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Characterizing the distribution of an endangered salmonid using environmental DNA analysis

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ABSTRACT

Determining species distributions accurately is crucial to developing conservation and management strategies for imperiled species, but a challenging task for small populations. We evaluated the efficacy of environmental DNA (eDNA) analysis for improving detection and thus potentially refining the known distribution of Chinook salmon (*Oncorhynchus tshawytscha*) in the Methow and Okanogan Subbasins of the Upper Columbia River, which span the border between Washington, USA and British Columbia, Canada. We developed an assay to target a 90 base pair sequence of Chinook DNA and used quantitative polymerase chain reaction (qPCR) to quantify the amount of Chinook eDNA in triplicate 1-L water samples collected at 48 stream locations in June and again in August 2012. The overall probability of detecting Chinook with our eDNA method in areas within the known distribution was 0.77 (±0.05 SE). Detection probability was lower in June (0.62, ±0.08 SE) during high flows and at the beginning of spring Chinook migration than during base flows in August (0.93, ±0.04 SE). In the Methow subbasin, mean eDNA concentration was higher in August compared to June, especially in smaller tributaries, probably resulting from the arrival of spring Chinook adults, reduced discharge, or both. Chinook eDNA concentrations did not appear to change in the Okanogan subbasin from June to August. Contrary to our expectations about downstream eDNA accumulation, Chinook eDNA did not decrease in concentration in upstream reaches (0–120 km). Further examination of factors influencing spatial distribution of eDNA in lotic systems may allow for greater inference of local population densities along stream networks or watersheds. These results demonstrate the potential effectiveness of eDNA detection methods for determining landscape-level distribution of anadromous salmonids in large river systems.

1. Introduction

Salmon populations once abundant throughout the Pacific Northwest have declined dramatically, due largely to hydropower development, habitat degradation and overharvest (Mullan, 1987; Nehlsen et al., 1991; FR 76:42658, 2011). The Columbia River drainage once supported some of the largest known runs of Chinook salmon (*Oncorhynchus tshawytscha*) (Chapman, 1986; Utter et al., 1989). Spring Chinook of the Upper Columbia River Evolutionarily Significant Unit (ESU) are now among the most imperiled North American salmon and are currently listed as Endangered under the Endangered Species Act (ESA) (FR 64:41839, 1999). Costly conservation efforts such as hatchery supplementation, habitat restoration and harvest management have been implemented to conserve remaining populations (LCFRB, 2010; GAO RCED-93-41, 1993). The ability to accurately monitor changes in distribution and to rapidly track responses to management strategies is important for assessing the status and effectiveness of conservation efforts and informs effective decision making (Hernandez et al., 2006; Stem et al., 2005). One major challenge of determining or confirming the distribution of an aquatic species such as Chinook across large landscapes is the low detection rate with conventional methods, especially when the species is present at low densities.

An emerging method that improves detection of many aquatic species is environmental DNA (eDNA) analysis. This method determines presence of a species based on the collection, extraction, and amplification of DNA from the environment (Ficetola et al., 2008;

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Goldberg et al., 2011; Jerde et al., 2011). Recent studies have demonstrated that eDNA detection can be a reliable method for determining the distribution of various species of fish in freshwater ecosystems (Jerde et al., 2011; Dejean et al., 2011; Minamoto et al., 2012; Takahara et al., 2012; Thomsen et al., 2012a; Takahara et al., 2013; Wilcox et al., 2013) as well as in oceans (Thomsen et al., 2012b). eDNA detection methods have been shown to be more sensitive than traditional sampling methods, such as electrofishing or visual surveys, particularly when determining presence of rare or low-density species. Studies have also shown positive correlation between eDNA concentration and relative abundance of the target organism (Thomsen et al., 2012b; Takahara et al., 2012; Goldberg et al., 2013; Pilliod et al., 2013). Inference to the upstream location of stream organisms detected using eDNA is uncertain (Pilliod et al., 2014), but a recent study showed that invertebrate DNA can be transported and detected downstream from known populations as far as 12 km (Deiner and Altermatt, 2014).

Despite the demonstrated effectiveness of detecting fish with eDNA detection methods, few fisheries management programs are currently taking advantage of this state-of-the-art tool for determining the presence of sensitive, native species. This study was designed to test the effectiveness of eDNA detection methods for determining the distribution of threatened and endangered Chinook salmon populations in the Methow and Okanogan Subbasins of the Columbia River by comparing a distribution resulting from eDNA detection to the current, known distribution of the species. We also examined several factors that may influence Chinook eDNA concentrations, such as time of sampling, water temperature as it relates to Chinook habitat preference and tolerance, and sample location along a stream.

2. Methods

2.1. Study species

Interior Columbia River Chinook are comprised of two lineages, described as ocean- and stream-type, each with a different life history strategy (Healey, 1991; Waples et al., 2004). Ocean-type Chinook adults migrate to freshwater throughout summer and fall and spawn primarily in mainstem rivers. Stream-type Chinook migrate upstream during peak spring flows, which allow them to access preferred spawning habitat in higher headwater tributaries. Spawning takes place in the late summer and fall for both strains, but in different habitats resulting in near complete reproductive isolation (Waples et al., 2004; Beacham et al., 2006; Narum et al., 2007). Upon emergence, juveniles of ocean-type Chinook migrate to the ocean their first spring, as sub-yearlings, while stream-type juveniles remain in freshwater until their second spring before migrating to the ocean as yearlings (Healey, 1991). Therefore, stream-type Chinook are likely present in freshwater systems throughout the year, while ocean-type Chinook are likely only present a portion of the year. Hereafter, we will refer to stream- and ocean-type Chinook by their more commonly used names: *spring* and *summer* Chinook, respectively.

2.2. Study area – Methow Subbasin

The Methow Subbasin in western Okanogan County, Washington USA drains 2900 km^2 via the Methow, Chewuch and Twisp Rivers before emptying into the Columbia River near Pateros, Washington (Fig. 1). The Methow contains both spring and summer Chinook (UCSRB, 2007). In 2012, 52,846 Chinook were counted as they migrated from the ocean upstream past Wells Dam, on their way to the Methow and Okanogan Subbasins (DeHart, 2013).

We used existing Chinook distribution maps (UCSRB, 2007) to select sites ($n = 32$) categorized a priori as (1) Chinook likely present (i.e. within the known distribution of Chinook, $n = 21$), or (2) Chinook likely absent (i.e. outside of the known distribution of Chinook, $n = 11$) (Fig. 1, Appendix A). These site-types will be referred to hereafter as *Chinook likely present* and *Chinook likely absent*. Three sample sites of the latter category were physically inaccessible to Chinook (above barriers to anadromy) and served as stream negative controls. All sites in the Methow Subbasin were sampled twice, once during high, spring flows from 22 to 27 June 2012, and again during reduced late-summer flows from 9 to 13 August. We also collected three water samples from a juvenile spring Chinook rearing tank at US Fish and Wildlife Service (USFWS) Winthrop National Fish Hatchery (WNFH, Winthrop, WA USA) on 26 June 2012. These samples served as laboratory positive controls, and were omitted from the distribution analysis. In general, stream flows were approximately 10 times higher during spring runoff in June than later in August, as flows approached base-flow. During June sampling, flows ranged from 242 m^3/s in the mainstem Methow River (USGS stream gage 12449950) to approximately <1 m^3/s in small tributaries (visual estimate).

2.3. Study area – Okanogan Subbasin

The Okanogan Subbasin is adjacent to and east of the Methow and spans the border between Washington, United States and British Columbia, Canada (Fig. 1). The Okanogan Subbasin is more than four times the size of the Methow, draining approximately 13,000 km^2. The Okanogan contains summer Chinook; spring Chinook were extirpated from this subbasin by the 1930s (UCSRB, 2007). Migrating spring Chinook adults from nearby subbasins may occasionally stray into the Okanogan, suggesting potential for presence of a very low-density population (J. Arterburn, CCT F&W biologist, personal communication). The Colville Confederated Tribes plan to reestablish spring Chinook throughout much of their historic range in the Okanogan as an experimental population under section 10(j) of the ESA (FR 76:42658, 2011). The source stock for the Okanogan reintroduction would initially come from the adjacent Methow Subbasin. We sampled 16 sites in the Okanogan Subbasin (Fig. 1, Appendix A), consisting of both Chinook likely present sites ($n = 7$) and Chinook likely absent sites ($n = 9$). All sites were sampled twice, once during high spring flows from 18 to 21 June 2012, and again during reduced late-summer flows from 14 to 17 August. These surveys will serve as the baseline distribution (prior to the reintroduction of spring Chinook to the Okanogan Subbasin) and can be used as part of a monitoring program to track changes in Chinook distribution following their reintroduction.

As in the Methow Subbasin, stream flows in the Okanogan were approximately 10 times higher during spring runoff in June than in August, as flows approached base-flow. During June sampling, flows ranged from 390 m^3/s in the mainstem Okanogan River to 0.03 m^3/s in small tributaries (USGS stream gages 12447200 and 12438900, respectively).

2.4. Field methods

At each sample site, we filtered three 1-L stream water samples, treated as replicates, followed by one 1-L negative control composed of distilled water. Water was filtered through a Whatman Disposable Filter Funnel with 47 mm diameter, 0.45 μm pore size cellulose nitrate type WCN sterile filter membrane (Whatman International Ltd., England). The filter funnel was connected to a Masterflex L/S Econodrive peristaltic pump. We held the filter funnel just below the surface of the stream, facing upstream, into the current. The pump was engaged until 1-L of stream water was collected. We collected water samples at approximately an arm's

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