, eDNA sampling has received a great deal of attention for its potential to detect early invasions of nonnative species (Darling and Blum 2007, Ficetola et al. 2008, Jerde et al. 2011, Dejean et al. 2012, Goldberg et al. 2013) as well as the presence and distribution of rare, native species (Goldberg et al. 2011, Olson et al. 2012, Thomsen et al. 2012, McKelvey et al. 2016), such as species currently listed under the U.S. Endangered Species Act.

The chum salmon (*Oncorhynchus keta*) is an anadromous fish native to the coastal regions of the northern Pacific Ocean (Groot and Margolis 1991). Once abundant throughout their range, chum salmon populations have declined decidedly over the last century due to factors such as habitat loss and fragmentation, overexploitation, and impacts from nonnative fish species (Nehlsen et al. 1991). These declines have prompted the National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS) to designate numerous evolutionarily significant units within the continental U.S. for this species as threatened under the U.S. Endangered Species Act (USFWS 2014). As a result, the presence of this species can greatly dictate stream and land management decisions. However, determining their distribution using traditional sampling methods is often costly, time consuming, and challenging because capture efficiency for these species tend to be low. Environmental-DNA-based techniques are ideal for organisms like salmonids that present these sampling challenges.

Methods

Sampling materials, field equipment, and protocols were sent to Oregon Department of Fish and Wildlife for collection of eDNA samples at 51 locations in Oregon and Washington. Field samples were collected between 11 November and 1 December 2015 (Table 1, Figure 1). For each sample, up to 5 L of stream water were pumped through a glass filter (GE HealthCare) using a peristaltic pump (GeoTech Environmental Equipment, Inc.) following the protocol developed by Carim et al. (2016b). Used filters were individually placed in plastic bags with silica desiccant and packaged in individual envelopes labeled with field information (e.g., date and sampling location).

Upon receipt of samples at the National Genomics Center for Wildlife and Fish Conservation (NGCWFC; a part of the U.S. Forest Service Rocky Mountain Research Station in Missoula, MT) sampling data were catalogued and samples were stored at -20 °C until analyzed. We performed DNA extractions on half of the sample filter using the Qiagen DNEasy® Blood and Tissue Kit with a modified protocol described in Carim et al. (2016a). The other half of the sample filter was retained and stored at -20 °C. If more than one filter was used to collect the sample, DNA from all filters for a given sample was combined during DNA extraction. All 51 samples were analyzed for chum salmon DNA using eDNA markers developed at the NGCWFC (Franklin et al. *in prep*). Each sample was analyzed in triplicate on a StepOne Plus qPCR instrument (Life Technologies). A sample was considered positive

for the presence of the target species if at least one of the three PCR reactions amplified DNA of that species (see supplement below).

All reactions included an internal positive control to ensure that the reaction was effective and sensitive to the presence of the target species' DNA. If the internal positive control appeared inhibited (i.e., chemical compounds on the filter reduced amplification of the target DNA; Figure S2), we treated the sample with an inhibitor removal kit (Zymo Research) and re-analyzed the sample in triplicate. Removal of inhibitors may result in loss of DNA in a sample. To counter this effect, we extracted the second half of the sample filter. We then combined all extracted DNA from a given sample to obtain ~200 µl of extracted DNA. With elution volumes of 100–200 µl, loss of DNA during inhibitor removal is on average less than 10% (see http://www.zymoresearch.com for more details).

All laboratory experiments were conducted with negative controls to insure there was no contamination during DNA extraction or qPCR setup.

Table 1. Location and collection details of eDNA samples collected by the Oregon Department of Fish and Wildlife.

Map ID	Stream	Site #	Liters	Latitude	Longitude	State	Collection Date	Collector	Collection Notes
1	Ashes Lake North trib	01	5	45.6782	-121.908	WA	11/25/2015	KH	
2	Dog Creek	01	5	45.7105	-121.671	WA	11/29/2015	KH	
3	Dry Creek	01	5	45.677	-121.881	OR	11/22/2015	KH	MAYBE Chum
4	Eagle Creek	01	5	45.6408	-121.929	OR	11/22/2015	KH	
5	Eagle Creek	02	5	45.6363	-121.918	OR	11/22/2015	KH	
6	Eagle Creek	03	5	45.631	-121.907	OR	11/22/2015	KH	
7	Eightmile Creek	01	5	45.6063	-121.085	OR	11/23/2015	JT, ML	
8	Eightmile Creek	02	5	45.602	-121.078	OR	11/24/2015	ML	
9	Eightmile Creek	03	5	45.5973	-121.081	OR	11/30/2015	ES	
10	Eightmile Creek	04	5	45.592	-121.079	OR	11/30/2015	ES	
11	Eightmile Creek	05	5	45.587	-121.074	OR	11/30/2015	ES	
12	Eightmile Creek	06	5	45.5834	-121.065	OR	11/30/2015	ES	
13	Fifteenmile Creek	01	5	45.6122	-121.123	OR	11/23/2015	JT	
14	Fifteenmile Creek	02	5	45.6116	-121.116	OR	11/23/2015	JT, ML	
15	Fifteenmile Creek	03	5	45.6111	-121.104	OR	11/23/2015	JT, ML	
16	Fifteenmile Creek	04	5	45.6091	-121.095	OR	11/23/2015	JT, ML	
17	Fifteenmile Creek	05	5	45.6066	-121.086	OR	11/23/2015	JT, ML	
18	Fifteenmile Creek	06	5	45.6101	-121.078	OR	11/24/2015	ML	
19	Fifteenmile Creek	07	5	45.6177	-121.072	OR	11/24/2015	ML	
20	Fifteenmile Creek	08	5	45.625	-121.066	OR	11/24/2015	ML	
21	Gorton Creek	01	5	45.6924	-121.778	OR	11/22/2015	KH	
22	Hamilton Spring Creek	01	3.5; 1.5	45.6336	-121.983	WA	11/20/2015	KH	KNOWN CHUM SITE; All 4 samples are from the same site on the same day

23	Hamilton Spring Creek	02	3.5; 1.5	45.6336	-121.983	WA	11/20/2015	ML	KNOWN CHUM SITE; All 4 samples are from the same site on the same day
24	Hamilton Spring Creek	03	2.5; 2.5	45.6336	-121.983	WA	11/20/2015	KH	KNOWN CHUM SITE; All 4 samples are from the same site on the same day
25	Hamilton Spring Creek	04	2; 3	45.6336	-121.983	WA	11/20/2015	ML	KNOWN CHUM SITE; All 4 samples are from the same site on the same day
26	Herman Creek	01	5	45.6812	-121.861	OR	11/25/2015	KH	
27	Herman Creek	02	5	45.6788	-121.86	OR	11/25/2015	KH	
28	Herman Creek	03	5	45.6745	-121.853	OR	11/25/2015	KH	
29	Hood River	01	4; 1	45.7126	-121.508	OR	11/21/2015	KH	
30	Hood River	02	5	45.7045	-121.504	OR	11/21/2015	KH	
31	Hood River	03	4; 1	45.6985	-121.509	OR	11/21/2015	KH	
32	Hood River	04	4; 1	45.6919	-121.508	OR	11/21/2015	KH	
33	Hood River	05	5	45.6869	-121.51	OR	11/30/2015	KH	
34	Hood River	01B	5	45.7126	-121.507	OR	12/1/2015	KH	1B River Right
35	Hood River	01C	5	45.7126	-121.508	OR	12/1/2015	KH	1C CENTER
36	Hood River	01D	5	45.7112	-121.508	OR	12/1/2015	KH	1D THALWEG
37	Jewett Creek	01	5	45.7164	-121.477	WA	11/29/2015	KH	
38	Kanaka Creek	01	5	45.6954	-121.877	WA	11/25/2015	KH	
39	Klickitat	02	4.5; 0.5	45.7016	-121.285	WA	11/29/2015	KH	(former site 2)
40	Little White Salmon River	01	5	45.7224	-121.641	WA	11/29/2015	KH	
41	Mill Creek	01	5	45.6065	-121.187	OR	11/28/2015	KH	
42	Mosier Creek	01	5	45.6848	-121.395	OR	11/28/2015	KH	
43	Rock Creek	01	5	45.6937	-121.893	WA	11/25/2015	KH	
44	Ruckle Creek	01	5	45.6465	-121.922	OR	11/21/2015	KH	
45	Viento Creek	01	5	45.6964	-121.673	OR	11/22/2015	KH	
46	White Salmon River	01	5	45.7295	-121.522	WA	11/30/2015	KH	
47	White Salmon River	02	5	45.7392	-121.523	WA	11/30/2015	KH	
48	White Salmon River	04	5	45.7544	-121.529	WA	11/30/2015	KH	
49	White Salmon River	07	5	45.7801	-121.515	WA	11/30/2015	KH	
50	Wind River	01	5	45.7199	-121.789	WA	11/25/2015	KH	
51	Wind River	02	5	45.7272	-121.794	WA	11/29/2015	KH	

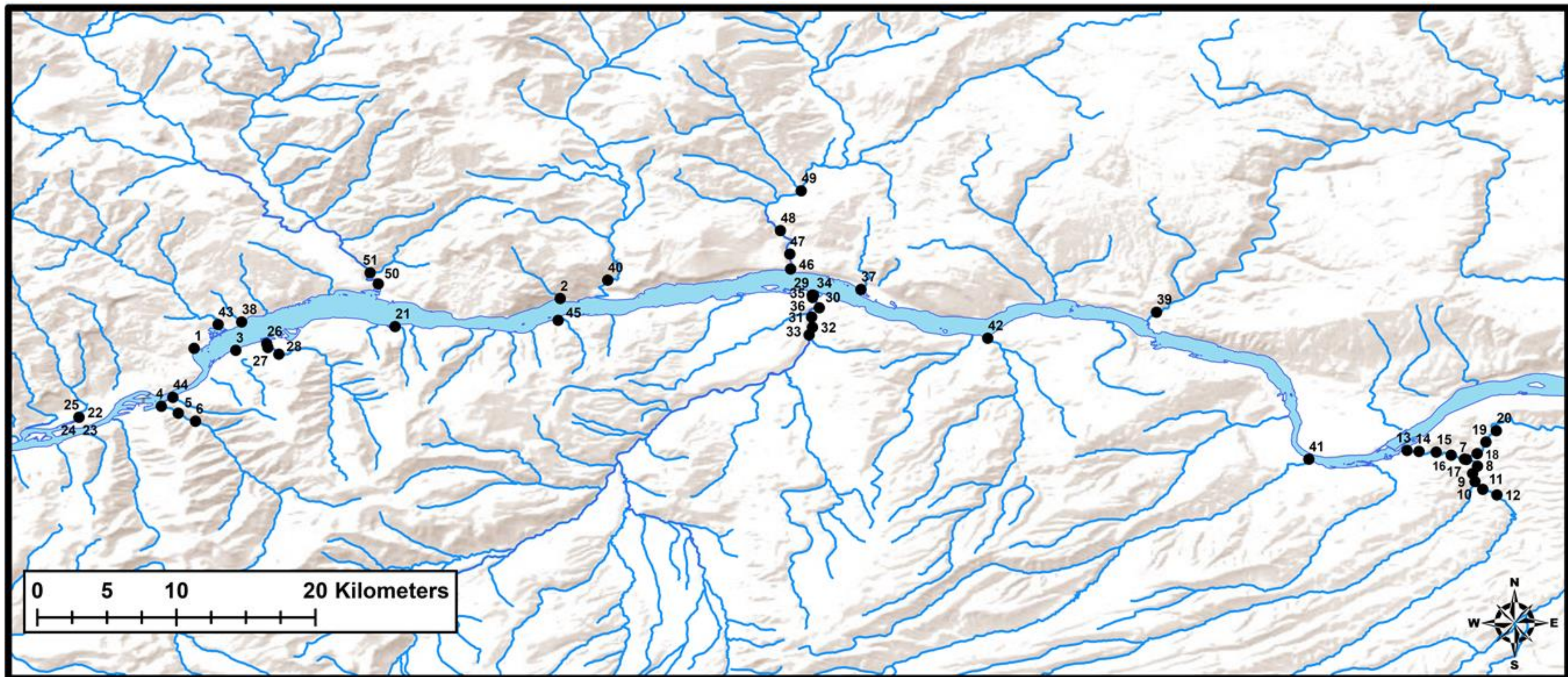

Figure 1. Locations of eDNA samples collected by during fall 2015. Site labels correspond to the Map ID column in Table 1.

Results and Discussion

Chum salmon DNA was detected in the four samples where Chum salmon were known to exist (Hamilton Spring Creek 1 – 4; Table 2, Figure 2). Further, chum salmon DNA was detected in five of the 47 experimental samples (Table 2, Figure 2). The number of triplicate PCR reactions for each sample that amplified can be used as a rough estimate of relative DNA concentration. Samples with amplification in all three reactions usually have more DNA than those with amplification in only one or two reactions.

There was no amplification of laboratory negative controls in this dataset indicating that these results were not influenced by laboratory contamination. The presence of PCR inhibitors was not detected in any of the 51 samples.

Although eDNA detection is generally more sensitive than traditional methods, it may still fail to detect an organism that is present. A variety of factors influence the detection and abundance of DNA in an environmental sample. For example, greater animal abundance and sampling proximity may increase probability of detection. Furthermore, eDNA production rate may vary with life history stage (e.g., high production during the breeding season; NGCWFC unpublished data; Turner et al. 2014). DNA detected in samples may also come from the carcass of an individual, particularly following eradication efforts, or from DNA sequestered in sediment (Merkes et al. 2014). Degradation of eDNA is influenced by factors such as water temperature and UV exposure (Pilliod et al. 2014). Additionally, some types of chemical compounds naturally found in streams may inhibit laboratory detection of eDNA (Jane et al. 2015). Field and laboratory methods can also influence eDNA detection (Renshaw et al. 2015).

The eDNA program at the National Genomics Center for Wildlife and Fish Conservation is actively investigating many of these unknowns, but has not presently quantified probability of detection in your ecological system. Additionally, because eDNA techniques detect an organism's DNA and not the organism itself, the precise nature of what is being detected is context dependent. For example, even the temporary presence of a single individual can produce a positive detection. This, along with occurrence of false negative detections, can be resolved through repeated sampling. The probability of receiving a false negative result will decline exponentially with multiple sampling visits across time. Furthermore, populations will produce repeated, positive detections in a basin, whereas individual migrants produce local and ephemeral detections.

Conclusions

Chum salmon DNA was detected in nine of the 51 samples analyzed, including four samples of known chum occupancy. Given the present methods, our interpretation of these results is that one or more individuals of the target species were present upstream from the sampling location with positive detections. Locations in which eDNA of any target species was not detected may have lacked that species, or that species may have been present in very low numbers or at distance upstream from the sampling point.

If you have any questions about the results or would like help interpreting these data, please contact us. We look forward to working with you in the future.

Table 2. Results of eDNA analysis to determine the presence of chum salmon for samples collected by Oregon Department of Fish and Wildlife.

Map ID	Stream	Site #	Chum Salmon Detected?[1]	# of Positive Replicates[2]	Collection Notes
1	Ashes Lake North trib	01	N	0	
2	Dog Creek	01	N	0	
3	Dry Creek	01	N	0	MAYBE Chum
4	Eagle Creek	01	**Y**	**3**	
5	Eagle Creek	02	N	0	
6	Eagle Creek	03	N	0	
7	Eightmile Creek	01	N	0	
8	Eightmile Creek	02	N	0	
9	Eightmile Creek	03	N	0	
10	Eightmile Creek	04	N	0	
11	Eightmile Creek	05	N	0	
12	Eightmile Creek	06	N	0	
13	Fifteenmile Creek	01	N	0	
14	Fifteenmile Creek	02	N	0	
15	Fifteenmile Creek	03	N	0	
16	Fifteenmile Creek	04	N	0	
17	Fifteenmile Creek	05	N	0	
18	Fifteenmile Creek	06	N	0	
19	Fifteenmile Creek	07	N	0	
20	Fifteenmile Creek	08	N	0	
21	Gorton Creek	01	N	0	
22	Hamilton Spring Creek	01	**Y**	**3**	KNOWN CHUM SITE; All 4 samples are from the same site on the same day
23	Hamilton Spring Creek	02	**Y**	**3**	KNOWN CHUM SITE; All 4 samples are from the same site on the same day
24	Hamilton Spring Creek	03	**Y**	**3**	KNOWN CHUM SITE; All 4 samples are from the same site on the same day
25	Hamilton Spring Creek	04	**Y**	**3**	KNOWN CHUM SITE; All 4 samples are from the same site on the same day
26	Herman Creek	01	N	0	
27	Herman Creek	02	N	0	
28	Herman Creek	03	N	0	
29	Hood River	01	N	0	
30	Hood River	02	N	0	
31	Hood River	03	N	0	
32	Hood River	04	N	0	
33	Hood River	05	N	0	
34	Hood River	01B	N	0	1B River Right
35	Hood River	01C	**Y**	**2/6**	1C CENTER
36	Hood River	01D	**Y**	**3**	1D THALWEG
37	Jewett Creek	01	N	0	
38	Kanaka Creek	01	N	0	
39	Klickitat	02	N	0	(former site 2)
40	Little White Salmon River	01	**Y**	**2/6**	

41	Mill Creek	01	N	0
42	Mosier Creek	01	N	0
43	Rock Creek	01	N	0
44	Ruckle Creek	01	N	0
45	Viento Creek	01	N	0
46	White Salmon River	01	N	0
47	White Salmon River	02	N	0
48	White Salmon River	04	N	0
49	White Salmon River	07	N	0
50	Wind River	01	**Y**	**3**
51	Wind River	02	N	0

[1]N, not detected; Y, detected

[2]Number of runs in each triplicate in which DNA of the target species was detected. Each sample was run three times unless otherwise noted

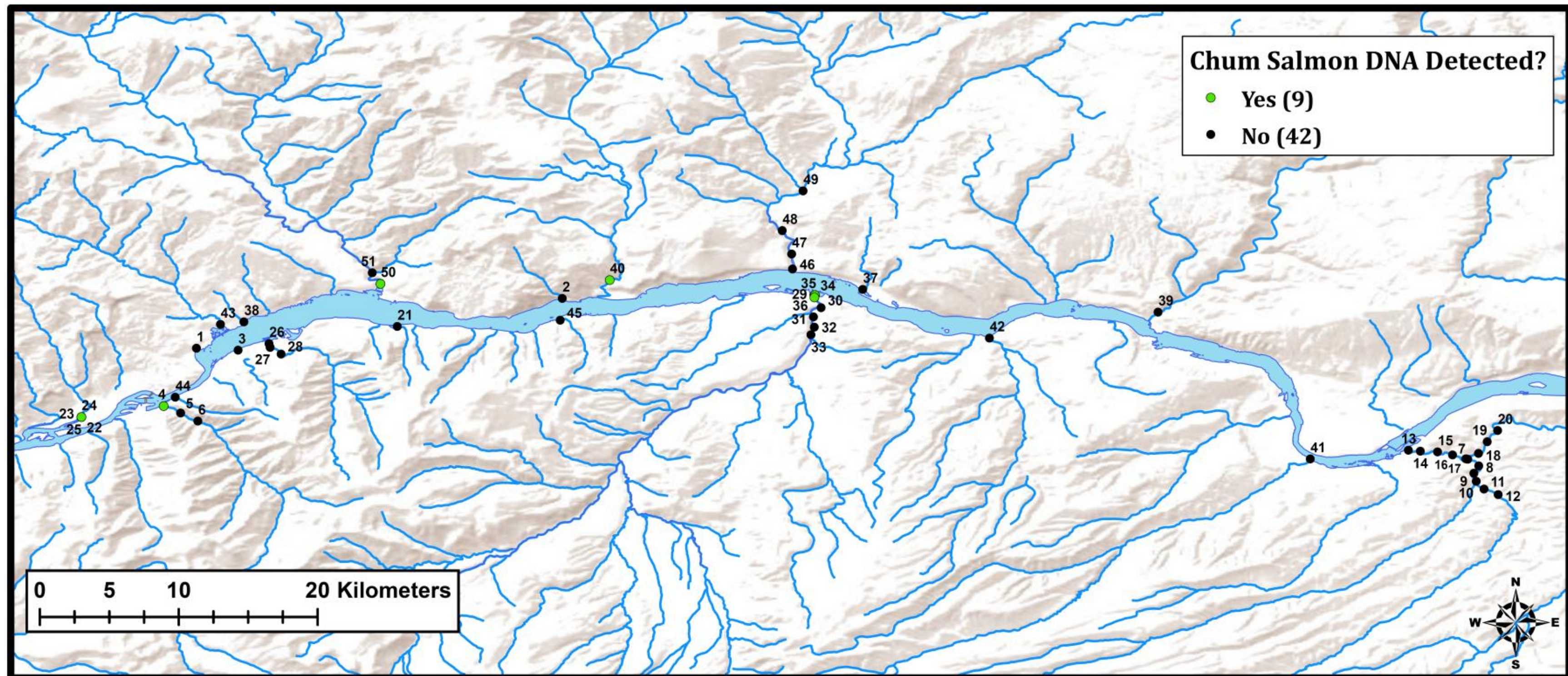

Figure 5. The eDNA results for samples analyzed for chum salmon DNA. Site labels correspond to the Map ID column in Tables 1 & 2.

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* Indicates publications from the RMRS eDNA program.

Supplemental Information

In a quantitative PCR reaction, light is emitted when DNA from a target species is present. This light is captured by the PCR instrument and plotted on a figure to help visualize the amount of DNA present in a sample (Figure S1). PCR is performed in cycles, wherein the amount target DNA (and thus fluorescence) is doubled with each cycle. Fluorescence will occur at an earlier cycle when there is more DNA present in a sample. If target DNA is not present in a sample, there will be no fluorescent light emitted during the qPCR reaction.

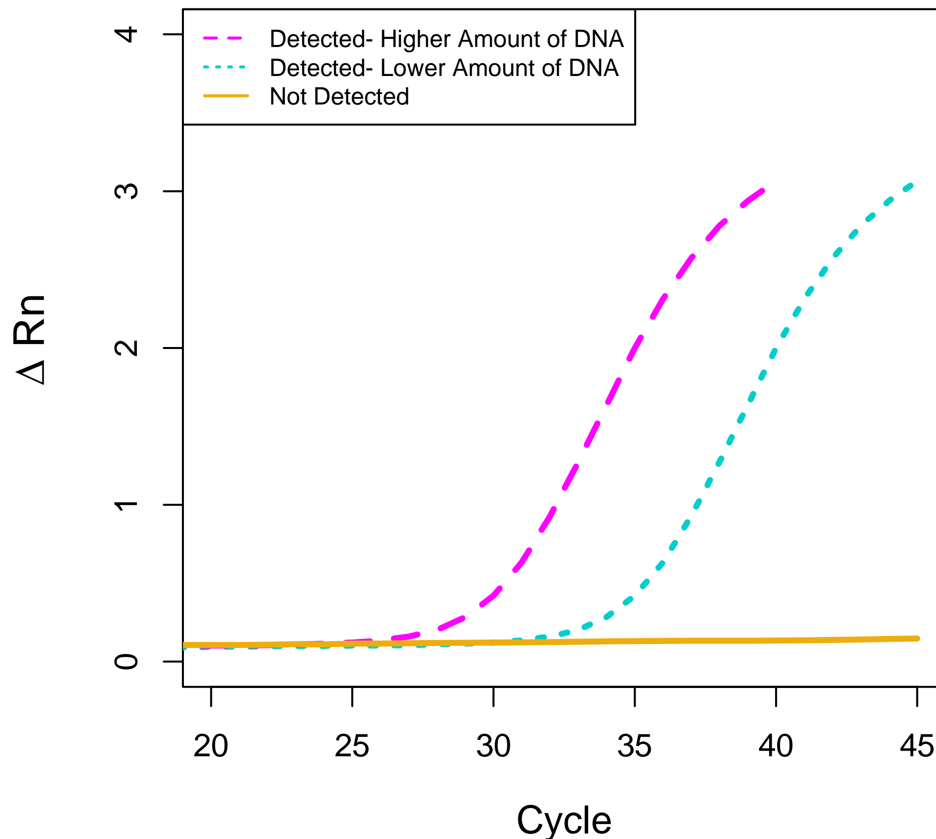

Figure S1. Amplification plot showing the change in normalized fluorescence (ΔRn) versus PCR cycle. When DNA from the target species is present, there is an increase in the amount of fluorescence (pink and blue dashed lines). Conversely, when there is no target DNA present in the sample, there is no increase in fluorescence above background levels (solid yellow line).

Each sample is also run with an internal positive control (IPC). The IPC is a separate, smaller reaction that is added to each sample, and used to determine if inhibitors are present in the sample that might alter our ability to detect DNA from the target species. For example, these inhibitors may be tannins or other compounds that lower the pH of water. If inhibition is detected in a sample (Figure S2), it is treated to remove inhibitors and re-analyzed with qPCR.

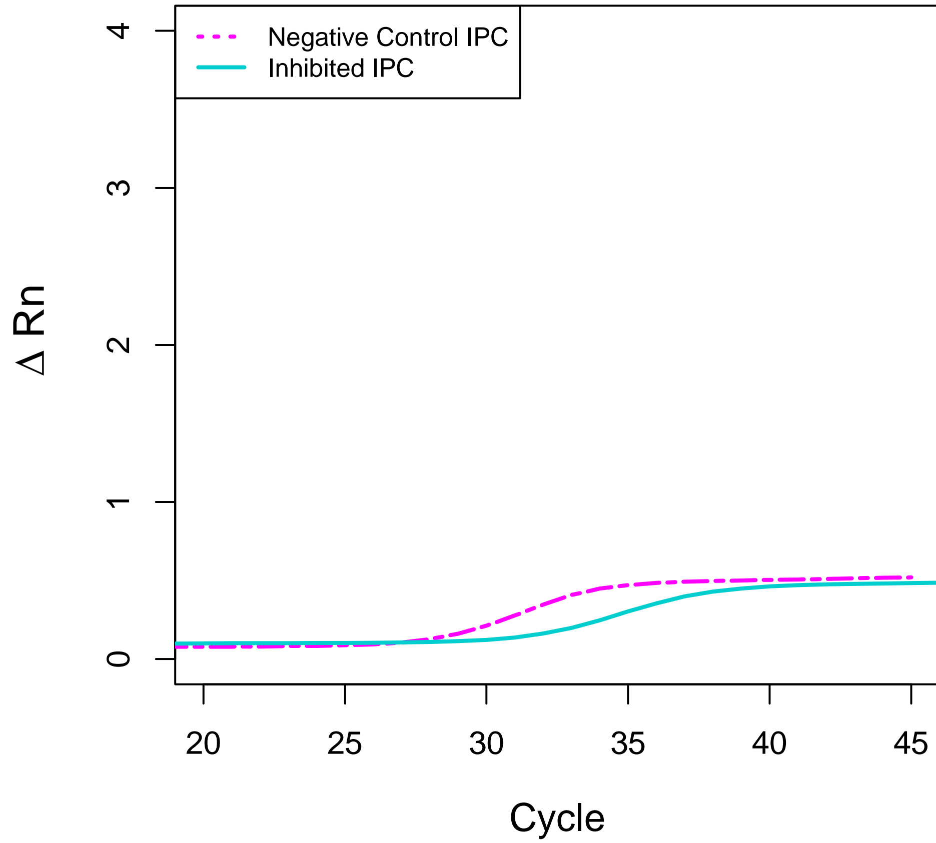

Figure S2. Amplification plot showing the change in normalized fluorescence (ΔRn) versus PCR cycle for the IPC reaction. When inhibitors are present in a sample, the IPC curve will be shifted compared to the negative control.